NANOTECHNOLOGY APPLIED TO BIOFILMS I

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QUÍMICO FARMACÉUTICO

UNU BIOLAC · Ministerio de Educación y Cultura · iibce INSTITUTO DE INVESTIGACIONES BIOLÓGICAS CLEMENTE ESTABLE · LBM LABORATORIO DE BIOFILMS MICROBIANOS · SCIAN Laboratory for Scientific Image Analysis · BNI CHILE · ICBM INSTITUTO DE CIENCIAS BIOMÉDICAS · FACULTAD DE MEDICINA UNIVERSIDAD DE CHILE

2025

October, 13-24

Innovative Biotechnological Approaches for Biofilm Control and Characterization

NANOTECHNOLOGY

- **“There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics” — Richard Feynman** at the annual American Physical Society meeting at Caltech on December 29, 1959.
- ***“Nano-technology' mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule.”*** **— Norio Taniguchi** International Conference on Production Engineering, 1974, Tokyo.

NNI
National Nanotechnology Initiative

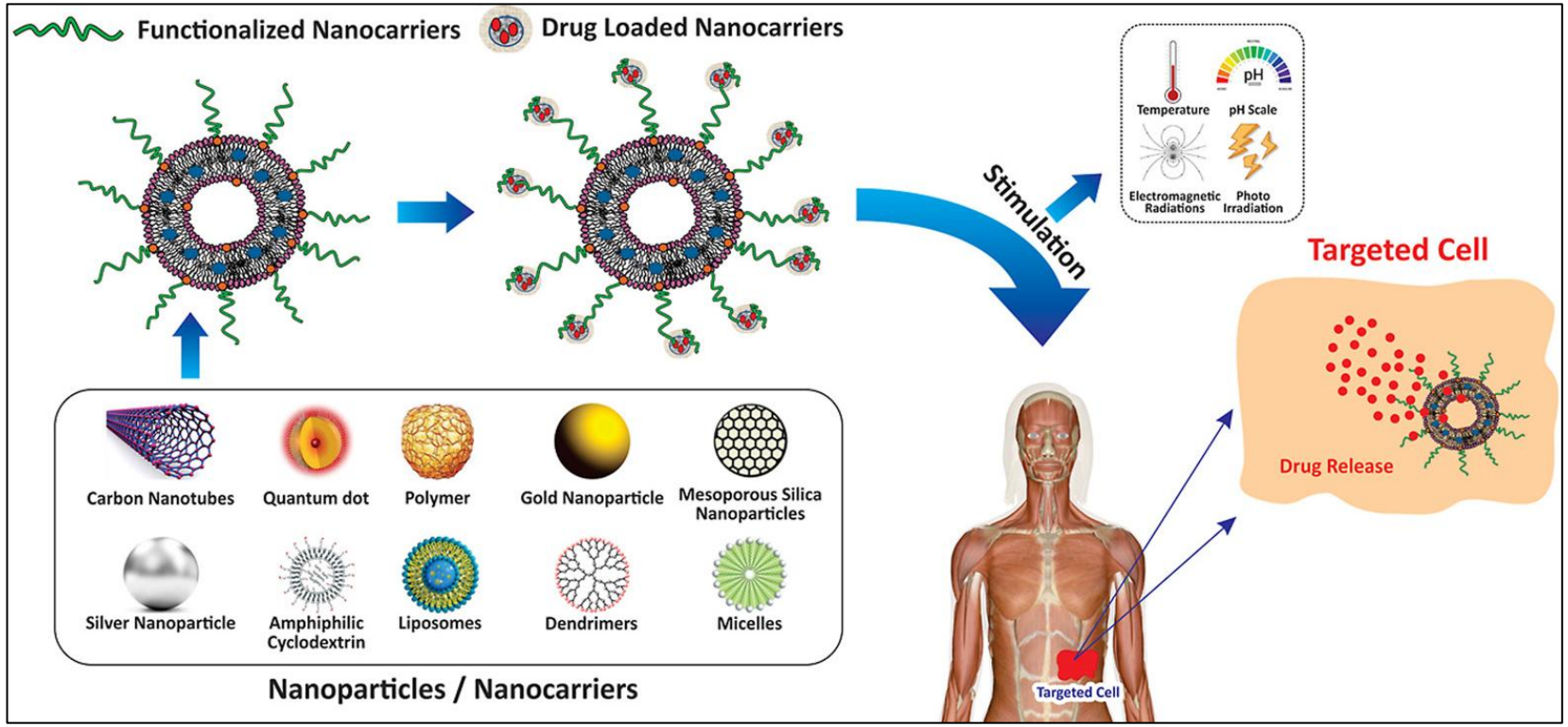
NANOTECHNOLOGY

Nanotechnology is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications.

Matter can exhibit unusual physical, chemical, and biological properties at the nanoscale, differing in important ways from the properties of bulk materials, single atoms, and molecules.

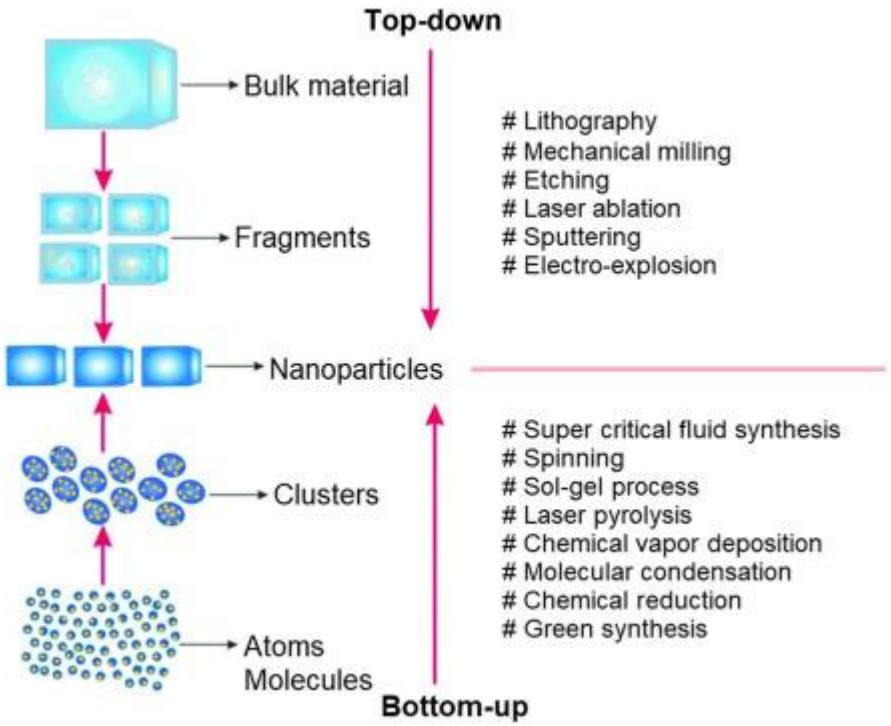
Some nanostructured materials are stronger or have different magnetic properties compared to other forms or sizes of the same material. Others are better at conducting heat or electricity. They may become more chemically reactive, reflect light better, or change color as their size or structure is altered.

NANOTECHNOLOGY APPLIED TO MEDICINE



Shah A. *et al.*, J Drug Deliv Sci Technol. 2021;61; 102426.

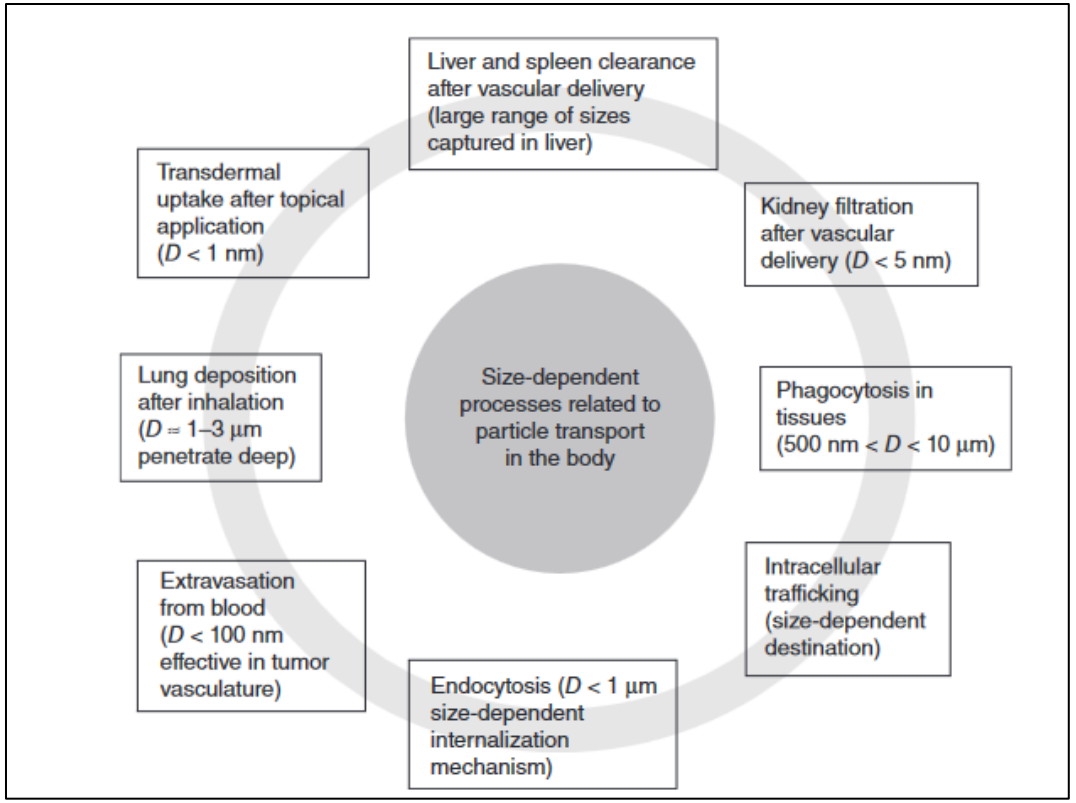
NANOCARRIERS



They are colloidal systems on the nanometric scale (1 - 100 nm), capable of transporting drugs or other substances of different nature.

Chitra Devi Venkatachalam, et al. Chapter 5 - Green synthesis of nanoparticles—metals and their oxides, Editor(s): R. Praveen Kumar, B. Bharathiraja, Nanomaterials, Academic Press, 2021, Pages 79-96, ISBN 9780128224014, doi.org/10.1016/B978-0-12-822401-4.00012-X.

NANOCARRIERS

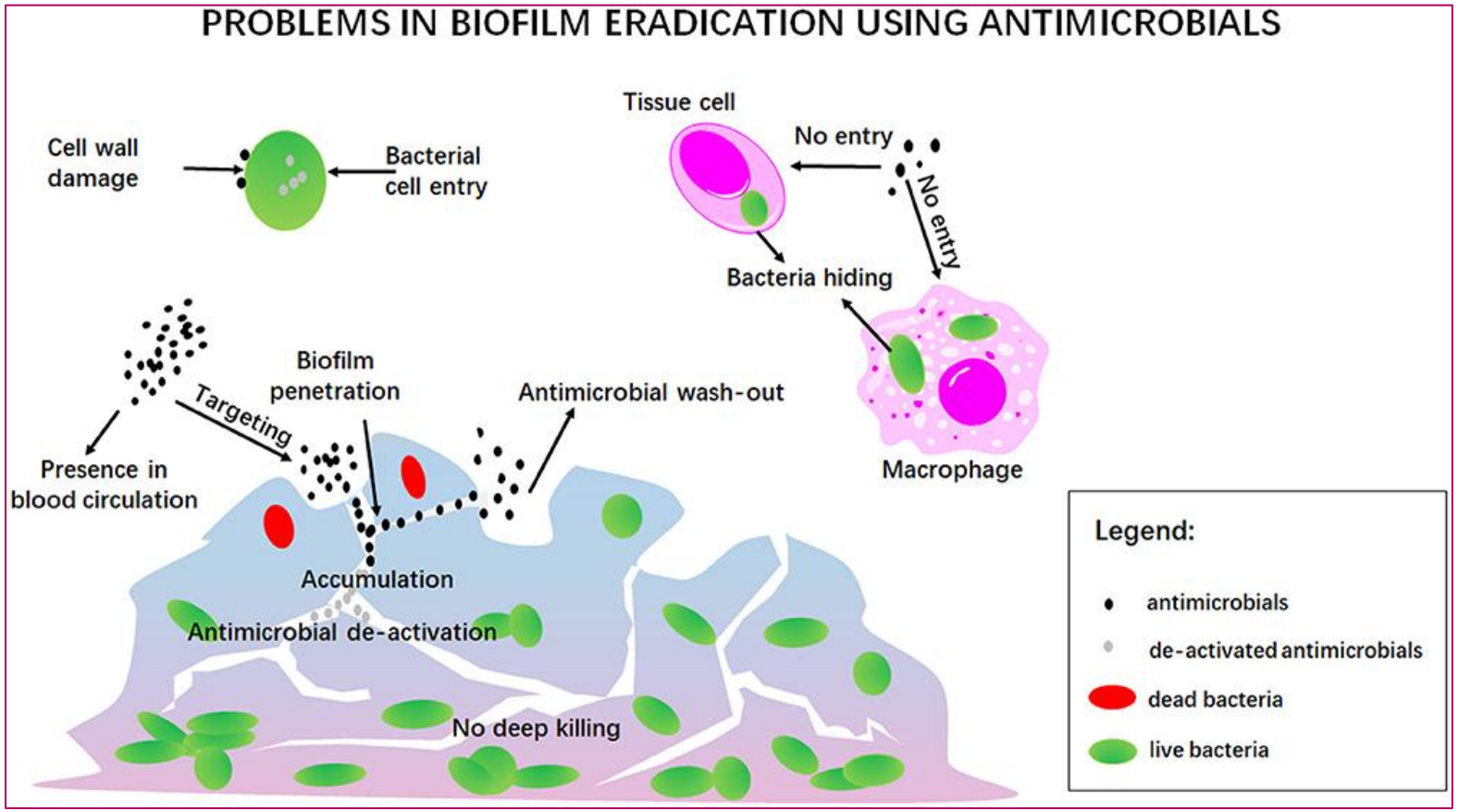


Particle Size Relative to Clearance and Applications

Particle Size (Based on Rigid Sphere)	Nanomedicine Applications
<10 nm	Rapidly cleared through extravasation or renal clearance
10–20 nm	Detection, imaging, potential to cross blood–brain barrier (BBB)
20–100 nm	Drug/gene delivery, cancer therapy, sites of inflammation (optimal range to escape physiological barriers; high circulation potential, reduced filtration by liver and spleen)
100–200 nm	Drug/gene delivery (high potential for prolonged circulation)
200 nm–1 µm	Generally cleared by the spleen
>1 µm	Usually opsonized and accumulate in liver and spleen, cleared from circulation almost immediately

Mitragotri S, Lahann J. Physical approaches to biomaterial design. Nat Mater 2009, 8:15–23. doi:10.1038/nmat2344

ATB NANOCARRIERS



Wang DY, van der Mei HC, Ren Y, Busscher HJ, Shi L. Lipid-Based Antimicrobial Delivery-Systems for the Treatment of Bacterial Infections. Front Chem. 2020

ATB NANOCARRIERS

Use lower concentrations of ATB (rational use)	• ATB Protection/Shielding
Surface Modifications	• Site-Specific Action
BF Disruption	• Planktonic Cells • Interaction/Denaturation EPS

Din, F. et al. (2017). Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *International journal of nanomedicine*, *12*, 7291–7309.

ATB NANOCARRIERS

Use lower concentra
of ATB (rational u

Surface Modificat

BF Disruption

ding

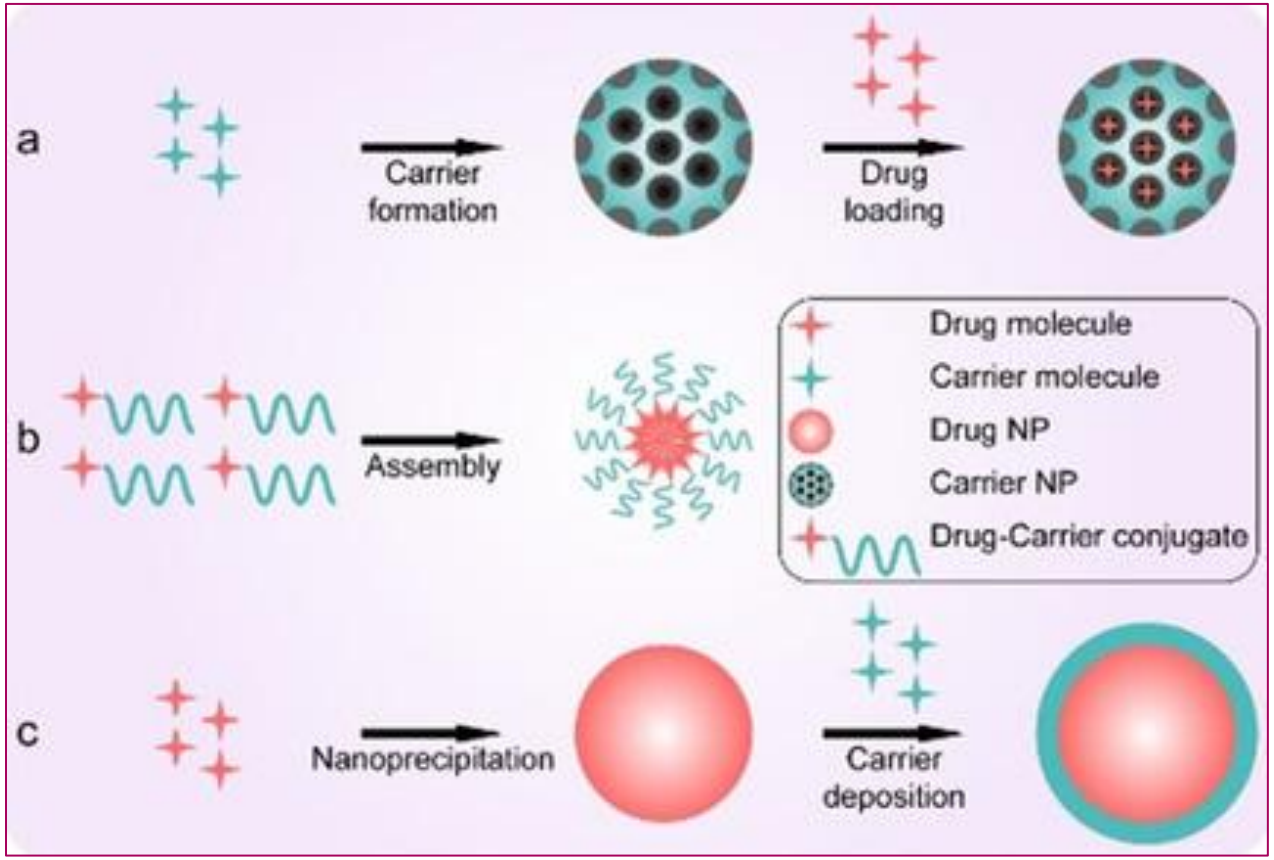
n

interaction/ Denaturation EPS

Strain	MIC against free antibiotics (mg/L)	MIC against liposomal encapsulated antibiotics (mg/L)	References
Vancomycin			
E. coli	512	6–25	Nicolosi et al., 2010
	512	10.5	
Klebsiella	512	25–50	
P. aeruginosa	512	50	
	512	83.7	
Acinetobacter baumanii	512	6–125	
S. aureus (MRSA)	1	0.5	Bhise et al., 2018
Amikacin			
P. aeruginosa	8	4	Mugabe et al., 2006

Din, F. et al. (2017). Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *International journal of nanomedicine, 12*, 7291–7309.

ATB NANOCARRIERS



Y. Liu, G. Yang, S. Jin, L. Xu, C.-X. Zhao, *ChemPlusChem* **2020**, *85*, 2143.

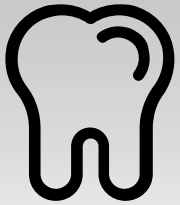
HOW CAN WE USE NATOTECHNOILOGY TO CONTROL BF?

SURFACE MODIFICATION

ATB/ATM NANOCARRIERS

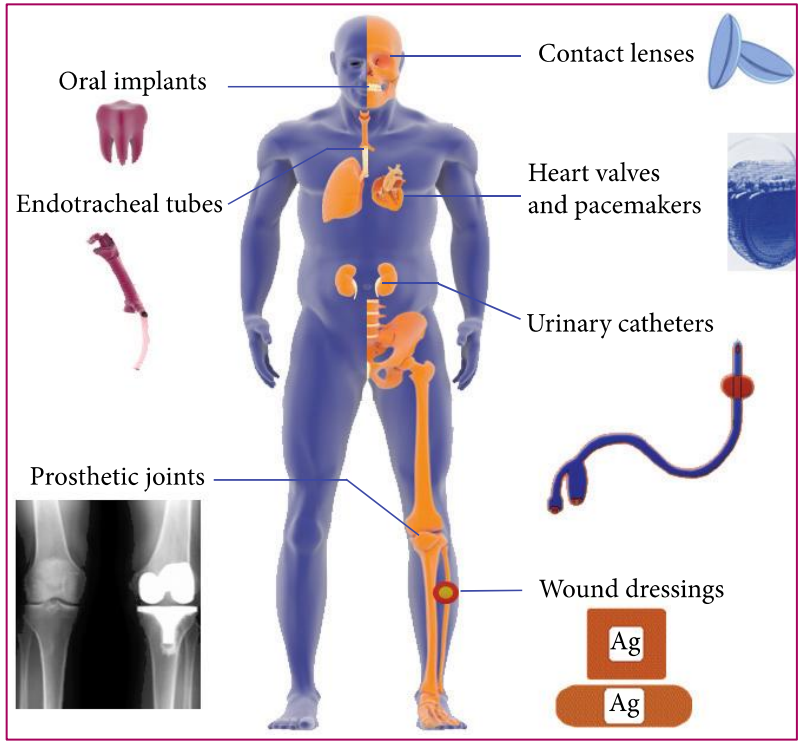
HOW CAN WE USE NATOTECHNOILOGY TO CONTROL BF?

SURFACE MODIFICATION



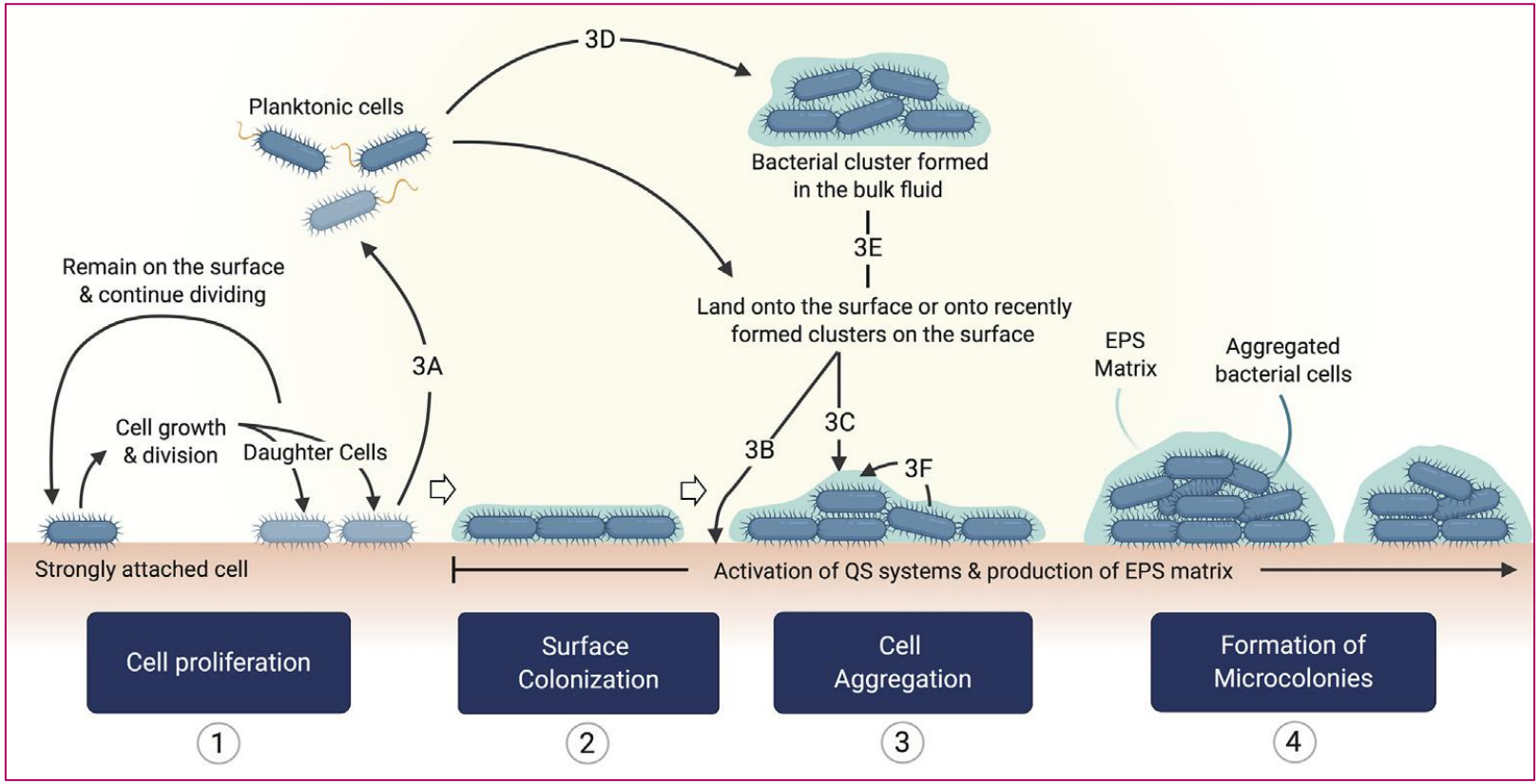
SURFACE MODIFICATION

- Central venous access catheters
- Urinary catheters
- Endotracheal tubes
- Dental implants
- Joint implants
- Pacemakers and Heart Valves



Ramasamy M, Lee J. Recent Nanotechnology Approaches for Prevention and Treatment of Biofilm-Associated Infections on Medical Devices. Biomed Res Int. 2016.

SURFACE MODIFICATION



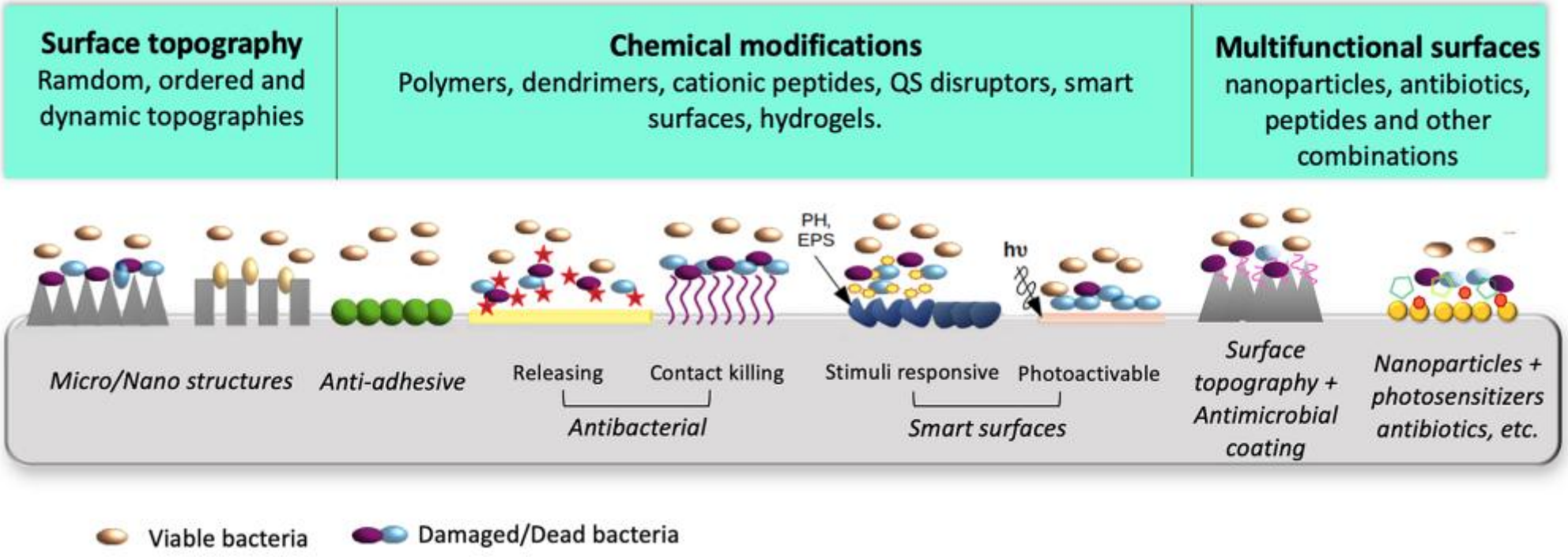
Guzmán-Soto I. et al. Mimicking biofilm formation and development: Recent progress in *in vitro* and *in vivo* biofilm models. iScience. 2021

SURFACE MODIFICATION

- Antifouling coatings (hardness, filler, hydrophobicity)
- Anti-adhesive modifications (polymers)
- Addition of antimicrobials.
- Chemical modification.
- Addition of bioactive molecules (QSi).

Ramasamy M, Lee J. Recent Nanotechnology Approaches for Prevention and Treatment of Biofilm-Associated Infections on Medical Devices. Biomed Res Int. 2016.

SURFACE MODIFICATIONS



Ghilini F, Pissinis DE, Miñán A, Schilardi PL, Diaz C. How Functionalized Surfaces Can Inhibit Bacterial Adhesion and Viability. ACS Biomater Sci Eng. 2019 Oct 14;5(10):4920-4936. doi: 10.1021/acsbiomaterials.9b00849.

SURFACE MODIFICATION

Implants
Metallic Surface

Catheters
Polymers

METALLIC SURFACE MODIFICATIONS

Table 2 Summary of Implant Coatings

Implant coating	Example	Studies	Outcome
Carbon coating[15–18]	Currently not on the market; still being investigated	In vitro, in vivo studies, and clinical studies	Improved biologic properties and histocompatibility but studies are still under way
Bisphosphonates[19–22]	Currently not on the market; still being investigated	No long-term studies available	No long-term studies available
Bone stimulating Factors[23–27]	Currently not on the market; still being investigated	Pilot animal studies and clinical studies	Studies are still under way
Bioactive glasses and ceramics[28–30]	Currently not on the market; still being investigated	Chemical, in vivo, and in vitro studies	Studies are still under way
Fluoride coatings[31]	OsseoSpeed	In vitro studies	Selective osteoblast differentiation results
Hydroxyapatite (HA)[32–36]	Restore Implant system	In vivo, in vitro, and retrieval studies	Most commonly used type of implant coating; other implant coating studies mainly use HA as a control
Titanium/titanium nitride[37–41]	IonFusion	In vitro, in vivo, and clinical studies	Titanium mechanical properties are considered in relation to the degree of osseointegration

Xuereb M, et al. Int J Prosthodont. 2015 Jan-Feb;28(1):51-9.

METALLIC SURFACE MODIFICATIONS

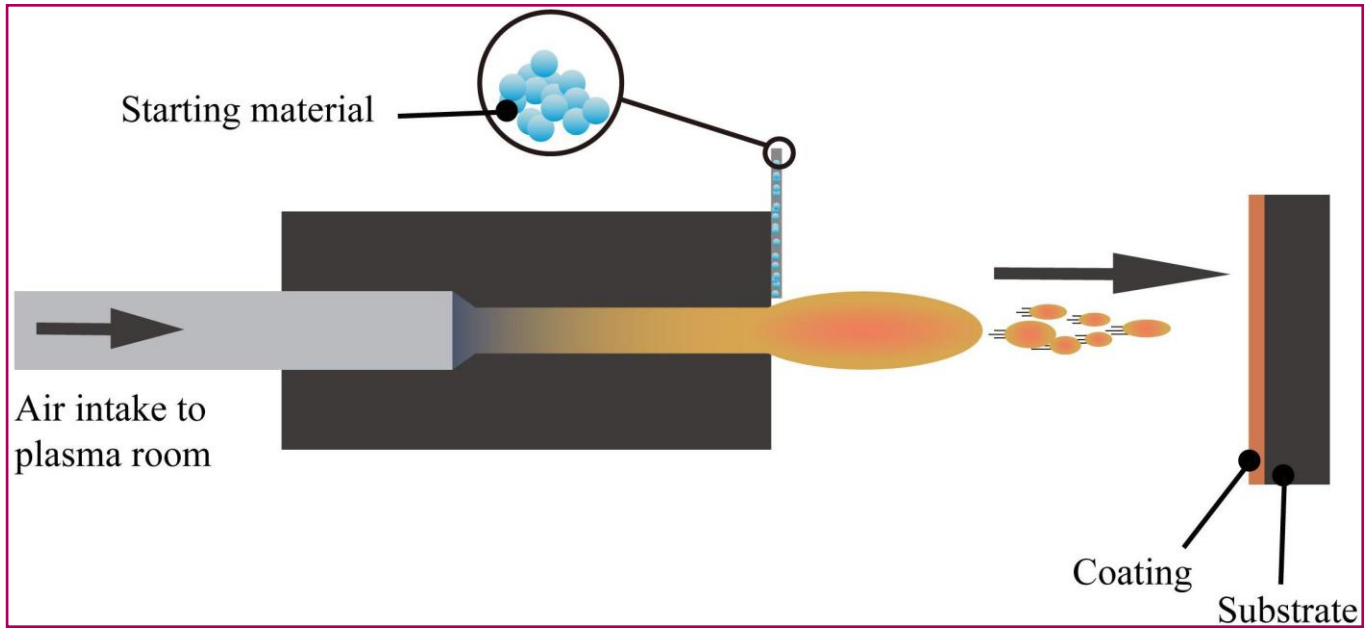
Plasma Spraying

Electrochemical Anodization

Xuereb M, et al. Int J Prosthodont. 2015 Jan-Feb;28(1):51-9.

PLASMA SPRAYING

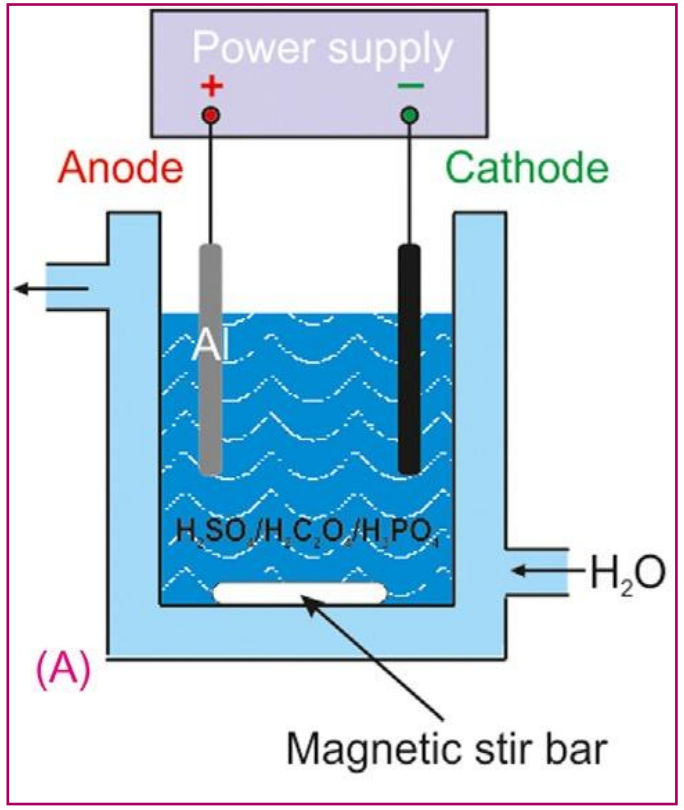
- Gold Standard.
- Ceramics (HA) and Metals.
- Nanometer Coating.
- High stability and control.
- High deposition efficiency and low porosity.



Liu Jianqiao, et al. Frontiers in Bioengineering and Biotechnology, (8), 1314, 2020.

ELECTROCHEMICAL ANODIZATION

- Anodic oxide films.
- Compact, nanoporous, or nanotubular morphology.
- Metal doping (Zn, Ag, Au, Cu, etc.).
- Structural modification.
- Low cost.
- Technical scalability.



Grzegorz D. Sulka, Chapter one - Introduction to anodization of metals, In Micro and Nano Technologies, Nanostructured Anodic Metal Oxides,Elsevier,2020,Pages 1-34.

ELECTROCHEMICAL ANODIZATION

Nanotube formation on the surface of titanium implants.

- Low cost
- Control of surface characteristics

  - Nanotube diameter can be controlled by oxidation voltage.

  - Nanotube length is controlled by a combination of oxidation time, oxidation temperature, and electrolyte pH.

Liu Jianqiao, et al. Frontiers in Bioengineering and Biotechnology, (8), 1314, 2020.

ELECTROCHEMICAL ANODIZATION

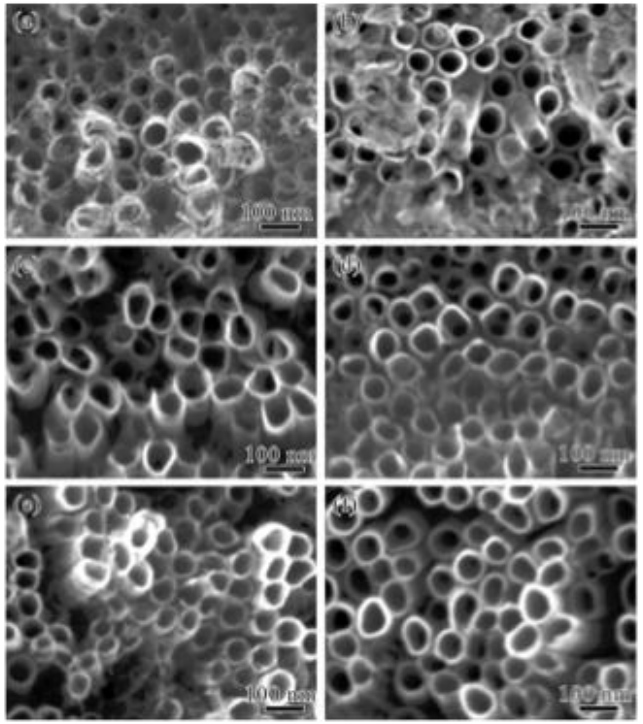


Figure 4. FE-SEM images representing the top view of ZnO-TNTs. (a) Undoped TNTs (spray-gold), (b) 1 mM · L^{-1} ZnO-TNTs, (c) 2 mM · L^{-1} ZnO-TNTs, (d) 3 mM · L^{-1} ZnO-TNTs, (e) 4 mM · L^{-1} ZnO-TNTs, (f) 5 mM · L^{-1} ZnO-TNTs.

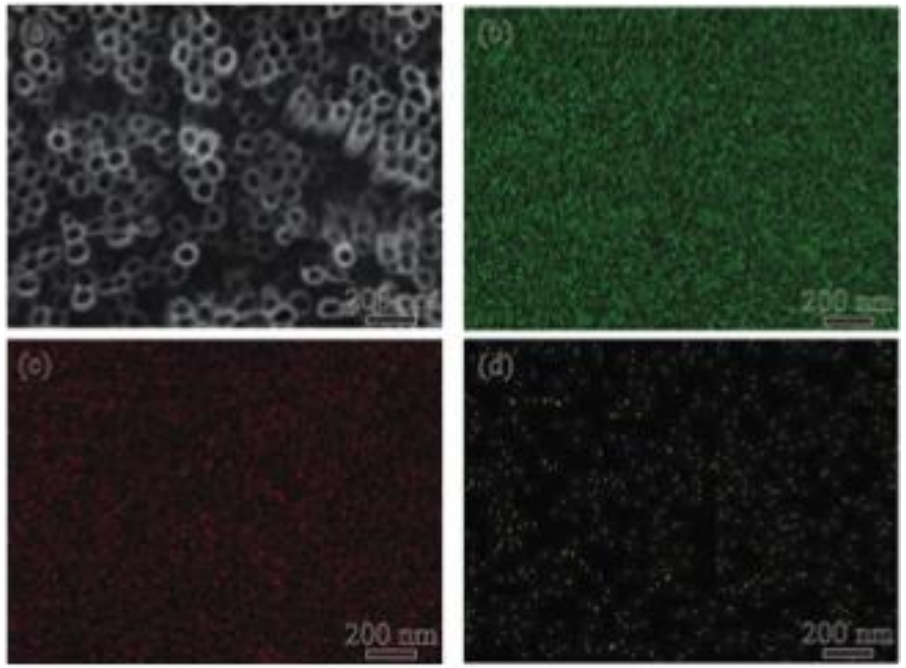
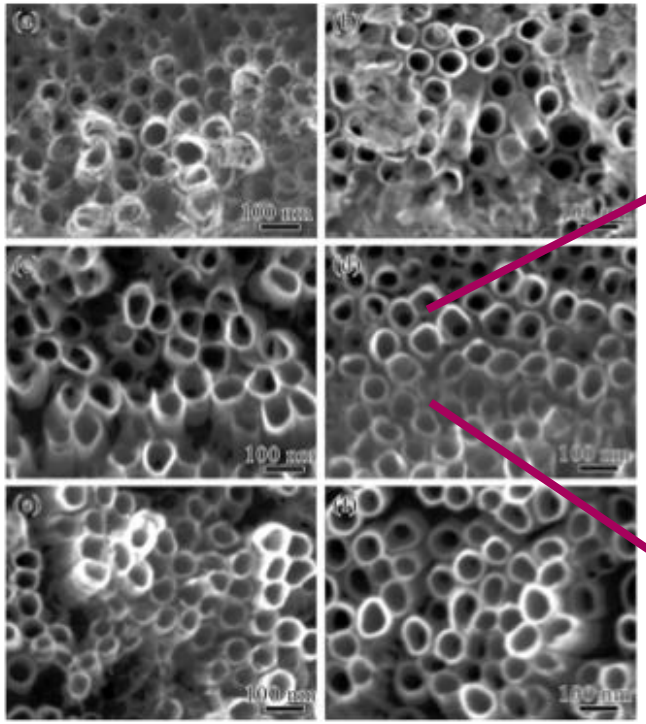


Figure 8. EDS analysis of ZnO-TNTs. (a) The selected area, (b) the distribution of Ti, (c) the distribution of O, (d) the distribution of Zn.

Chang F, et al. J Nanosci Nanotechnol. 2019 Apr 1;19(4):2070-2077.

ELECTROCHEMICAL ANODIZATION



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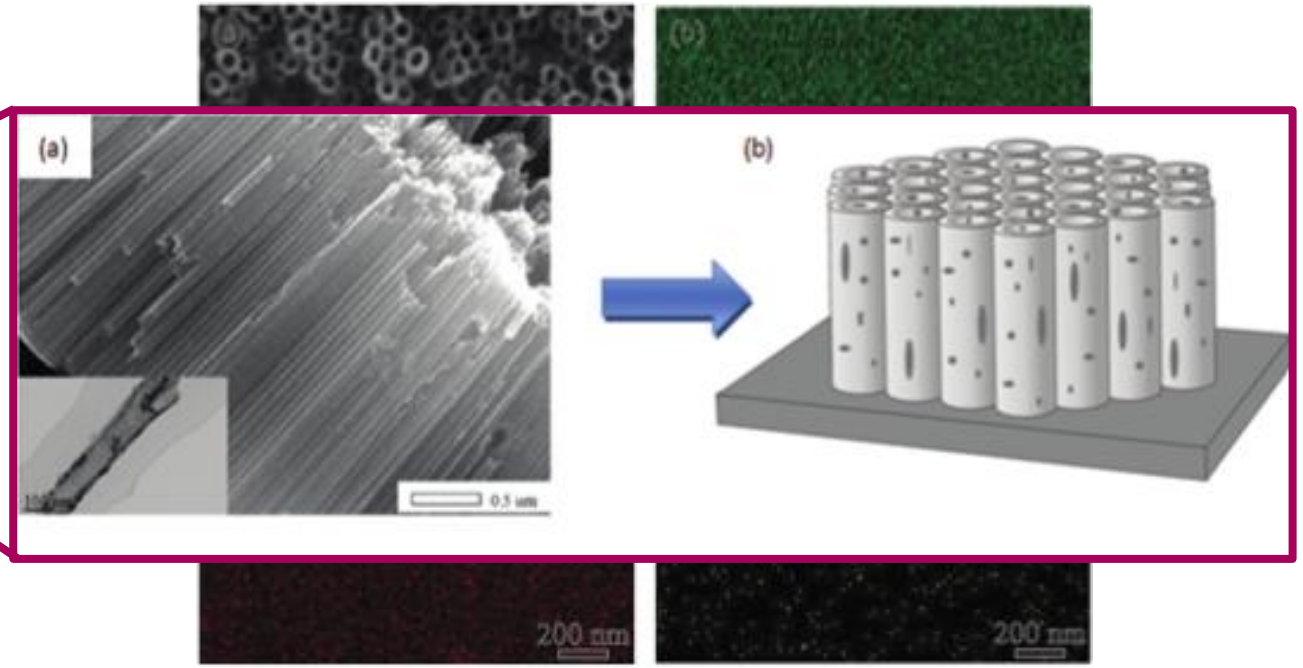

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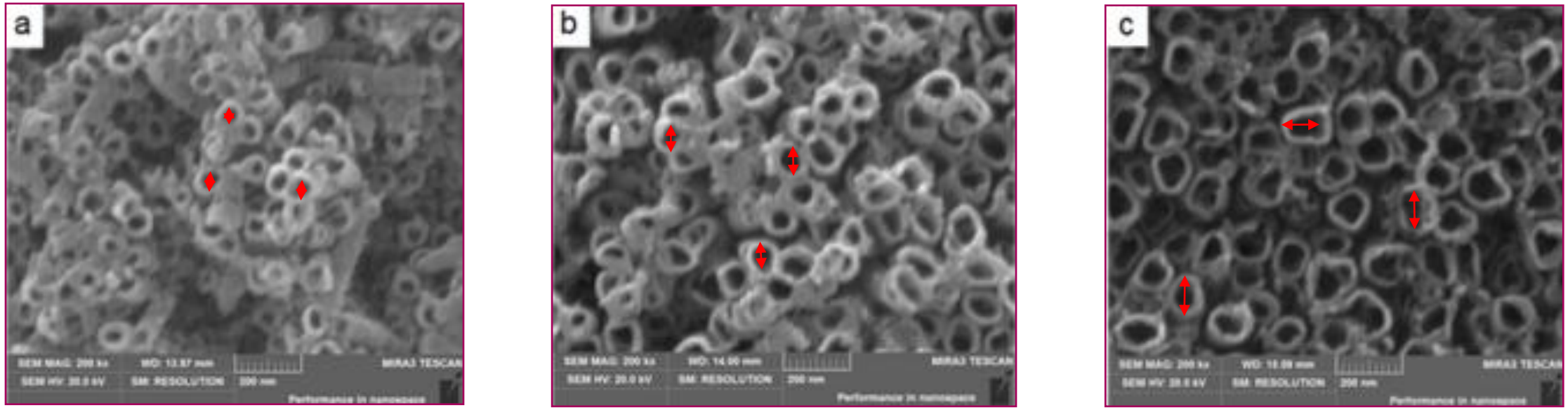


Figure 3. SEM images of nanotube arrays anodized at the following times: (**a**) 3 h, (**b**) 6 h and (**c**) 12 h, with inner diameters of approximately 44.5, 71.2 and 136.8 nm, respectively.

Chang F, et al. J Nanosci Nanotechnol. 2019 Apr 1;19(4):2070-2077.

FDA GUIDELINES

The FDA does **NOT** approve the individual use of materials or coatings, but rather the finished medical device as a whole.

FDA evaluates:

- Metallurgical analysis.
- Microstructure of the modified surface (thickness, shape, size, diameter or area, pore diameter, volume).
- Physical properties (hardness).
- Mechanical properties (strength/fatigue).
- Biocompatibility.
- Clinical information.

[online: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-document-testing-orthopedic-implants-modified-metallic-surfaces-apposing-bone-or-bone].

HOW CAN WE USE NATOTECHNOILOGY TO CONTROL BF?

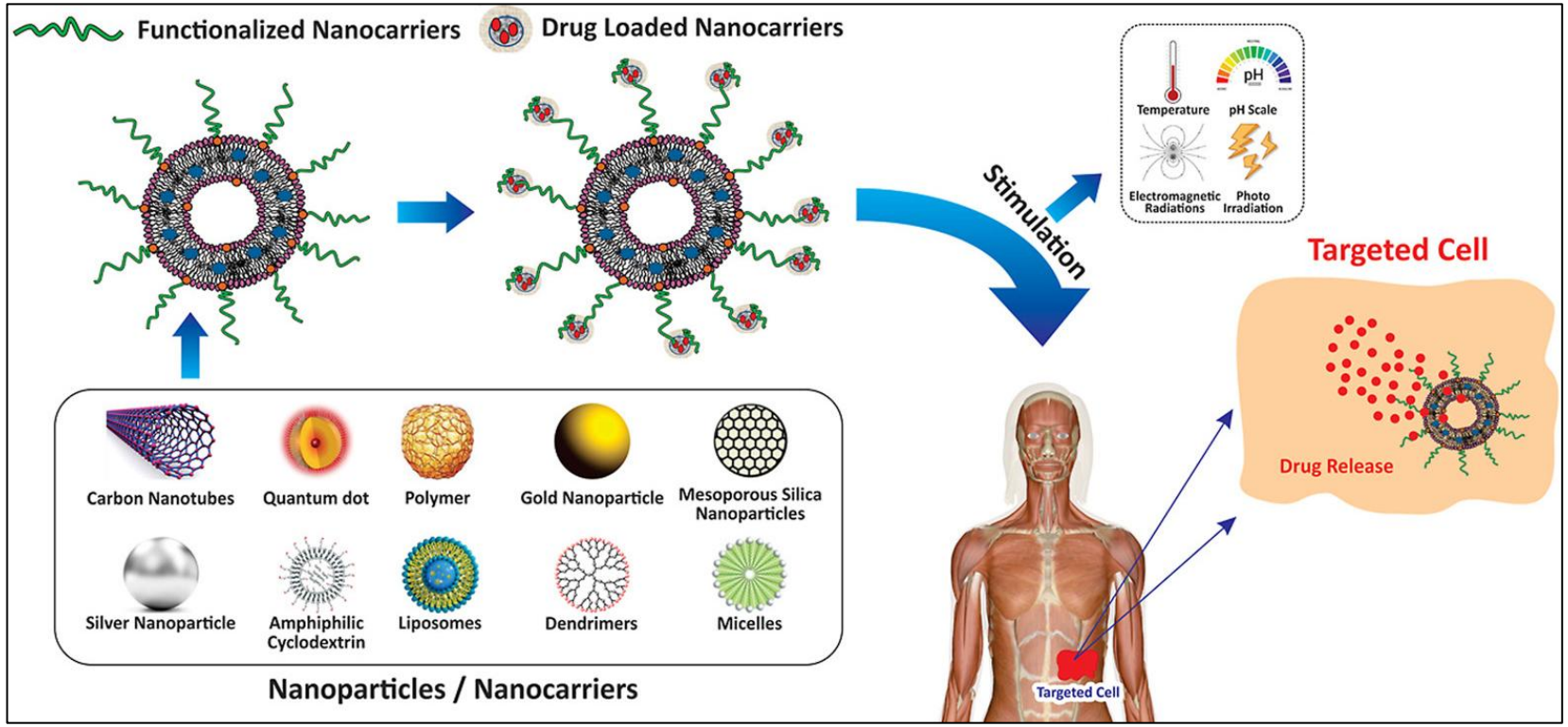
SURFACE MODIFICATION

ATB/ATM NANOCARRIERS

HOW CAN WE USE NATOTECHNOILOGY TO CONTROL BF?

ATB/ATM NANOCARRIERS

NANOTECHNOLOGY APPLIED TO MEDICINE



Shah A. *et al.*, J Drug Deliv Sci Technol. 2021;61; 102426.

METALLIC NANOPARTICLES

They are nanomaterials made up of only one element.

- They can have individual atoms or groups of atoms.
- Au, Ag, Pt, Cu, Pd, Re, Zn, Ru, Co, Cd, Al, Ni y Fe.

Synthesis by different methods:

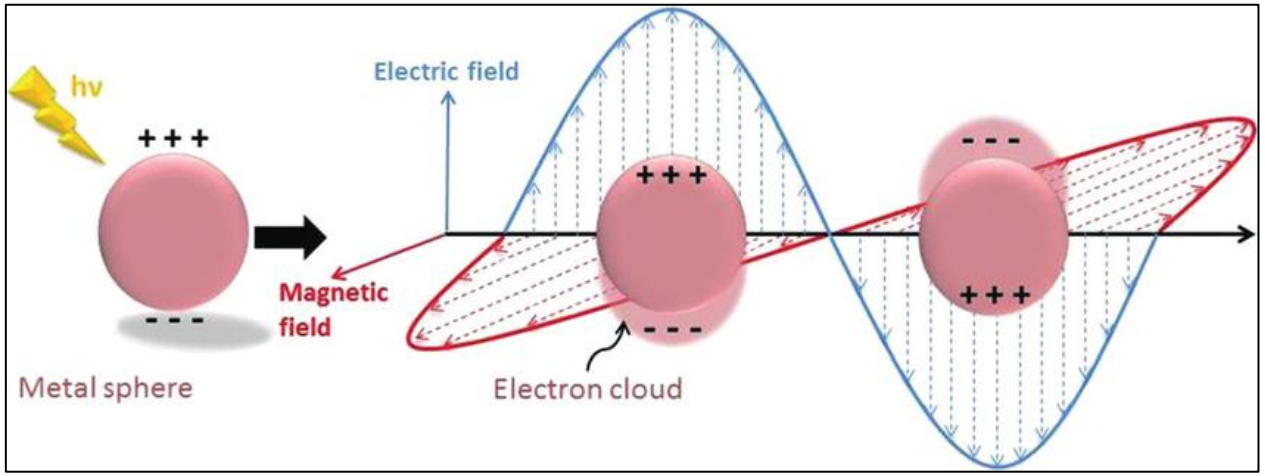
- Bio-assisted
- Hydrothermal
- Microwave
- Among others

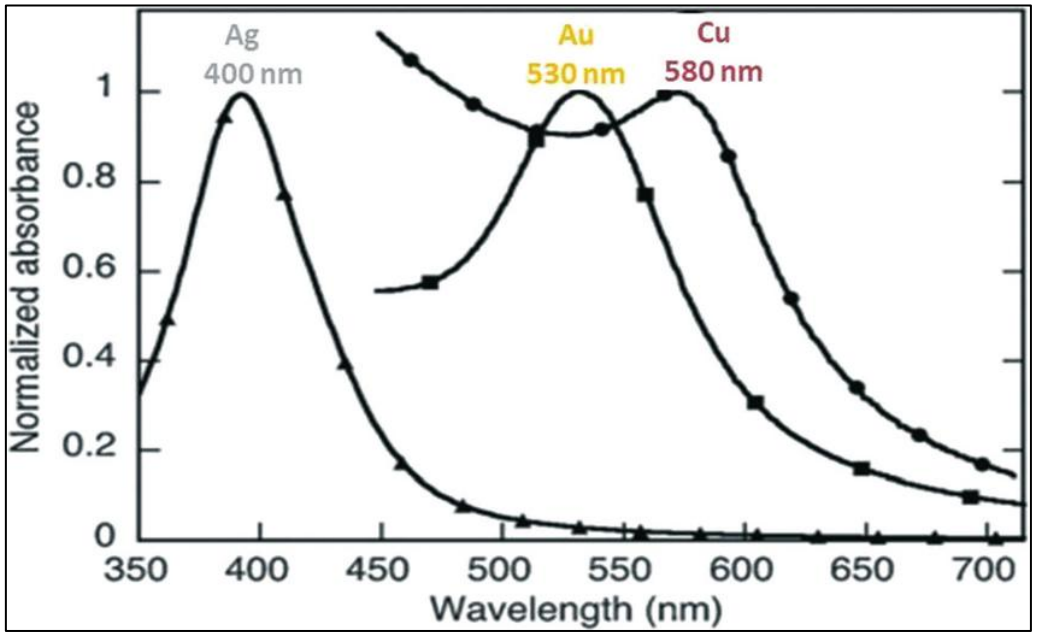
Different properties:

- Localized surface plasmon resonance (LSPR)
- Quantum confinement
- Optoelectrics
- Antimicrobials
- Anticancer
- Antivirals

Saleh, Tawfik. (2022). Chapter 8: Properties of nanoadsorbents and adsorption mechanisms. 10.1016/B978-0-12-849876-7.00010-5.

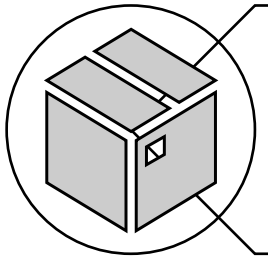
LOCALIZED SURFACE PLASMON RESONANCE (LSPR)



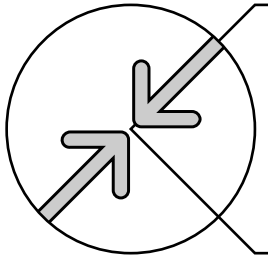


Peiris, Sunari & McMurtrie, John & Zhu, H.. (2015). Metal nanoparticle photocatalysts: Emerging processes for green organic synthesis. Catal. Sci. Technol.. 6. 10.1039/C5CY02048D.

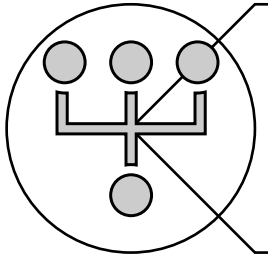
QUANTUM CONFINEMENT



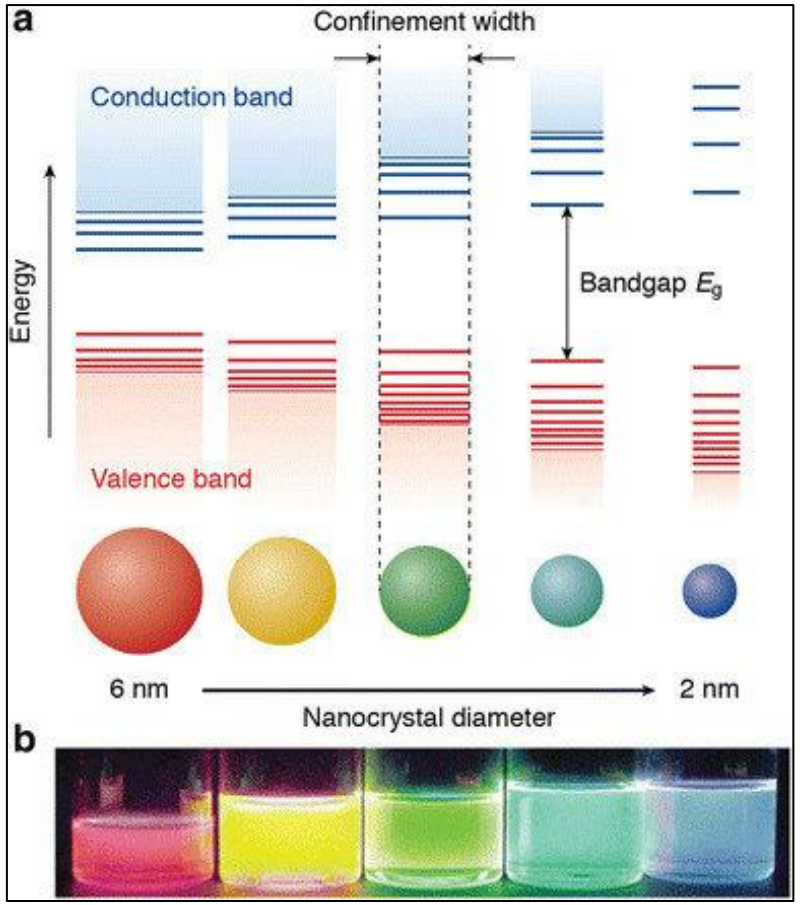
Confinamiento espacial de pares electrón-hueco (excitón) en una o más dimensiones dentro de un material.



Niveles de energía son discretos (≈λ de Broglie de e^-).



Semiconductores II – VI -> Band Gap.



Rabouw, Freddy & Donega, Celso. (2016). Excited-State Dynamics in Colloidal Semiconductor Nanocrystals. Topics in Current Chemistry. 374. 10.1007/s41061-016-0060-0.

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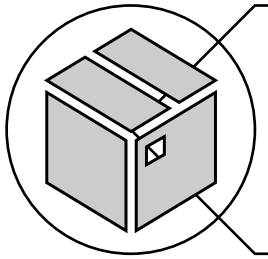
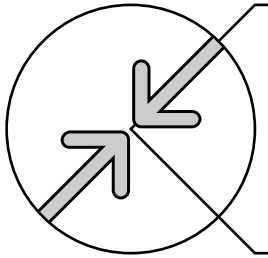


Confinamiento espacial de pares electrón-hueco (excitón) en una o más dimensiones dentro de un material.



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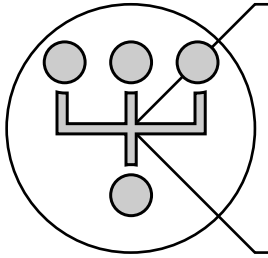

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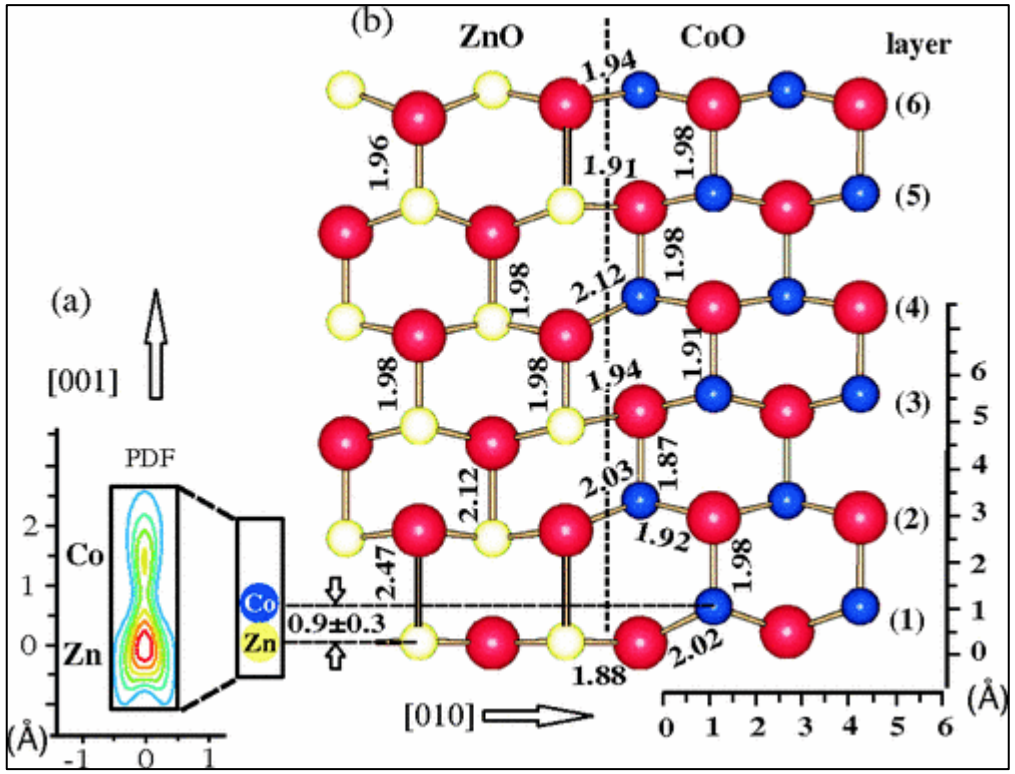

H. L. Meyerheim, C. Tusche, A. Ernst, et al. Wurtzite-Type CoO Nanocrystals in Ultrathin ZnCoO Films. Phys. Rev. Lett. **102**, 156102 – Published 13 April 2009

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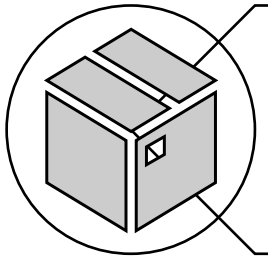

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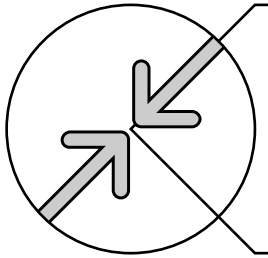

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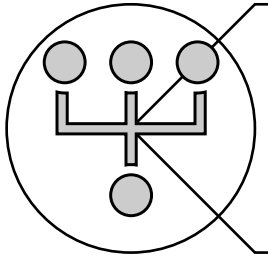
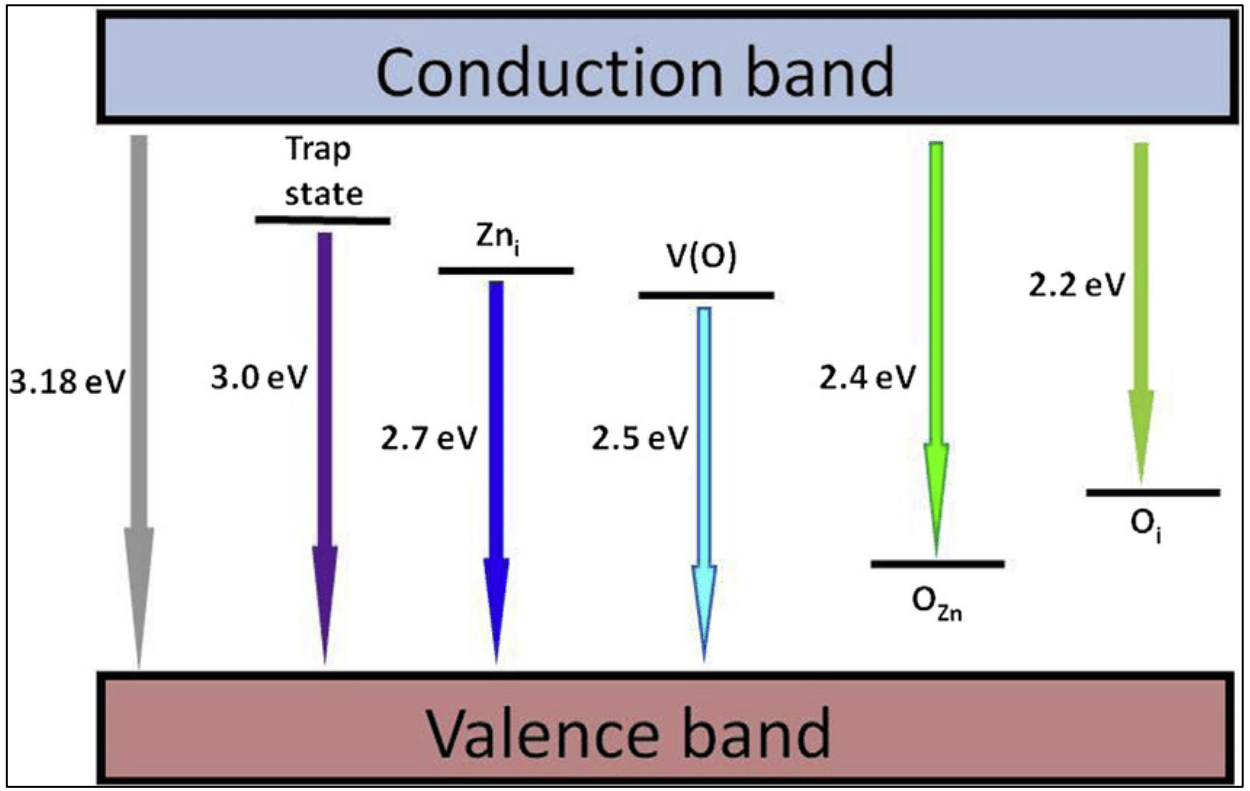
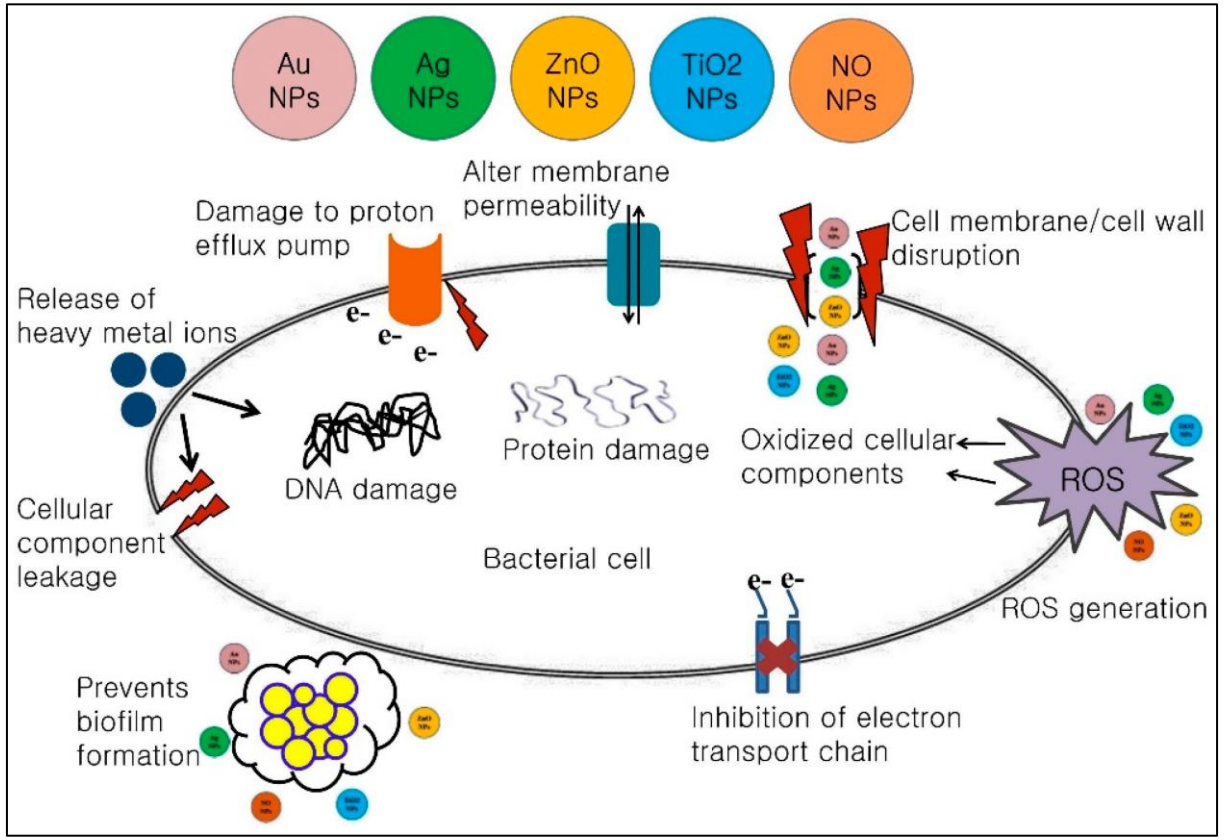


Semiconductores II – VI -> Band Gap.



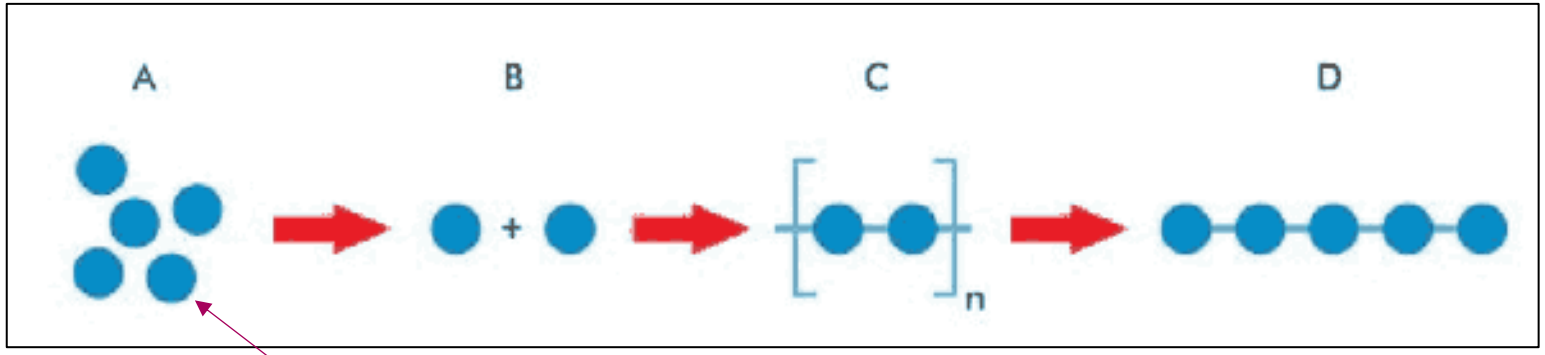
Mrabet, Chokri & Kamoun, Olfa & Boukhachem, A. & Amlouk, Mosbah & Tahar, Manoubi. (2015). Some physical investigations on hexagonal-shaped nanorods of lanthanum-doped ZnO. Journal of Alloys and Compounds. 648. 826-837. 10.1016/j.jallcom.2015.07.009.

METALLIC NPS: MECHANISMS



Shaikh S, Nazam N, Rizvi SMD, Ahmad K, Baig MH, Lee EJ, Choi I. Mechanistic Insights into the Antimicrobial Actions of Metallic Nanoparticles and Their Implications for Multidrug Resistance. *International Journal of Molecular Sciences*. 2019; 20(10):2468. https://doi.org/10.3390/ijms20102468

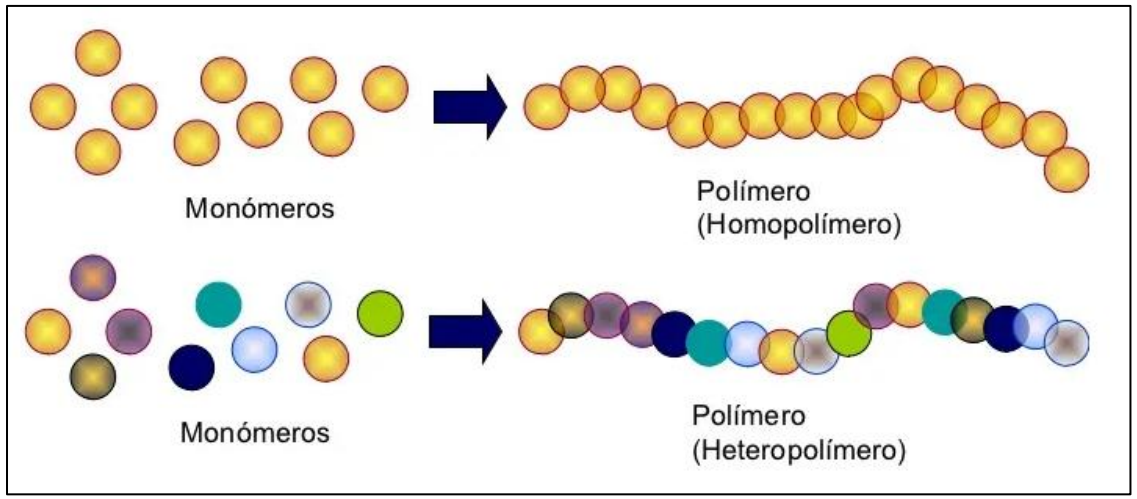
POLYMERIC NANOPARTICLES



Monomers

Greek

- poly: many
- meros: part or unit



Saleh, Tawfik. (2022). Chapter 8: Properties of nanoadsorbents and adsorption mechanisms. 10.1016/B978-0-12-849876-7.00010-5.

POLYMERIC NANOPARTICLES

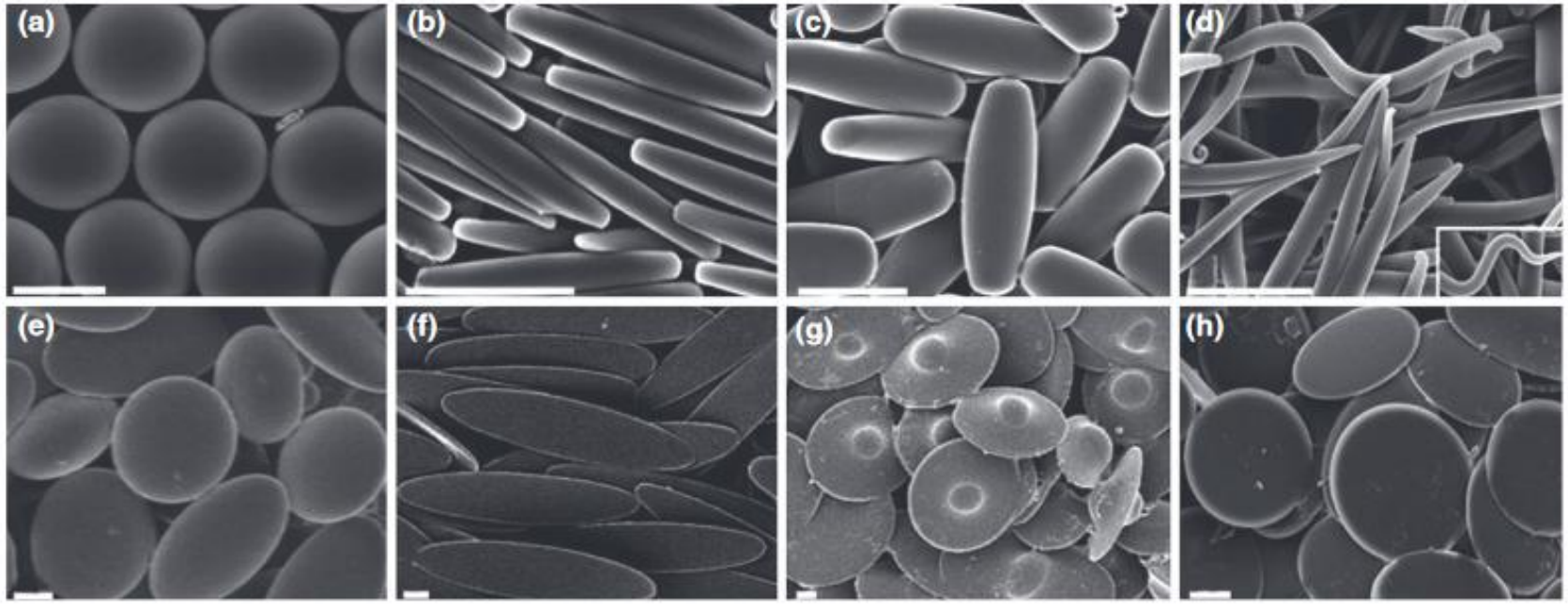
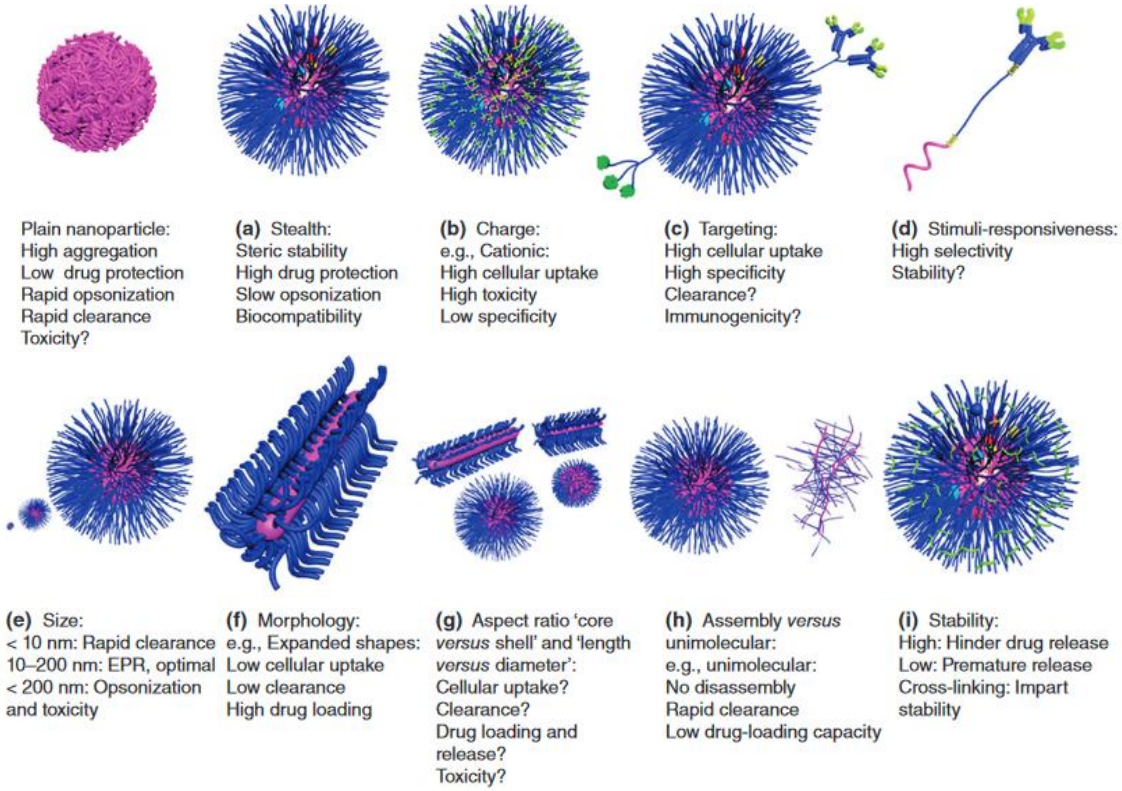


FIGURE 4 | Micrographs of particle shapes made by using a mechanical stretching technique. (a) Spheres, (b) rectangular disks, (c) rods, (d) worms, (e) oblate ellipses, (f) elliptical disks, (g) unidentified flying objects (UFOs), and (h) circular disks (scale bars: 2 μm). (Reprinted with permission from Ref 45. Copyright 2007 National Academy of Sciences, U.S.A.)

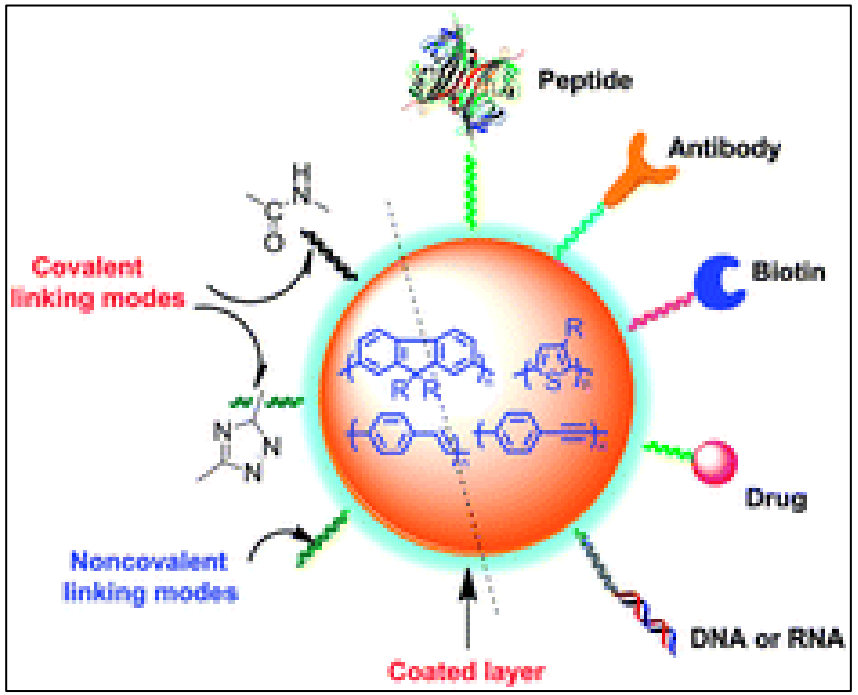
Champion JA, Katare YK, Mitragotri S. Making poly-meric micro- and nanoparticles of complex shapes. Proc Natl Acad Sci USA 2007, 104:11901–11904. doi:10.1073/pnas.0705326104.

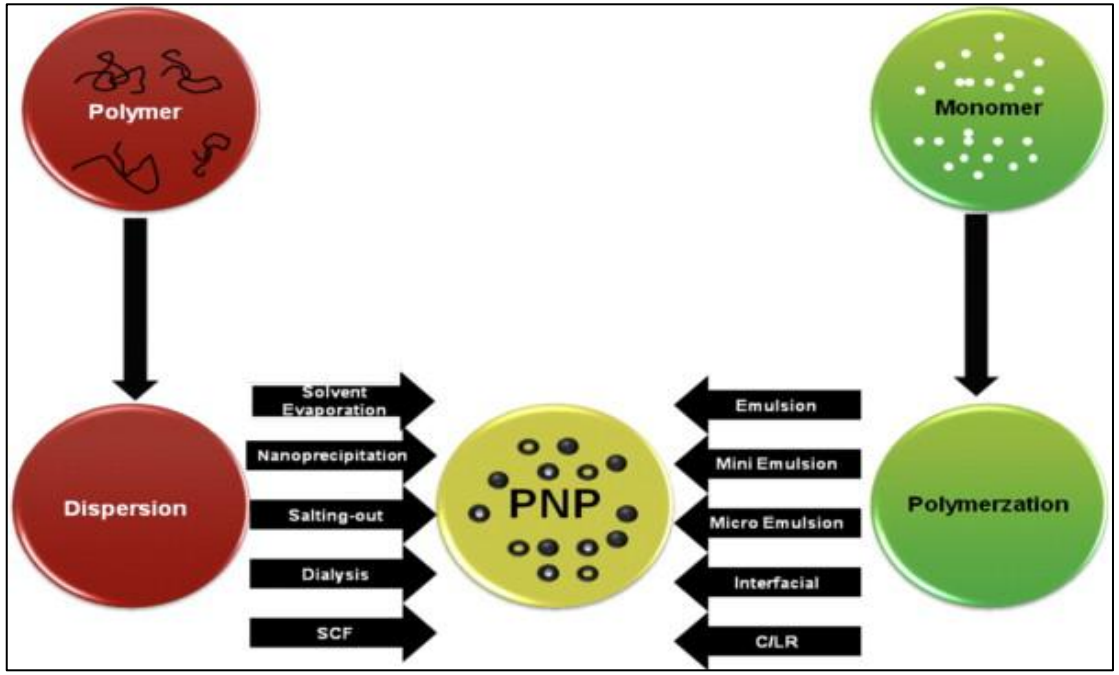
POLYMERIC NANOPARTICLES



Elsabahy M, Wooley KL. Design of polymeric nanoparticles for biomedical delivery applications. Chem Soc Rev 2012, 41:2545–2561. doi:10.1039/c2cs15327k

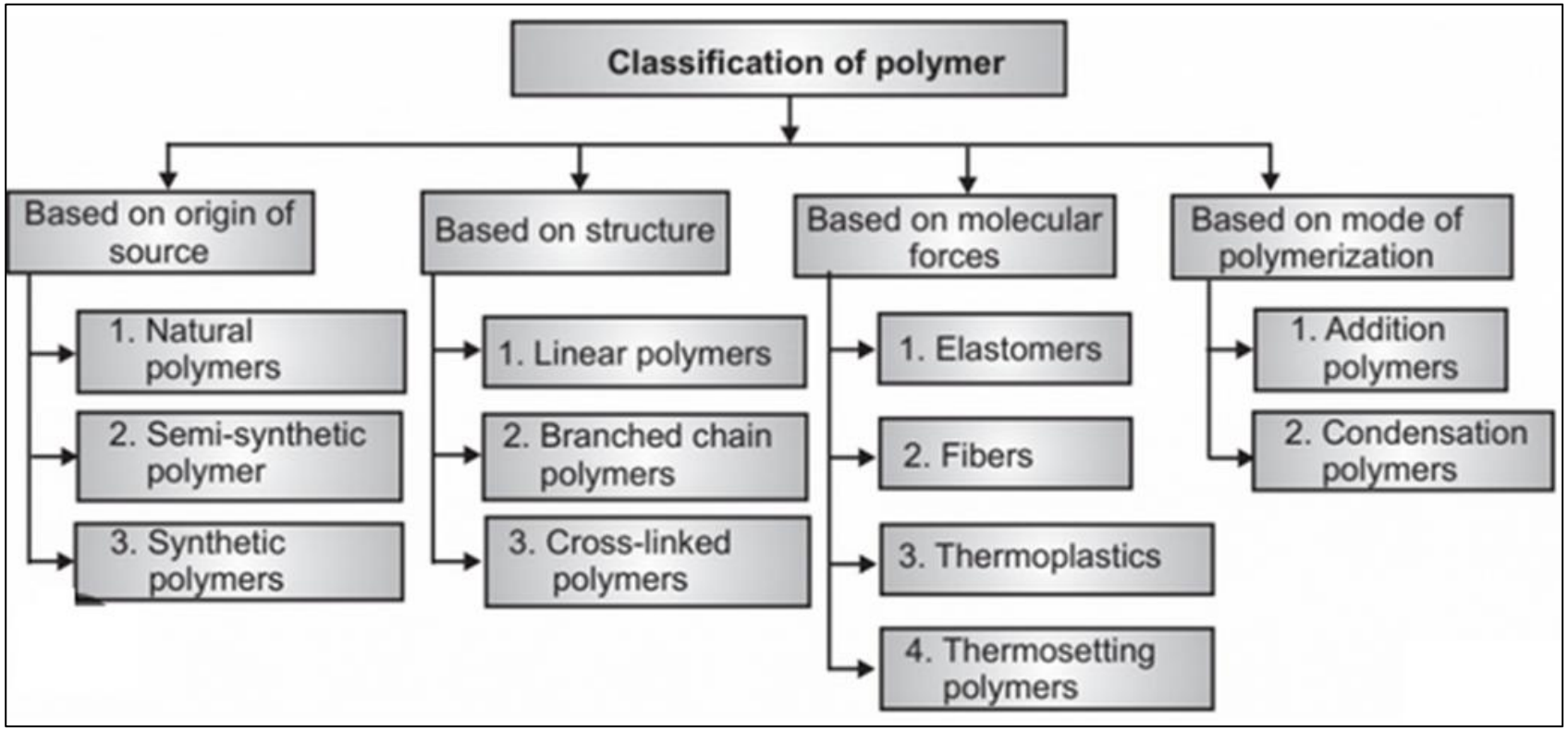
POLYMERIC NANOPARTICLES





Feng L, Zhu C, Yuan H, Liu L, Lv F, Wang S. Conjugated polymer nanoparticles: preparation, properties, functionalization and biological applications. Chem Soc Rev. 2013 Aug 21;42(16):6620-33. doi: 10.1039/c3cs60036j.
J. Prasad Rao, Kurt E. Geckeler. Polymer nanoparticles: Preparation techniques and size-control parameters, Progress in Polymer Science, Volume 36, Issue 7,2011,Pages 887-913. https://doi.org/10.1016/j.progpolymsci.2011.01.001.

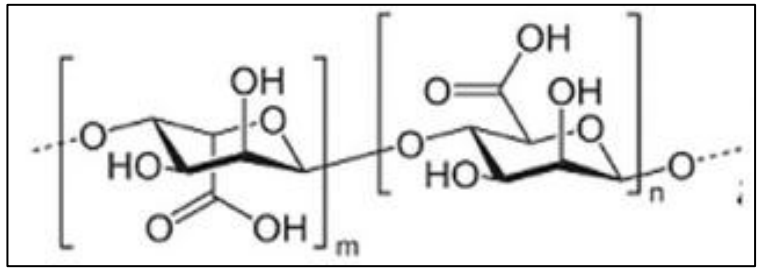
POLYMERIC NANOPARTICLES



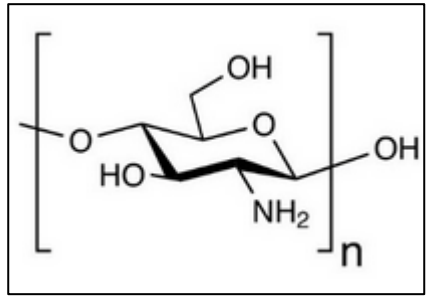
J. Prasad Rao, Kurt E. Geckeler. Polymer nanoparticles: Preparation techniques and size-control parameters, Progress in Polymer Science, Volume 36, Issue 7,2011,Pages 887-913. https://doi.org/10.1016/j.progpolymsci.2011.01.001.

POLYMERIC NANOPARTICLES

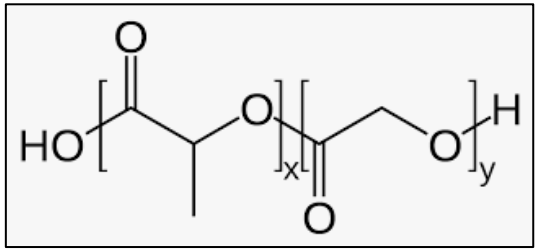
Biodegradable and biocompatible



Alginate



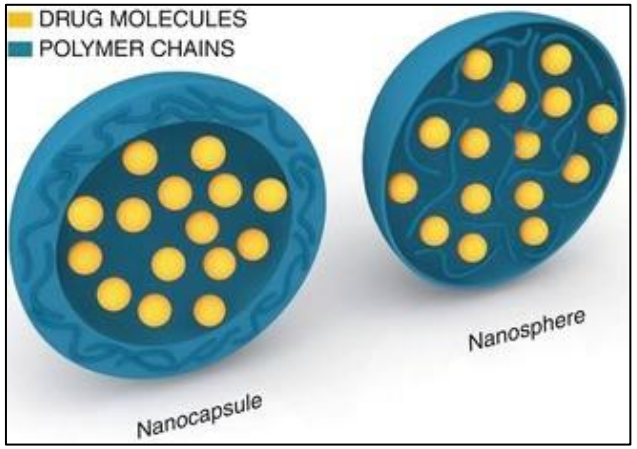
Chitosan

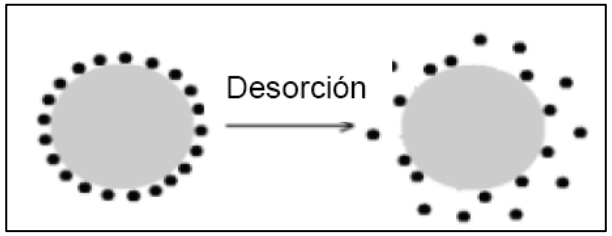


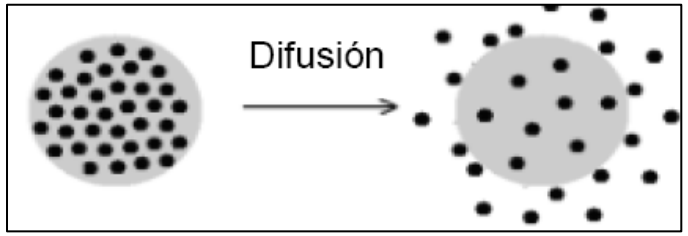
Polylactic co-glycolic acid

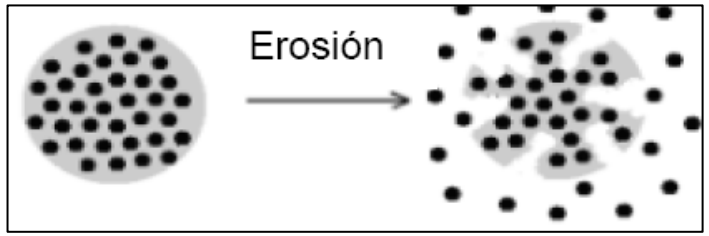
Del Amo L, Cano A, Ettcheto M, Souto EB, Espina M, Camins A, García ML, Sánchez-López E. Surface Functionalization of PLGA Nanoparticles to Increase Transport across the BBB for Alzheimer's Disease. *Applied Sciences*. 2021; 11(9):4305. https://doi.org/10.3390/app11094305

POLYMERIC NANOPARTICLES: CONTROLLED RELÉASE SYSTEMS



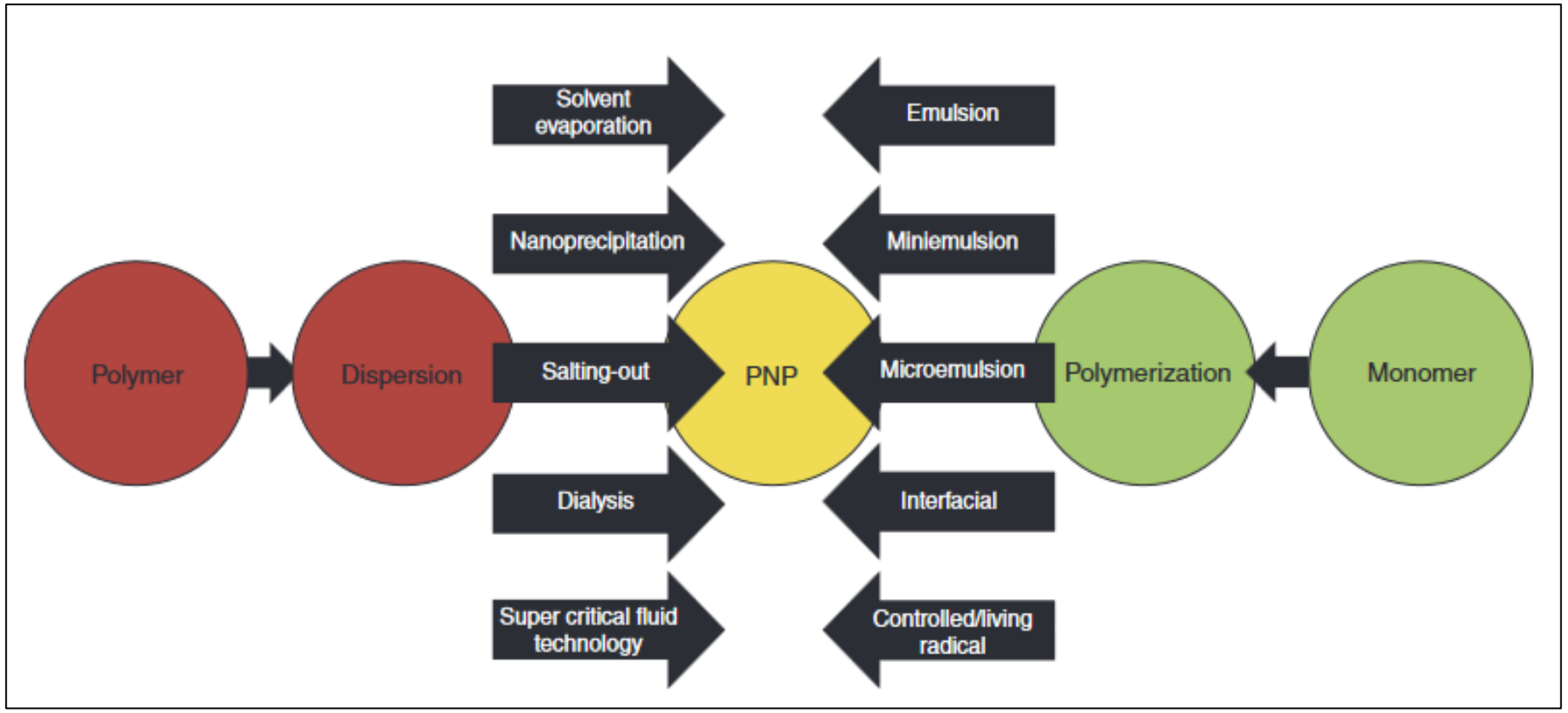






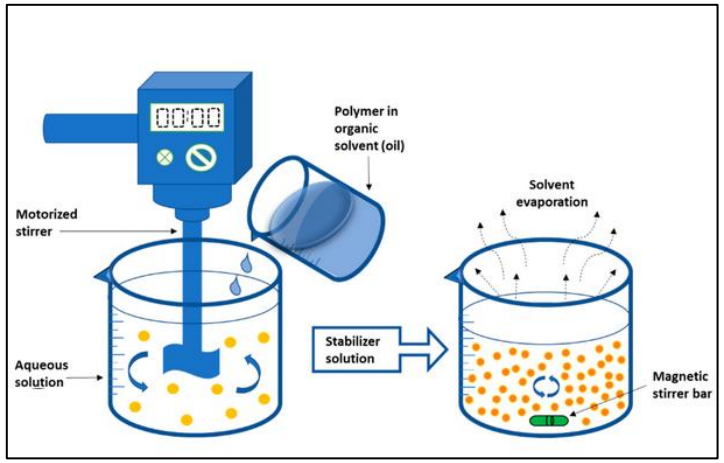
Sobot, D., Mura, S., Couvreur, P. (2014). Nanoparticles: Blood Components Interactions. In: Kobayashi, S., Müllen, K. (eds) Encyclopedia of Polymeric Nanomaterials. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-36199-9_227-1

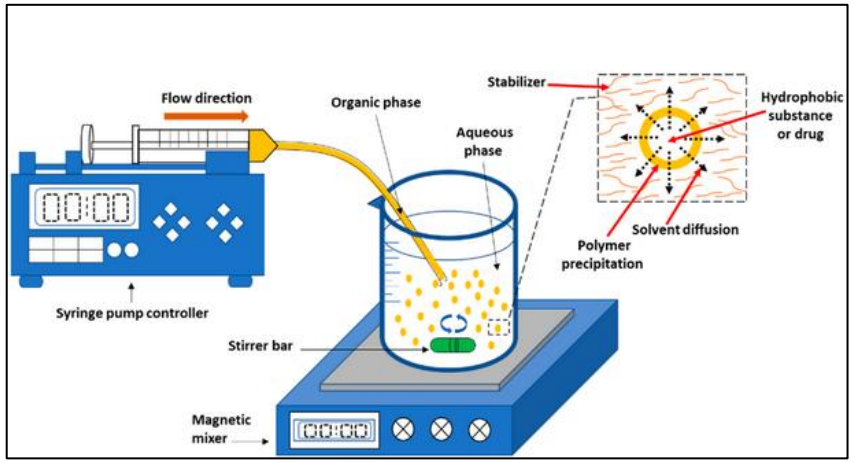
POLYMERIC NANOPARTICLES



Rao JP, Geckeler KE. Polymer nanoparticles: preparation techniques and size-control parameters. Prog Polym Sci 2011, 36:887–913. doi:10.1016/j.progpolymsci.2011.01.001.

POLYMERIC NANOPARTICLES SYNTHESIS





Solvent Evaporation

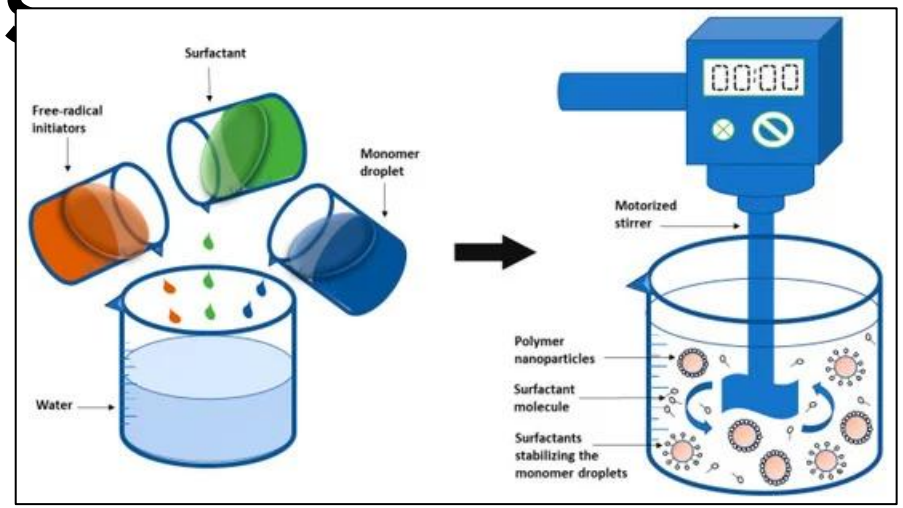
- Advantages: scalable, controllable size and morphology
- Disadvantage: slow process

Nanoprecipitation

- Advantages: Simple, rapid, and reproducible method
- Disadvantage: Limited water-miscible solvents

Pulingam T et all. Exploring Various Techniques for the Chemical and Biological Synthesis of Polymeric Nanoparticles. *Nanomaterials*. 2022; 12(3):576. https://doi.org/10.3390/nano12030576

POLYMERIC NANOPARTICLES SYNTHESIS



Emulsion

- Advantages: Produces polymers with high molar mass. Uses water as a dispersion medium. Excellent heat dissipation.
- Disadvantage: Requires surfactant removal.
- (micro: 10–100 nm, mini: 100 nm–1 µm, macro: >1 µm).

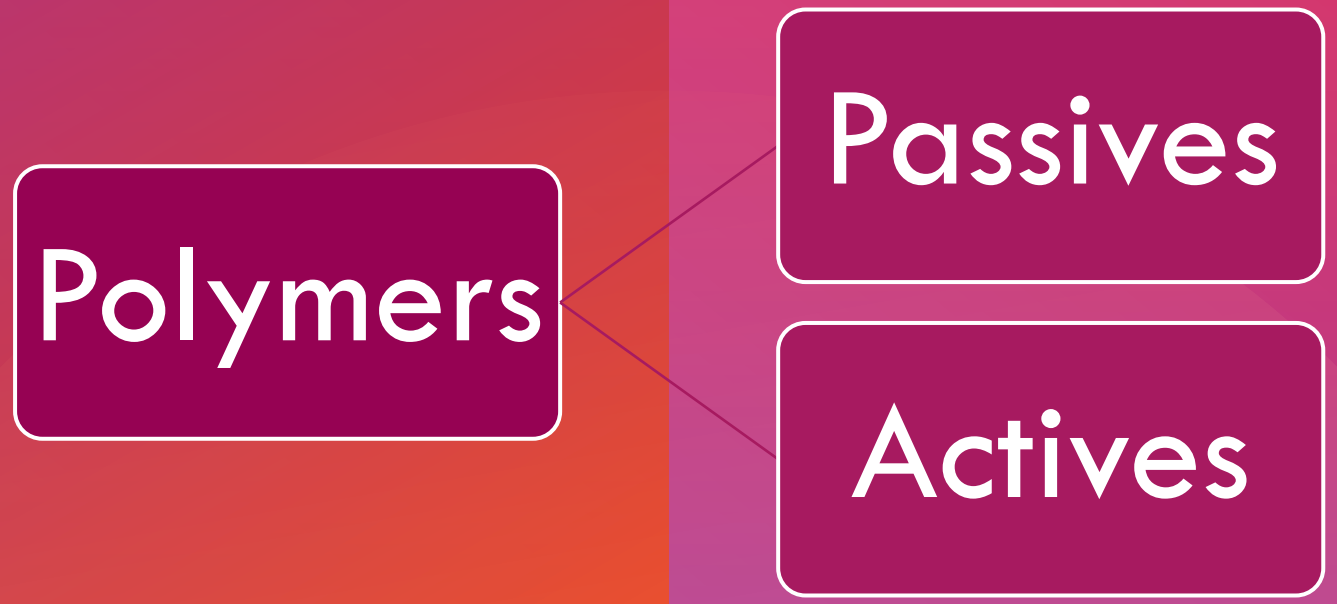
Pulingam T et all. Exploring Various Techniques for the Chemical and Biological Synthesis of Polymeric Nanoparticles. *Nanomaterials*. 2022; 12(3):576. https://doi.org/10.3390/nano12030576

HOW CAN WE USE NATOTECHNOILOGY TO CONTROL BF?

SURFACE MODIFICATION

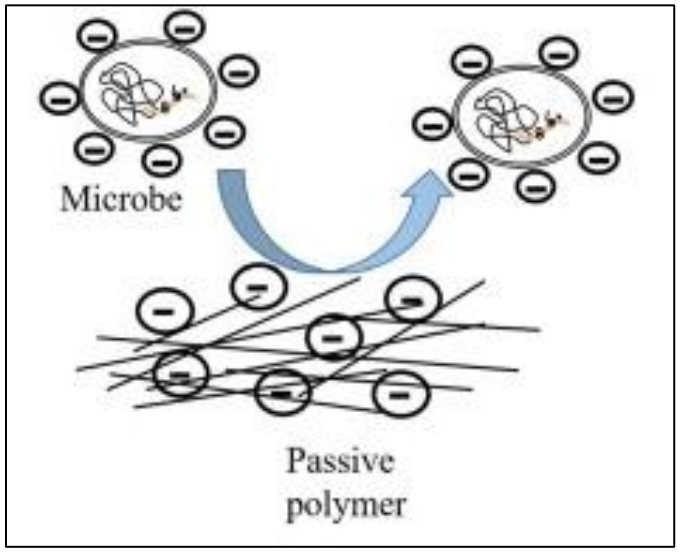
ATB/ATM NANOCARRIERS

ANTIBACTERIAL ACTIVITIES OF POLYMERS



ANTIBACTERIAL ACTIVITIES OF POLYMERS

Passive Polymers

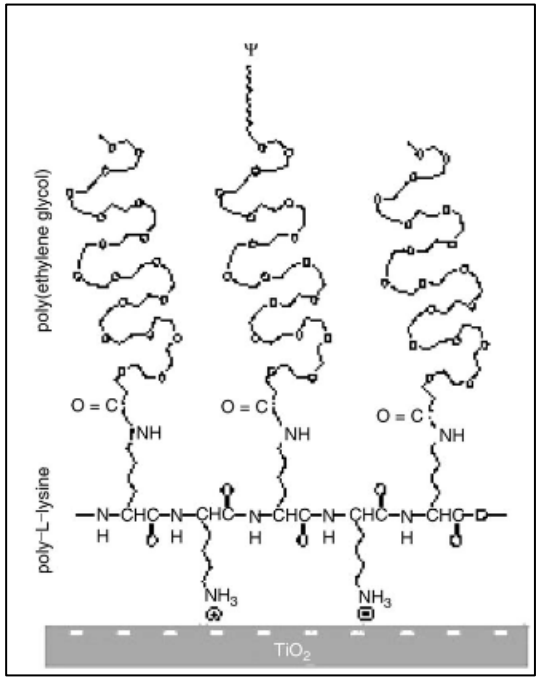


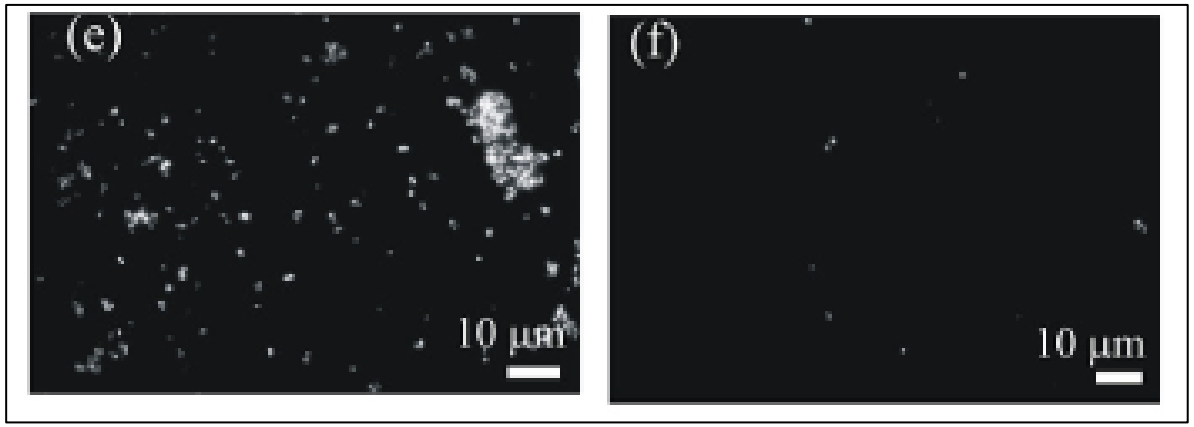
- Minimal protein adsorption
- Non cytotoxic
- Polymer with negative charge, hydrophilic or low surface free energy
- Electrostatic repulsions between the polymer and the bacterial cell wall prevent adhesion

Naveera Naeem, et al Chapter 7 - Antimicrobial Green Composites, Editor(s): Dr. Inamuddin, Tariq Altalhi, Green Sustainable Process for Chemical and Environmental Engineering and Science, Elsevier, 2023, Pages 187-206, ISBN 9780323951692doi.org/10.1016/B978-0-323-95169-2.00012-2.

ANTIBACTERIAL ACTIVITIES OF POLYMERS

Passive Polymers



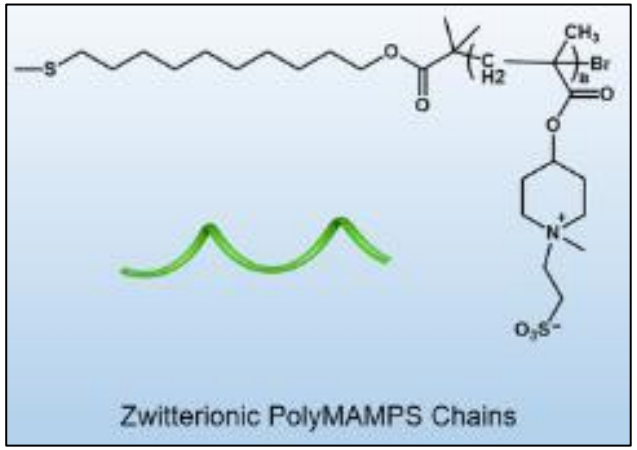


Representative fluorescence microscopy images of S. aureus adhering to (e) bare titanium surface, and (f) PLL-g-PEG coated titanium surface, incubated stationary at 37oC for 1h. Fluorescent redox dye, CTC

Harris, Llinos et al. (2004). Staphylococcus aureus adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly(L-lysine)-graft-poly(ethylene glycol) copolymers. Biomaterials. 25. 4135-48. 10.1016/j.biomaterials.2003.11.033.

ANTIBACTERIAL ACTIVITIES OF POLYMERS

Passive Polymers



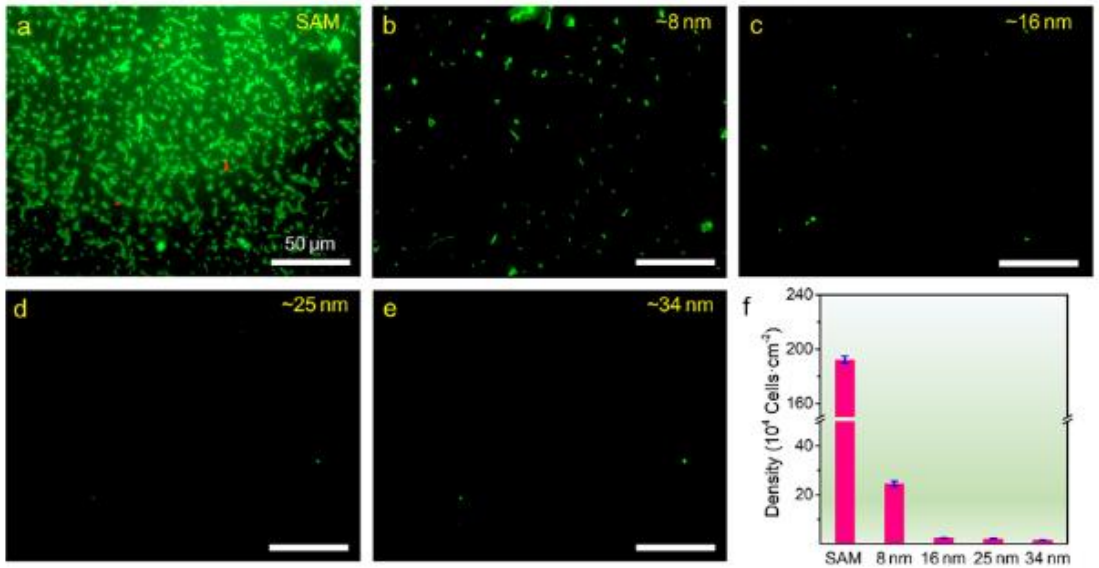
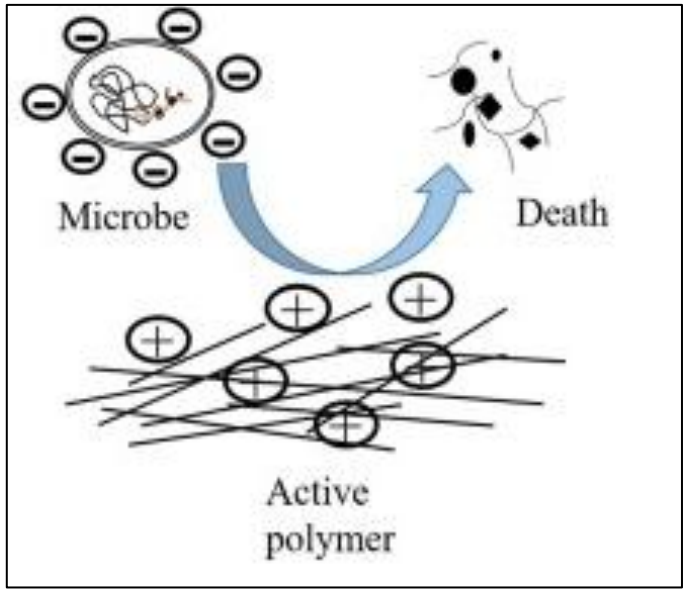


Fig. 6. Representative confocal microscopy of *E. coli* attachment on (a) initiator-coated surface and polyMAMPS brushes with different thicknesses of (b) ~8 nm, (c) ~16 nm, (d) ~25 nm, and (e) ~34 nm after 12 h incubation. (f) Statistical analysis of adhered *E. coli* density on different surfaces (n = 3).

Zhang D, Ren B, Zhang Y, Liu Y, Chen H, Xiao S, Chang Y, Yang J, Zheng J. Micro- and macroscopically structured zwitterionic polymers with ultralow fouling property. J Colloid Interface Sci. 2020 Oct 15;578:242-253. doi: 10.1016/j.jcis.2020.05.122. .

ANTIBACTERIAL ACTIVITIES OF POLYMERS

Active Polymer

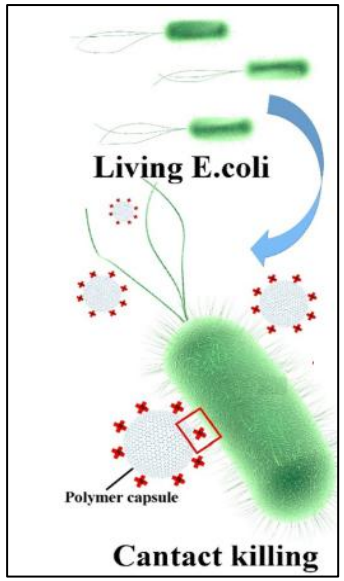


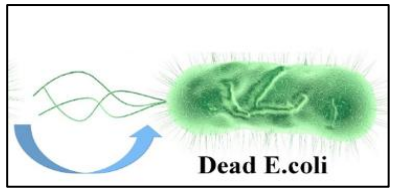
Membrane destabilization → Output of cellular content → Death

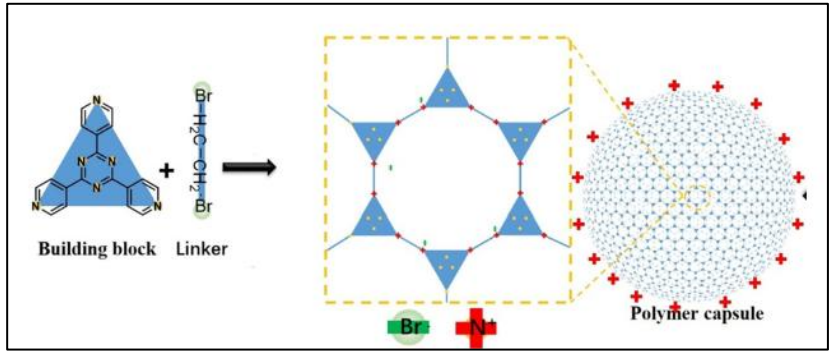
Electrostatic Interactions → Cell inactivation and membrane damage by disrupting calcium salt bridges and membrane receptors.

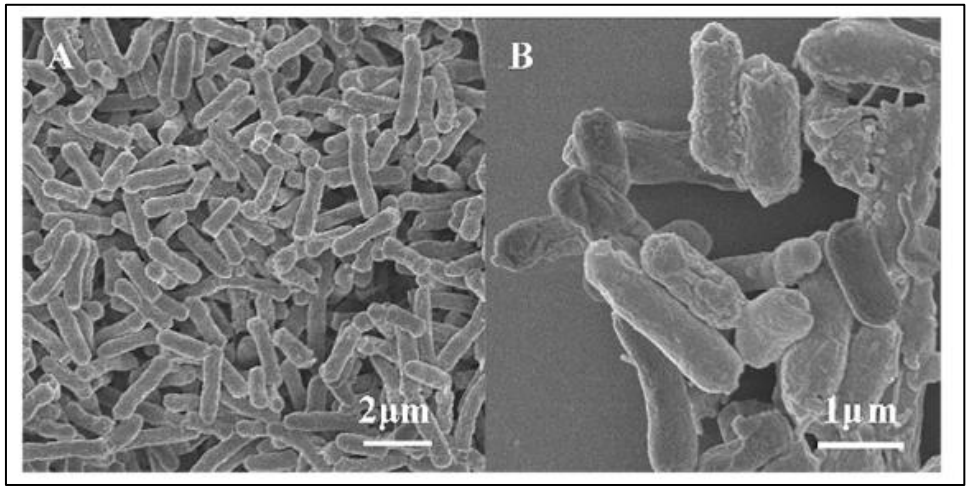
Naveera Naeem, et al Chapter 7 - Antimicrobial Green Composites, Editor(s): Dr. Inamuddin, Tariq Altalhi, Green Sustainable Process for Chemical and Environmental Engineering and Science, Elsevier, 2023, Pages 187-206, ISBN 9780323951692doi.org/10.1016/B978-0-323-95169-2.00012-2.

ANTIBACTERIAL ACTIVITIES OF POLYMERS





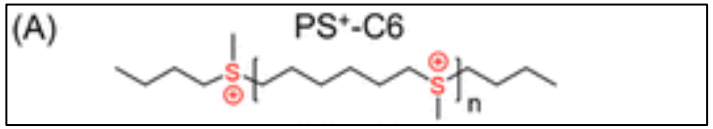




SEM observation of *E. coli* (A) untreated control (no polymer vesicles or monomers), (B) mixed with polymer nanocapsules.

Zeng M, Xu J, Luo Q, Hou C, Qiao S, Fu S, Fan X, Liu J. Constructing antibacterial polymer nanocapsules based on pyridine quaternary ammonium salt. Mater Sci Eng C Mater Biol Appl. 2020 Mar;108:110383. doi: 10.1016/j.msec.2019.11038

ANTIBACTERIAL ACTIVITIES OF POLYMERS



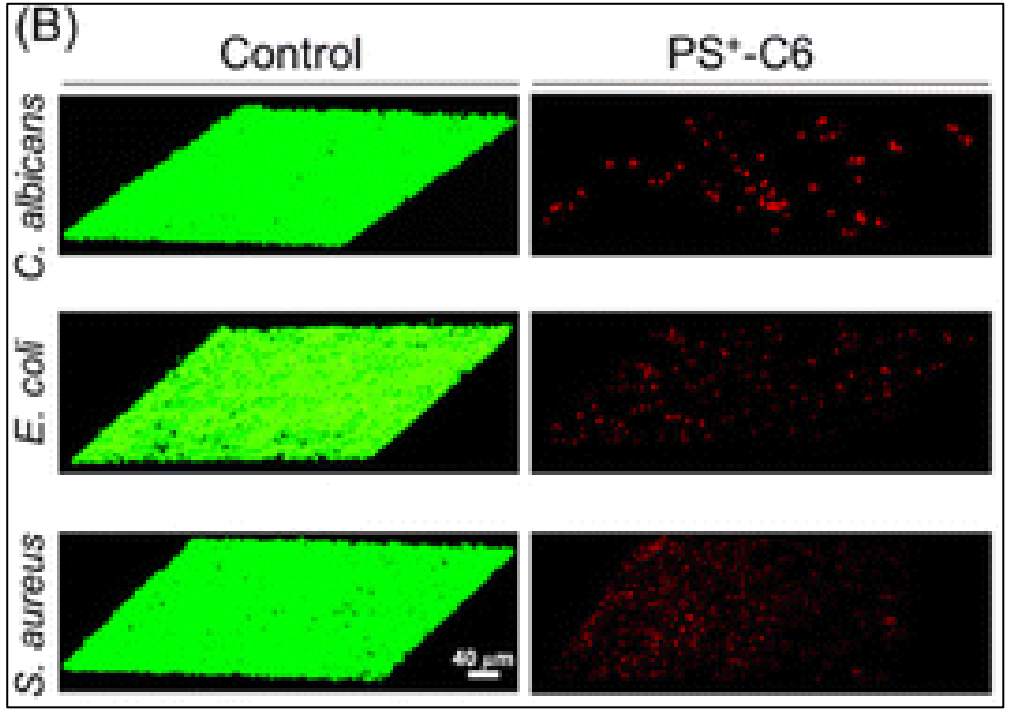
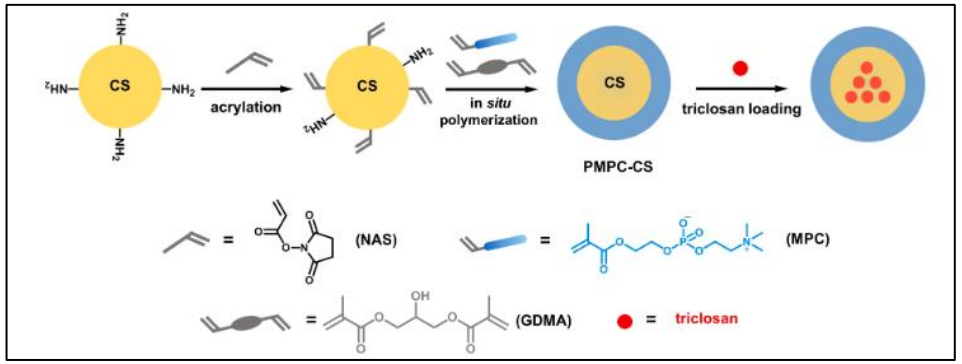


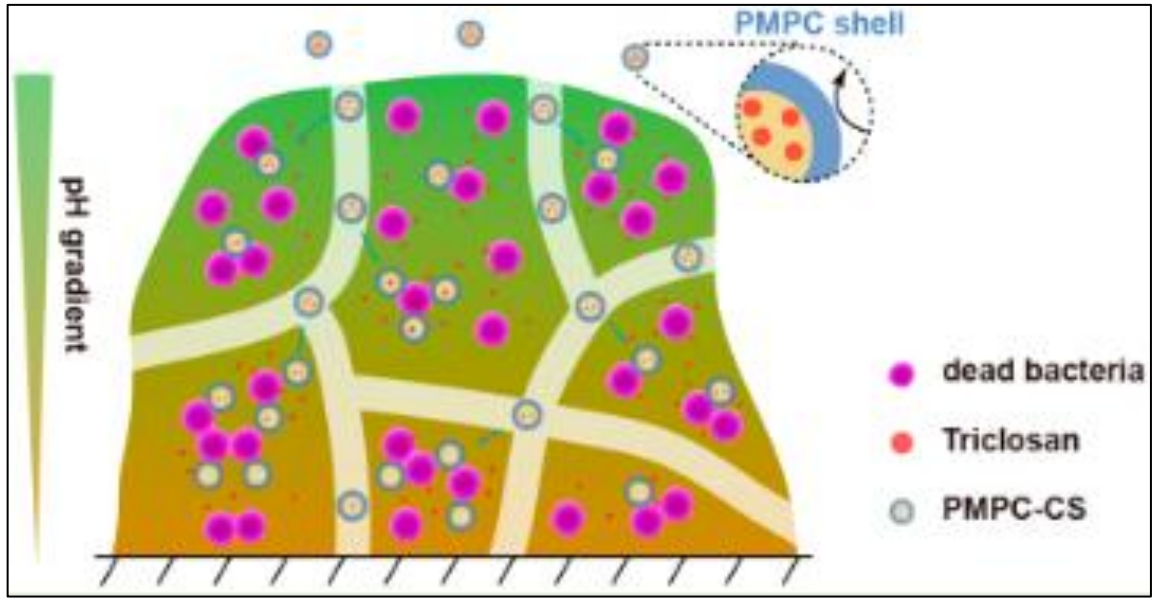
Fig. 8 (A) Chemical structure of main chain sulfonium-containing homopolymer. (B) CLSM images of C. *albicans*, *E. coli* and *S. aureus* constructed biofilms treated with the control solution and PS+-C6. Treatment with the PS+-C6 polymer for 24 h, thick biofilms produced mostly red fluorescent signals that signified the existence of dead microorganisms; however, most of the cells in the control groups were still alive

X. Wang, G. Wang, J. Zhao, Z. Zhu and J. Rao, ACS Macro Lett., 2021, 10, 1643–1649

ANTIBACTERIAL ACTIVITIES OF POLYMERS



Chitosan (CS) nanoparticles encapsulated with poly(2-methacryloyloxyethyl phosphorylcholine (PMPC)) and loaded with antimicrobial (triclosan)



ACS Macro Lett. 2019, 8, 651−657. 10.1021/acsmacrolett.9b00142

ANTIBACTERIAL ACTIVITIES OF POLYMERS

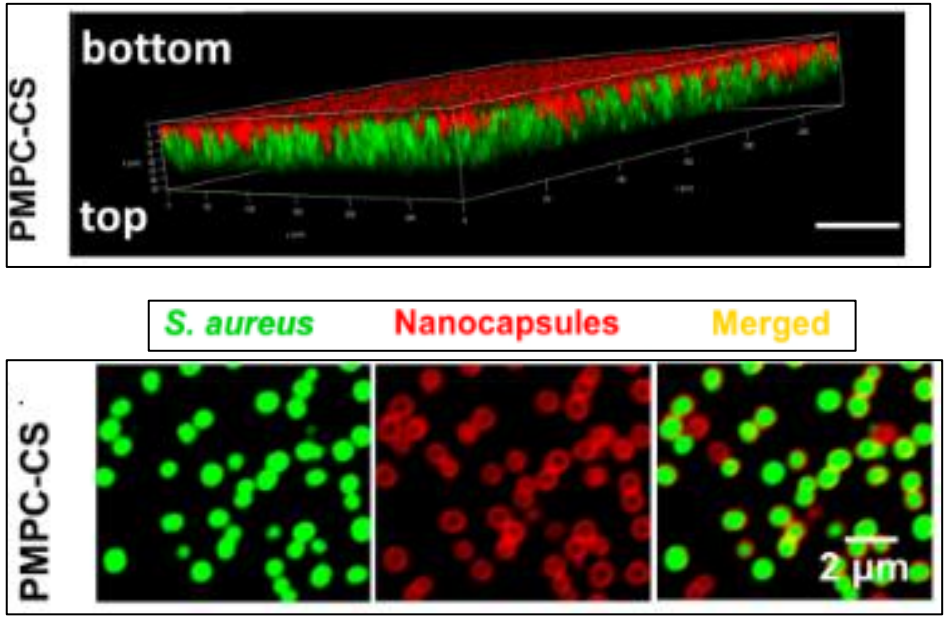
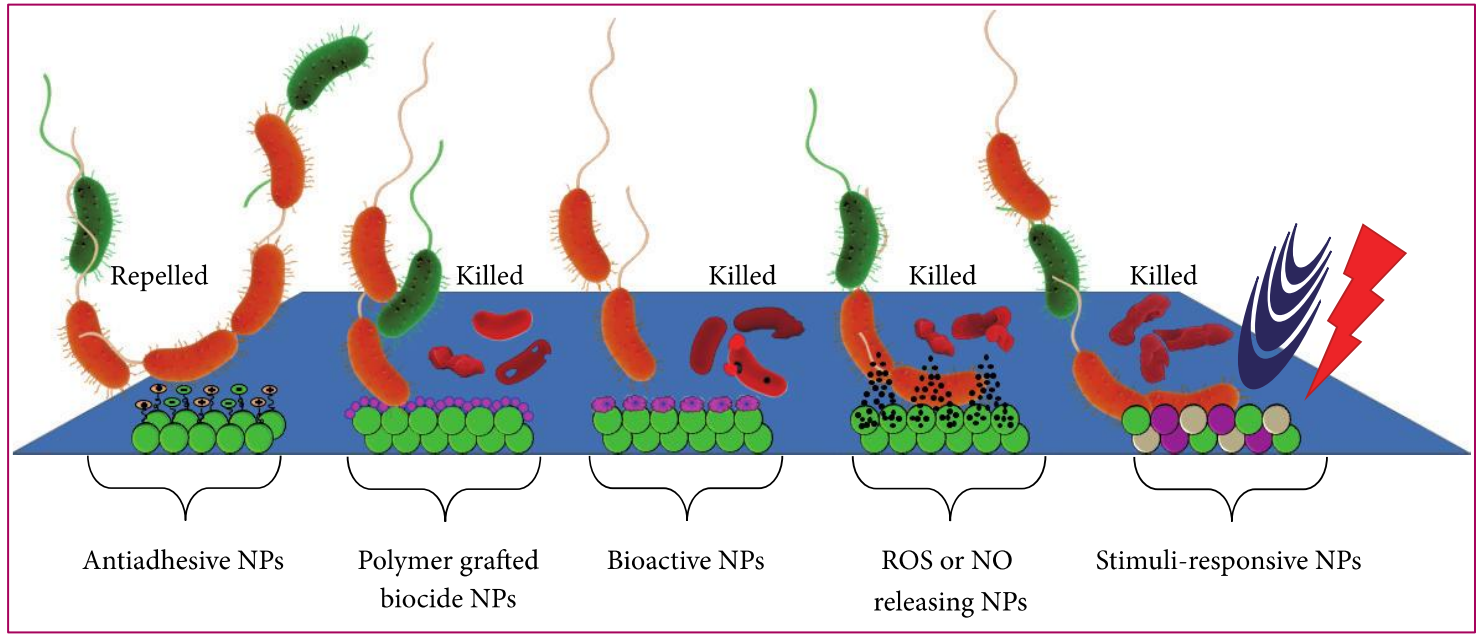


Figure 2. Penetration and accumulation of different Cy5-labeled nanocapsules (red) into a S. aureus ATCC12600 GFP biofilm (green). (a) Three-dimensional confocal laser scanning micrographs, showing penetration and accumulation of nanocapsules into a S. aureus biofilm (scale bar, 50µm (b) Fluorescence images of the attachment of different Cy5-labeled nanocapsules (red) to S. aureus ATCC12600GFP (green) cell surfaces in PBS at pH 5.0 (scale bar, 2 µm).

ACS Macro Lett. 2019, 8, 651−657. 10.1021/acsmacrolett.9b00142

CATHETERS SURFACE MODIFICATIONS

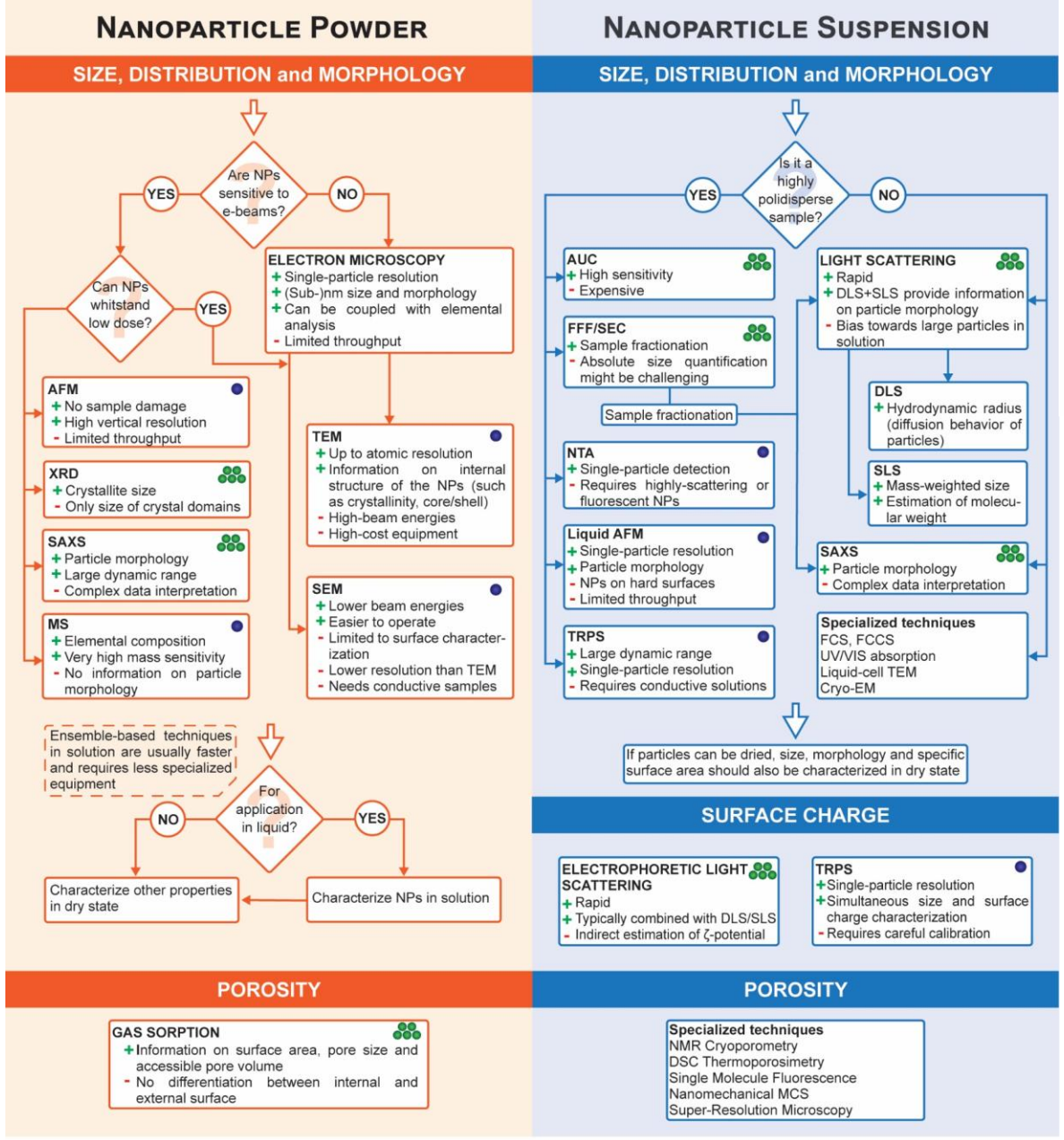
- Use of polymers with bactericidal properties.
- Low cost.
- Use of FDA-approved materials.
- Combined with active molecules (NPs/ATB).



Ramasamy M, Lee J. Recent Nanotechnology Approaches for Prevention and Treatment of Biofilm-Associated Infections on Medical Devices. Biomed Res Int. 2016.

CHALLENGES

- Biocompatibility
- Long-term cytotoxicity
- Tissue accumulation
- Metabolization/Excretion



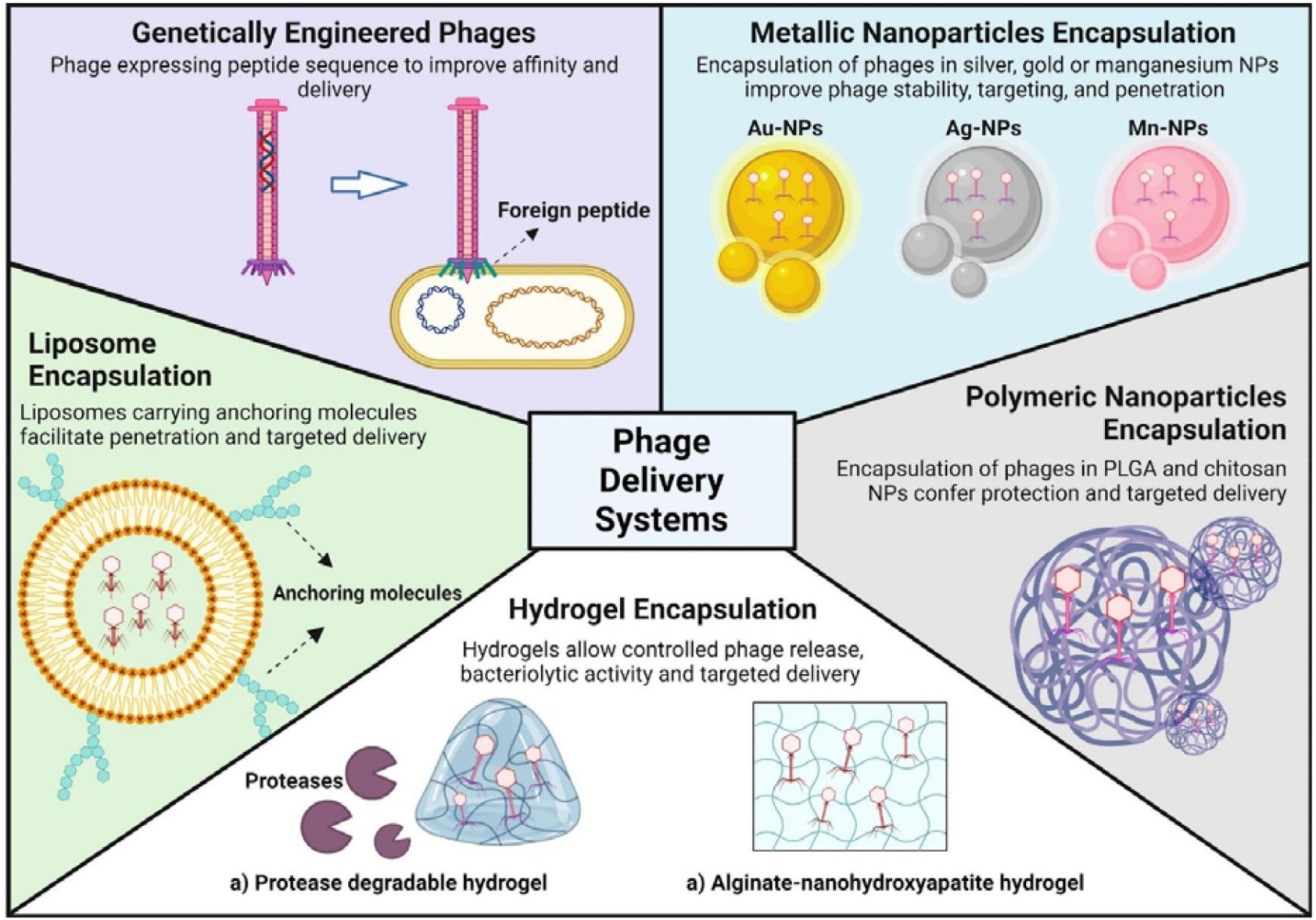
RECOMMENDATIONS

- Consider the intended application environment and use of the nanoparticle under examination
- Develop standard operating procedure (including data analysis) for nanoparticle characterization to be followed for *every* batch
- Calibration or reference particles should be used to characterize the accuracy of the measurement and compare different batches
- For single-particle techniques, a representative number of particles (~1000) should be measured to ensure statistical significance
- Multiple approaches should be used to characterize each nanoparticle property and increase the reliability of the results. Ideally, at least one single-particle and one ensemble-based method should be chosen
- Report all experimental parameters, including method-specific settings and data treatment procedures

LEGEND

- ● Single-particle technique
- Ensemble-based technique

Modena MM, Rühle B, Burg TP, Wuttke S. Nanoparticle Characterization: What to Measure? Adv Mater. 2019 Aug;31(32):e1901556. doi: 10.1002/adma.201901556.



Mayorga-Ramos A, Carrera-Pacheco SE, Barba-Ostria C, Guamán LP. Bacteriophage-mediated approaches for biofilm control. Front Cell Infect Microbiol. 2024 Oct 7;14:1428637. doi: 10.3389/fcimb.2024.1428637.

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FACULTAD DE MEDICINA UNIVERSIDAD DE CHILE

2025

October, 13-24

Innovative Biotechnological Approaches for Biofilm Control and Characterization

GRACIAS

NANOTECHNOLOGY APPLIED TO BIOFILMS II

2025
October, 13-24

Innovative Biotechnological Approaches for Biofilm Control and Characterization

PHD. ERLEN CRUZ JORGE

Research Article

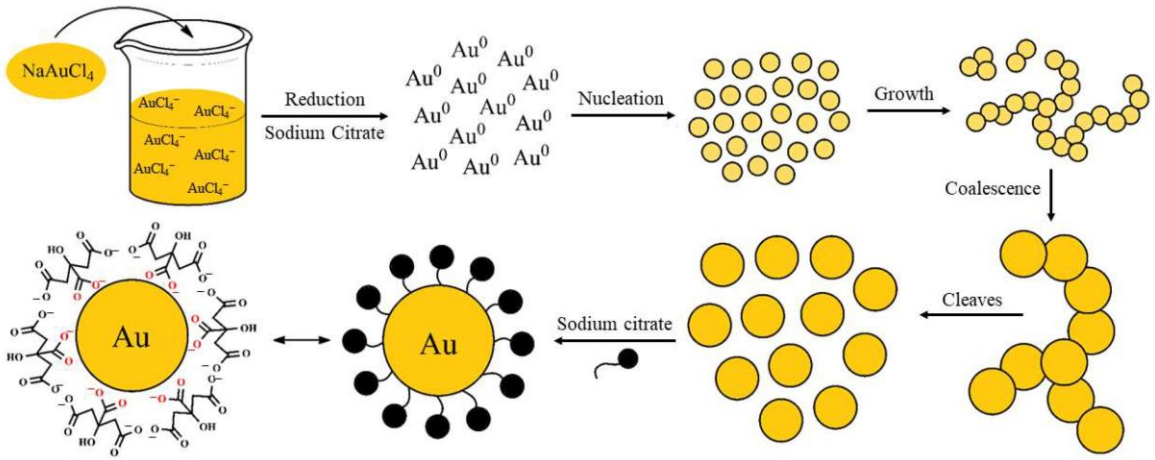
Gold-, Silver- and Magnesium-Doped Zinc Oxide Nanoparticles Prevents The Formation of and Eradicates Bacterial Biofilms

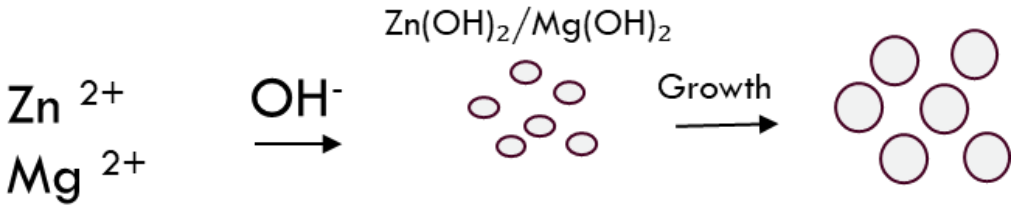
Erlen Cruz Jorge, Nicolás Navarro Martínez, María José González, Sofía V Sánchez, Luciana Robino, Javier O Morales & Paola Scavone ...show less

Pages 803-818 | Received 15 Sep 2022, Accepted 24 Apr 2023, Published online: 31 May 2023

Cite this article https://doi.org/10.2217/nnm-2022-0239 Check for updates

NANOPARTICLE SYNTHESIS





Oliveira, A. E. F., Pereira, A. C., Resende, M. A. C., & Ferreira, L. F. (2023). Gold Nanoparticles: A Didactic Step-by-Step of the Synthesis Using the Turkevich Method, Mechanisms, and Characterizations. Analytica, 4(2), 250-263. https://doi.org/10.3390/analytica4020020

CHARACTERIZATION

Figure 1. Physicochemical characterization of nanoparticles. (A) Absorption spectrum of gold Nps (AuT1-Nps, AuT2-Nps) and silver Nps. **(B)** Absorption and emission spectra of magnesium-doped ZnO Nps.
Np: Nanoparticle.

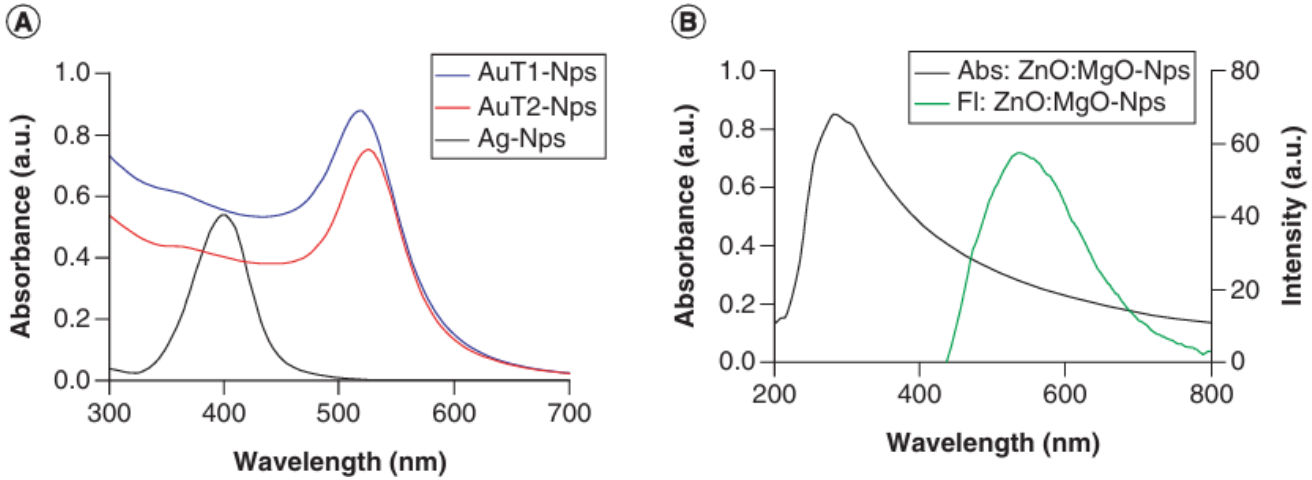


Table 1. Characterization summary of magnesium-doped ZnO nanoparticles, gold (AuT1-nanoparticles, AuT2-nanoparticles) and silver nanoparticles.

Nanoparticles	Hydrodynamic radius (d.nm)	Zeta potential (mV)	Polydispersity index
Magnesium-doped zinc oxide	209.82 ± 5.06	14.87 ± 1.64	0.45 ± 0.02
Gold (AuT1)	22.4 ± 1.01	-27.1 ± 1.70	0.34 ± 0.02
Gold (AuT2)	37.45 ± 0.76	-27.57 ± 5.76	0.24 ± 0.003
Silver	20.3 ± 2.53	-35.87 ± 1.87	0.32 ± 0.01

CHARACTERIZATION

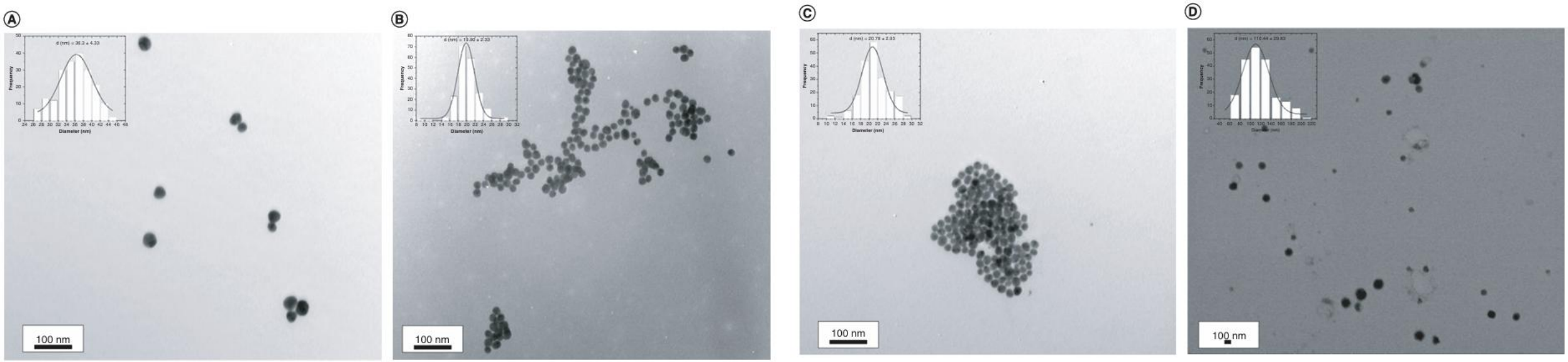
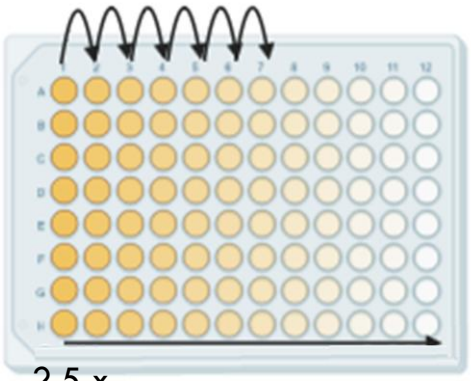


Figure 2. Transmission electron microscopy micrographs. (A) Gold AuT1 nanoparticles. **(B)** Gold AuT2 nanoparticles. **(C)** Silver nanoparticles. **(D)** Magnesium-doped zinc oxide nanoparticles. Silver and gold nanoparticles were obtained using a magnification of 120,000× and magnesium-doped zinc oxide nanoparticles with a magnification of 50,000×.

MIC AND MBC

LB or BHI, 37°C, 24h

Control: NPs, Culture medium



2,5 x

1,22 *10^{-3} x

LA, 37°C, 24h

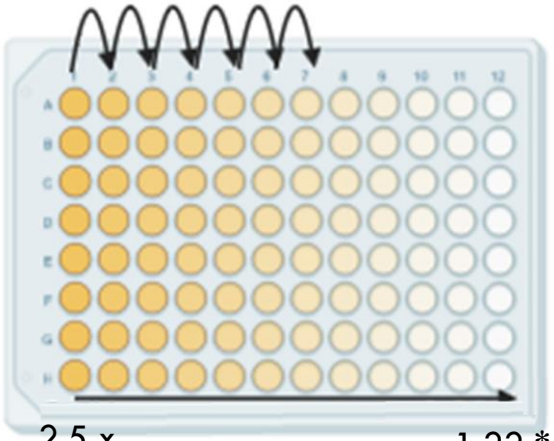
Table 2. Minimal inhibitory concentrations and minimal bactericidal concentrations for gold (AuT1, AuT2) nanoparticles, silver nanoparticles and magnesium-doped ZnO nanoparticles.

Strains	Gold AuT1 Nps		Gold AuT2 Nps		Silver Nps		Magnesium-doped zinc oxide Nps	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli 144	2.5×	>2.5×	>2.5×	>2.5×	2.5×	>2.5×	1.25×	1.25×
Proteus mirabilis 2921	2.5×	>2.5×	>2.5×	>2.5×	2.5×	>2.5×	1.25×	1.25×
Staphylococcus aureus 6538	2.5×	>2.5×	>2.5×	>2.5×	2.5×	>2.5×	1.25×	1.25×
Acinetobacter baumannii 19606	2.5×	>2.5×	>2.5×	>2.5×	1.25×	1,25x	1.25×	1.25×
Pseudomonas aeruginosa 9027	2.5×	>2.5×	2.5×	>2.5×	2.5×	>2.5×	1.25×	1.25×

MIC AND MBC: DOSE RESPONSE CURVES

Control: NPs, Culture médium, Strain

LB or BHI, 37°C, 24h

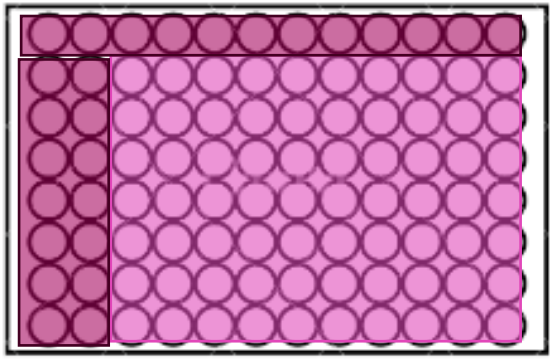


2,5 x

1,22 *10^{-3} x

Resazurin 0.5 mg/ml, 10 µL, 37°C, 2h

Ex = 530 nm and Em = 590 nm



MIC AND MBC: DOSE RESPONSE

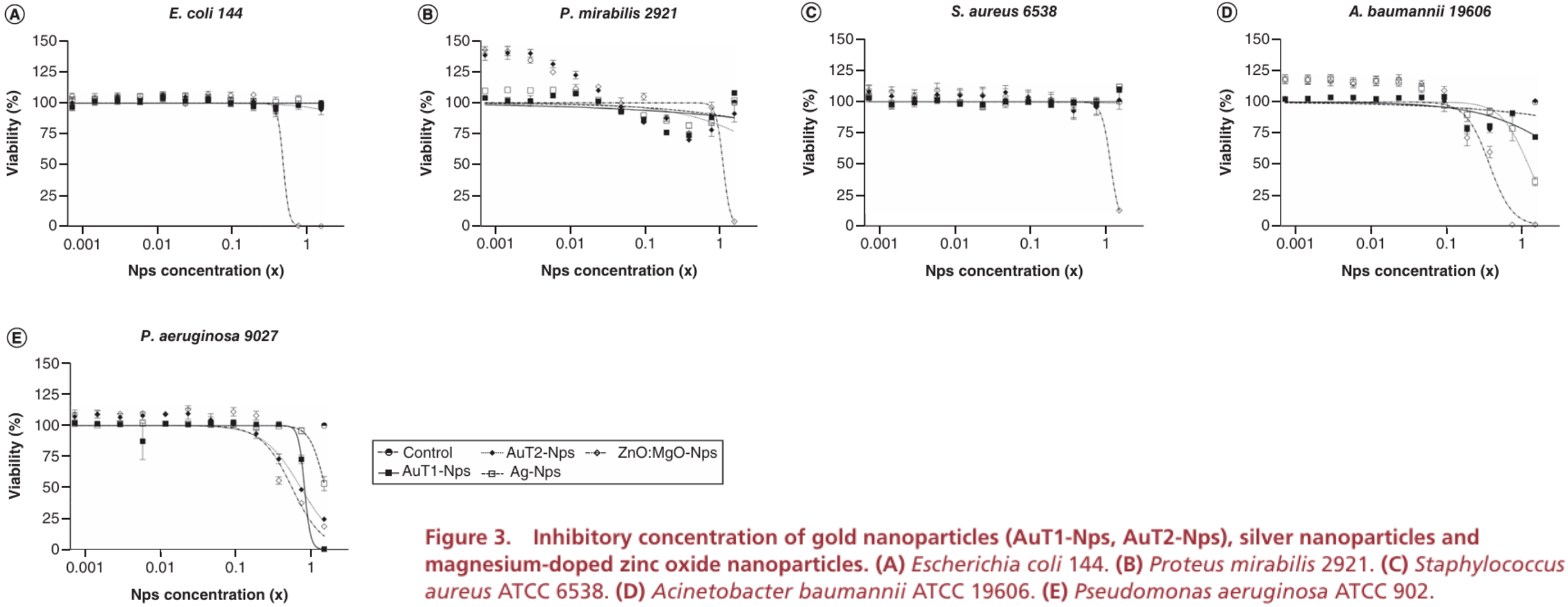


Figure 3. Inhibitory concentration of gold nanoparticles (AuT1-Nps, AuT2-Nps), silver nanoparticles and magnesium-doped zinc oxide nanoparticles. (A) *Escherichia coli* 144. **(B)** *Proteus mirabilis* 2921. **(C)** *Staphylococcus aureus* ATCC 6538. **(D)** *Acinetobacter baumannii* ATCC 19606. **(E)** *Pseudomonas aeruginosa* ATCC 902. Np: Nanoparticle.

PREVENTION OF BF FORMATION

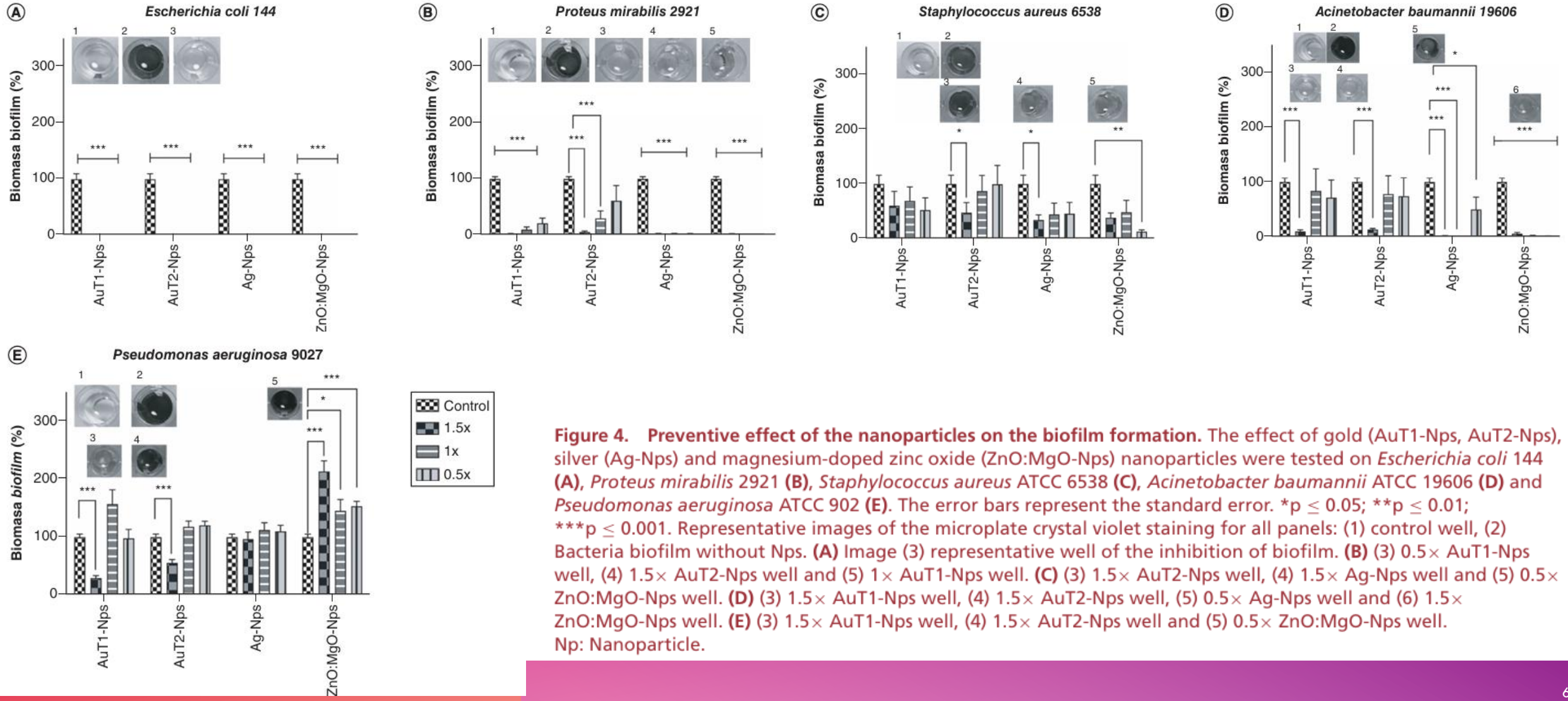


Figure 4. Preventive effect of the nanoparticles on the biofilm formation. The effect of gold (AuT1-Nps, AuT2-Nps), silver (Ag-Nps) and magnesium-doped zinc oxide (ZnO:MgO-Nps) nanoparticles were tested on *Escherichia coli* 144 **(A)**, *Proteus mirabilis* 2921 **(B)**, *Staphylococcus aureus* ATCC 6538 **(C)**, *Acinetobacter baumannii* ATCC 19606 **(D)** and *Pseudomonas aeruginosa* ATCC 902 **(E)**. The error bars represent the standard error. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Representative images of the microplate crystal violet staining for all panels: (1) control well, (2) Bacteria biofilm without Nps. **(A)** Image (3) representative well of the inhibition of biofilm. **(B)** (3) 0.5× AuT1-Nps well, (4) 1.5× AuT2-Nps well and (5) 1× AuT1-Nps well. **(C)** (3) 1.5× AuT2-Nps well, (4) 1.5× Ag-Nps well and (5) 0.5× ZnO:MgO-Nps well. **(D)** (3) 1.5× AuT1-Nps well, (4) 1.5× AuT2-Nps well, (5) 0.5× Ag-Nps well and (6) 1.5× ZnO:MgO-Nps well. **(E)** (3) 1.5× AuT1-Nps well, (4) 1.5× AuT2-Nps well and (5) 0.5× ZnO:MgO-Nps well.
Np: Nanoparticle.

ERRADICATION OF BF FORMATION

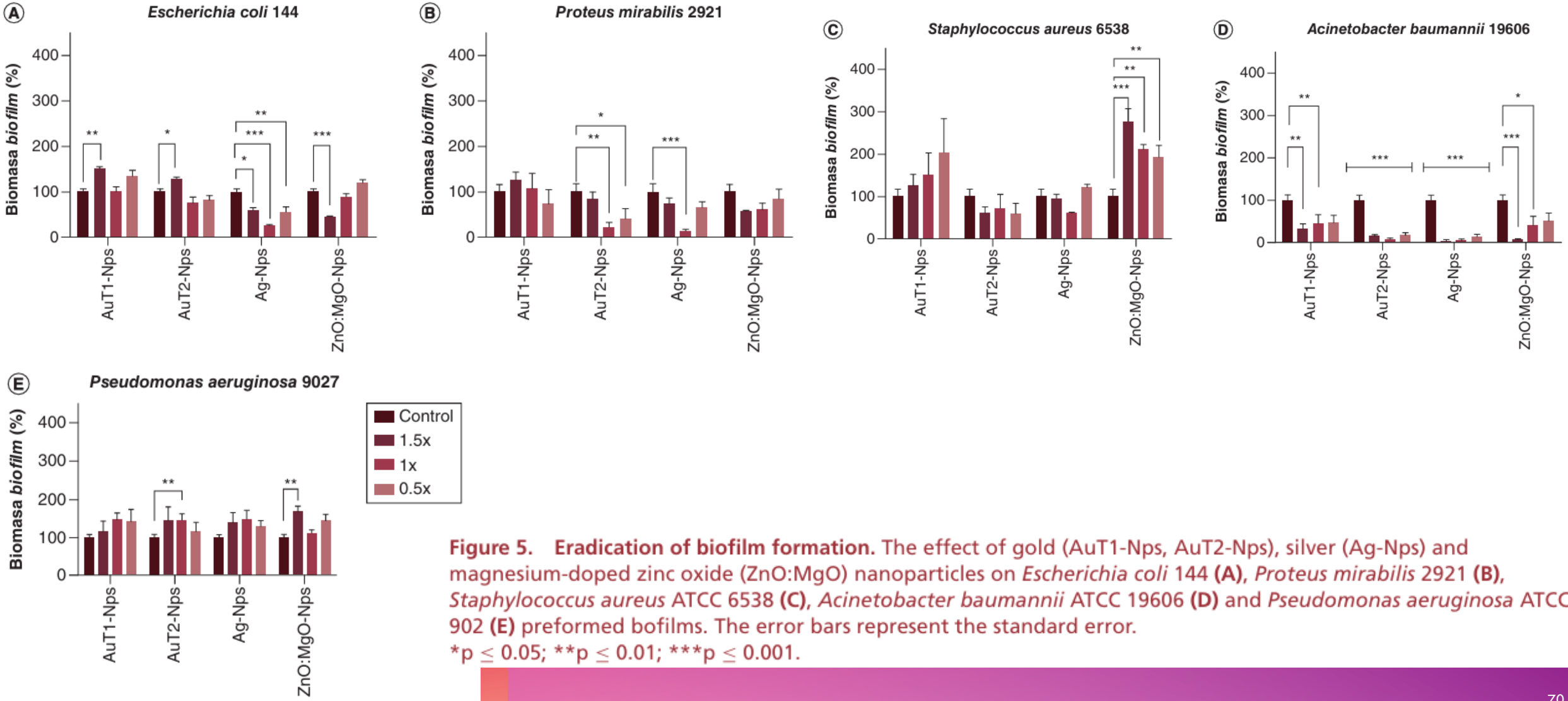


Figure 5. Eradication of biofilm formation. The effect of gold (AuT1-Nps, AuT2-Nps), silver (Ag-Nps) and magnesium-doped zinc oxide (ZnO:MgO) nanoparticles on *Escherichia coli* 144 **(A)**, *Proteus mirabilis* 2921 **(B)**, *Staphylococcus aureus* ATCC 6538 **(C)**, *Acinetobacter baumannii* ATCC 19606 **(D)** and *Pseudomonas aeruginosa* ATCC 902 **(E)** preformed bofilms. The error bars represent the standard error.
*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

ELSEVIER

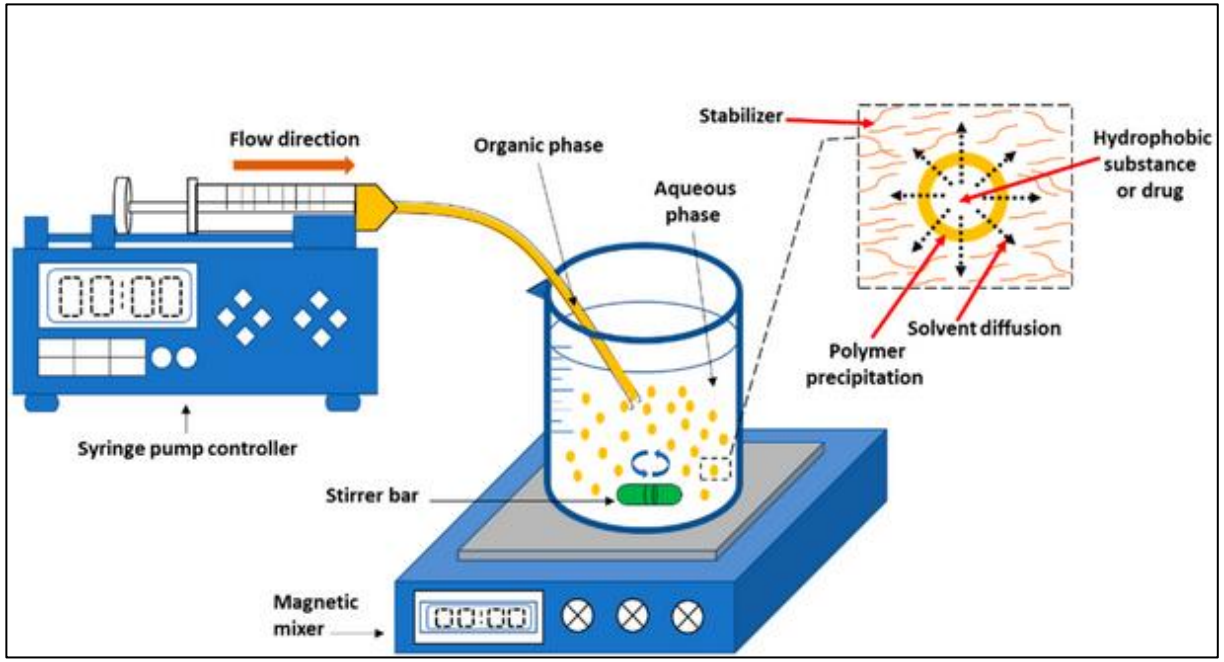
Journal of Drug Delivery Science and Technology

Volume 113, November 2025, 107330

Novel properties of poly(n-butyl cyanoacrylate) as antibacterial and antibiofilms nanoparticles

Erlen Yizenia Cruz Jorge [a], Nicolás Matías Navarro Martínez [a], María José González [a], Yaquelin Ramos Carriles [b], Luciana Robino [a c], Rubén Álvarez Brito [b], Paola Scavone [a]

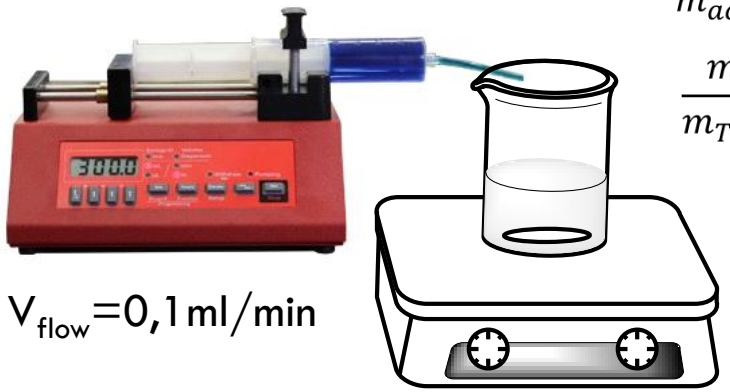
SYNTHESIS



ρ_{PBCA}=0,05%

$$\frac{m_{H_2O}}{m_{acetone}} = 1.5$$

$$\frac{m_{PBCA}}{m_{Tween80}} = 1$$



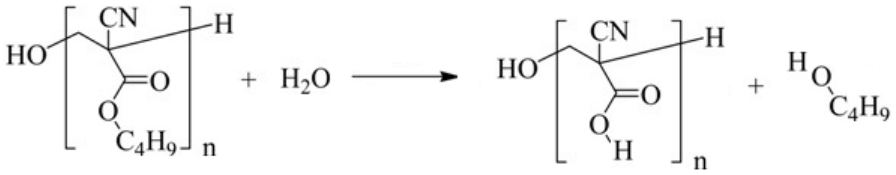
V_{flow}=0,1 ml/min

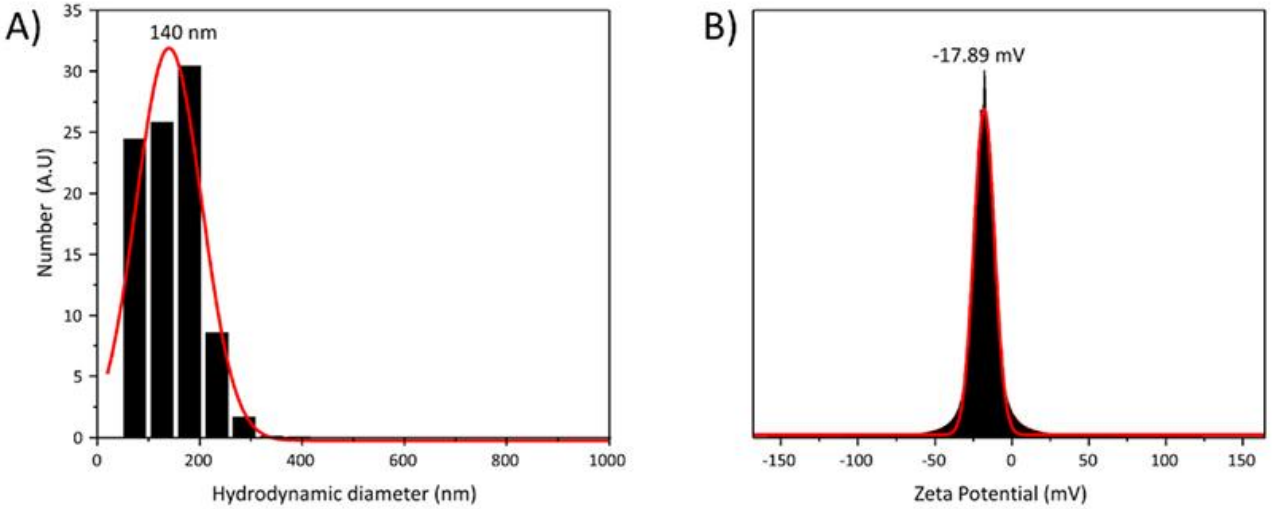
T atm

V=700 rpm

0,21 ± 0,02 mg/mL → **84 % yield**

CHARACTERIZATION





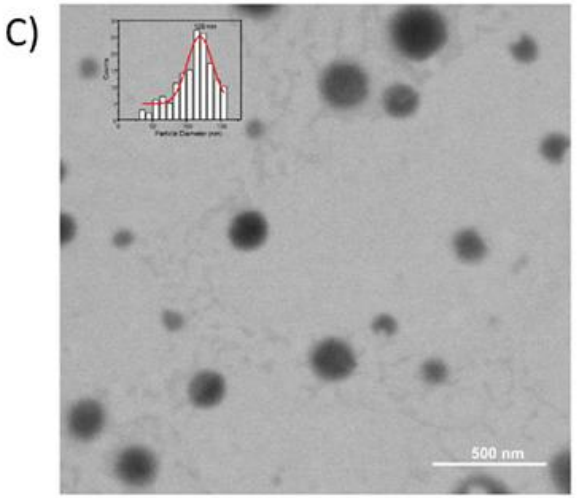


Fig. 1. (A) Size distribution obtained by DLS, (B) Zeta potential and (C) representative TEM micrograph of PBCA NPs.

MIC AND MBC: DOSE-RESPONSE CURVES

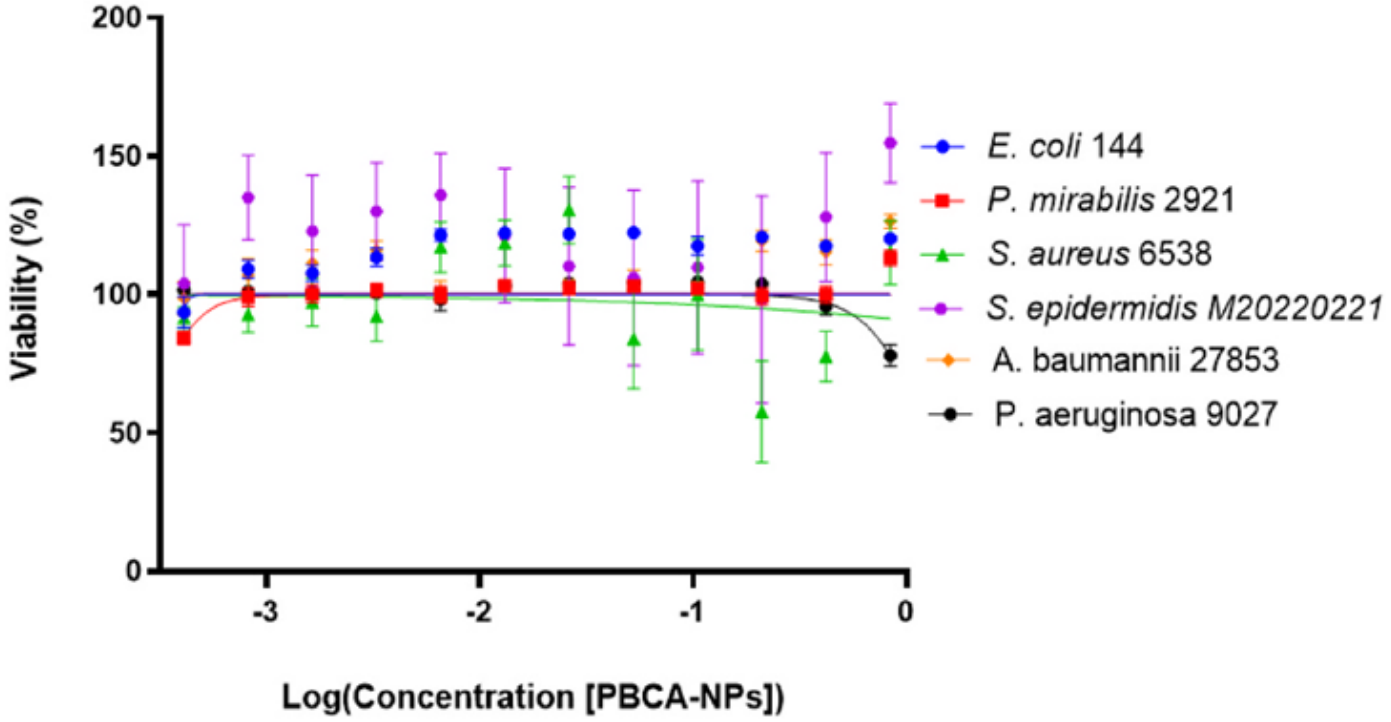


Fig. 2. Viability percent of *E. coli, P. mirabilis, S. aureus, S. epidermidis, A. baumannii* and *P. aeruginosa* against PBCA.

CYTOTOXICITY: T24 CELLS

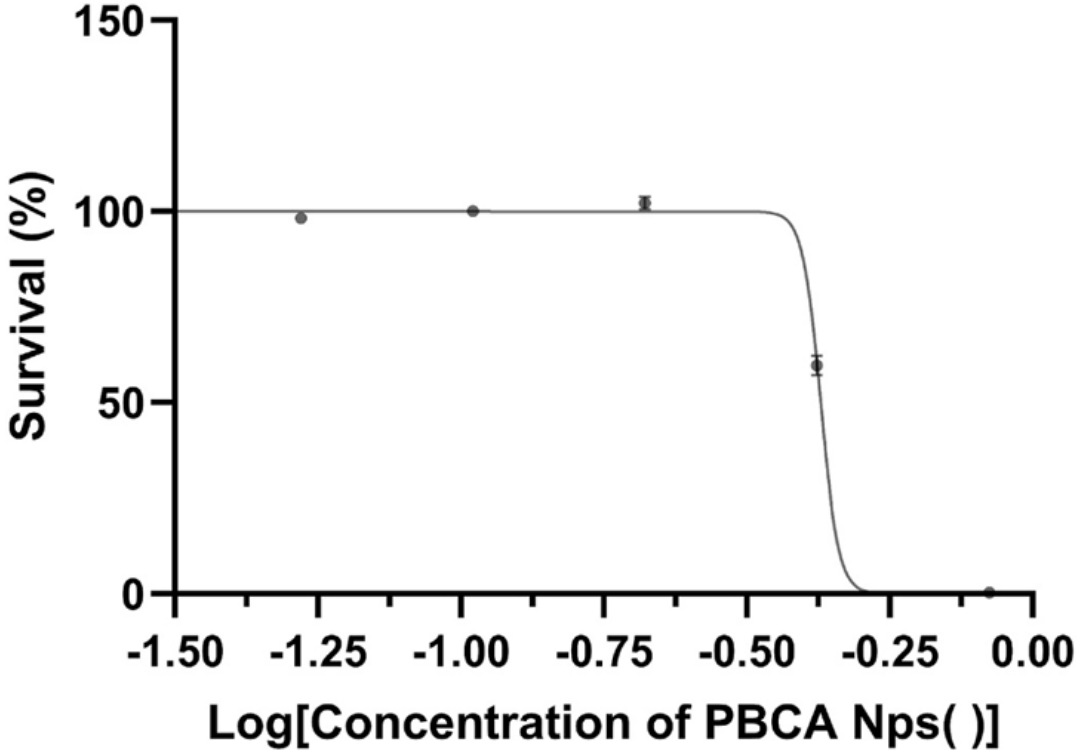
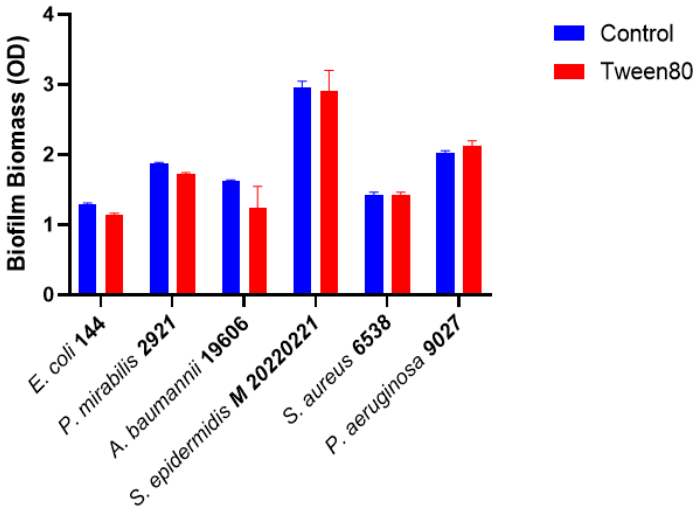


Fig. 3. IC_{50} graphs showing the inhibitory concentration of PBCA NPs on T24 bladder carcinoma cells (ATCC HTB-4).

PREVENTION AND ERADICATION OF BIF FORMATION



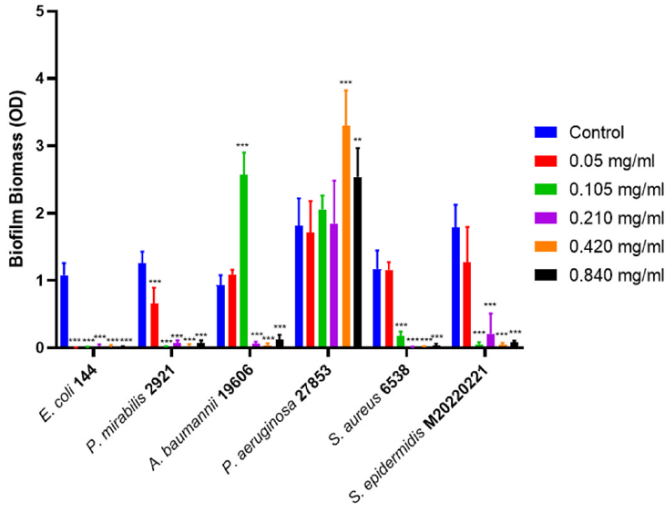


Fig. 4. Effect of NPs on the Inhibition of biofilm formation of *E. coli, P. mirabilis, S. aureus, S. epidermidis, A. baumannii* and *P. aeruginosa*. Error bars represent standard error. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

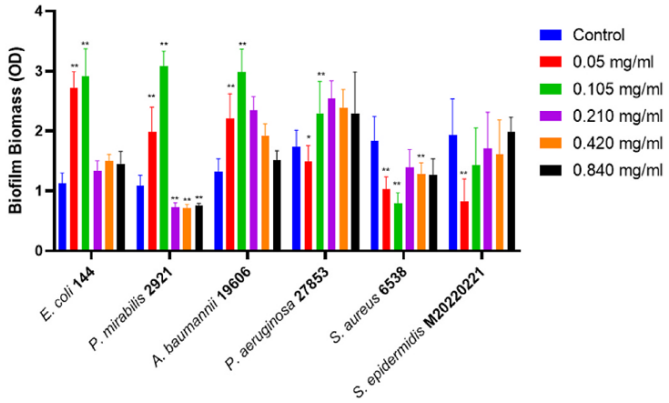
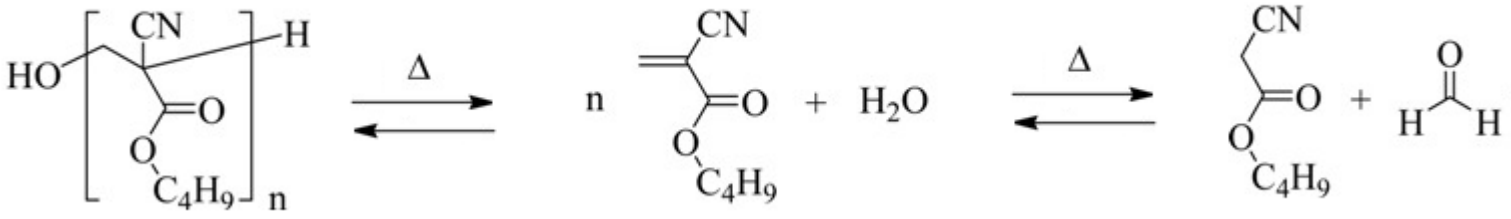


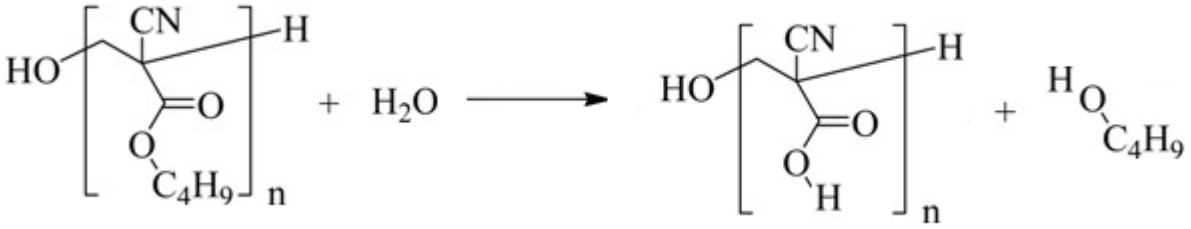
Fig. 5. Effect of NPs on the eradication of mature biofilms of *E. coli, P. mirabilis, S. aureus, S. epidermidis, A. baumannii* and *P. aeruginosa*. Error bars represent standard error. *$p \leq 0.05$; **$p \leq 0.01$.

DEGRADATION OF PBCA IN AN AQUEOUS MEDIUM

1- Knoevenagel reverse reaction



2- Hydrolysis of the side chain



Advanced Drug Delivery Reviews 55(4):519-48

CUANTIFICATION OF EPS: CONGO RED

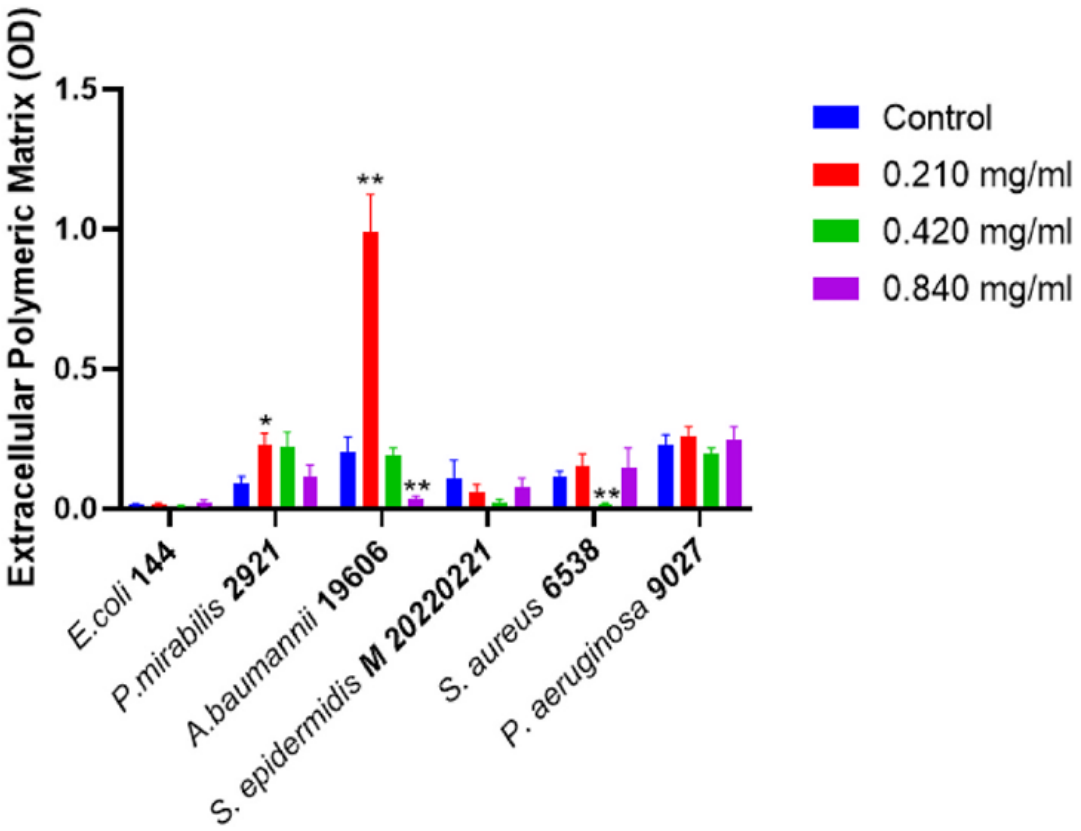


Fig. 6. Effect of NPs on extracellular polymeric substances of *E. coli, P. mirabilis, S. aureus*, *S. epidermidis*, *A. baumannii* and *P. aeruginosa*. Error bars represent standard error. *$p \leq 0.05$; **$p \leq 0.01$.

PREVENTION OF BF FORMATION: COATED SURFACES

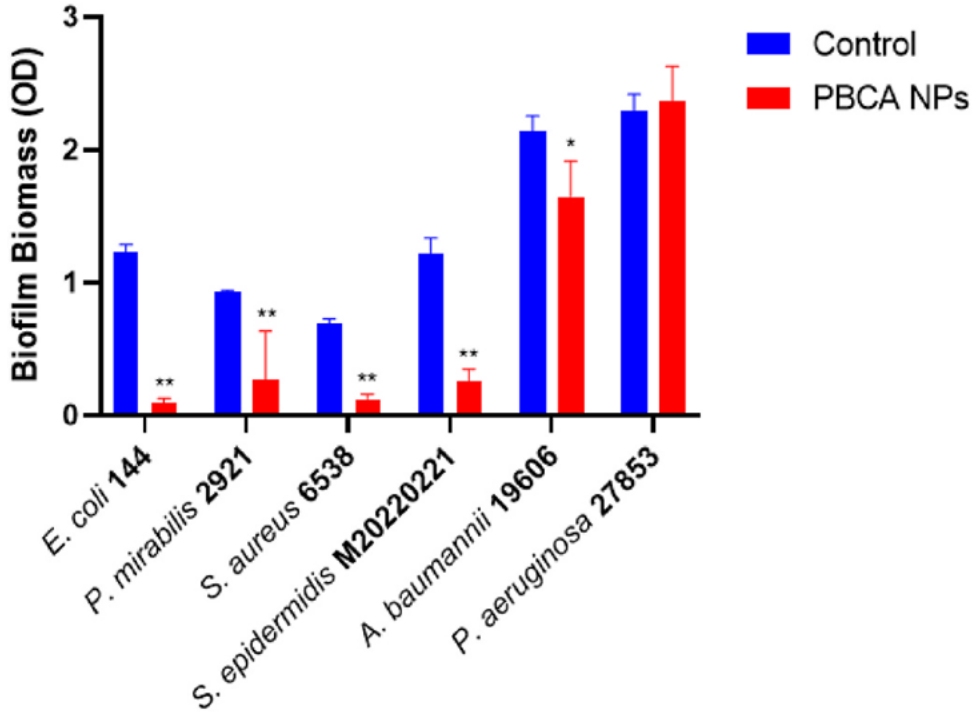


Fig. 7. Effect of NPs on the adhesion of biofilms of *E. coli, P. mirabilis, S. aureus, S. epidermidis, A. baumannii* and *P. aeruginosa*. Error bars represent standard error. *$p \leq 0.05$; **$p \leq 0.01$.

PREVENTION OF BF FORMATION: COATED SURFACES

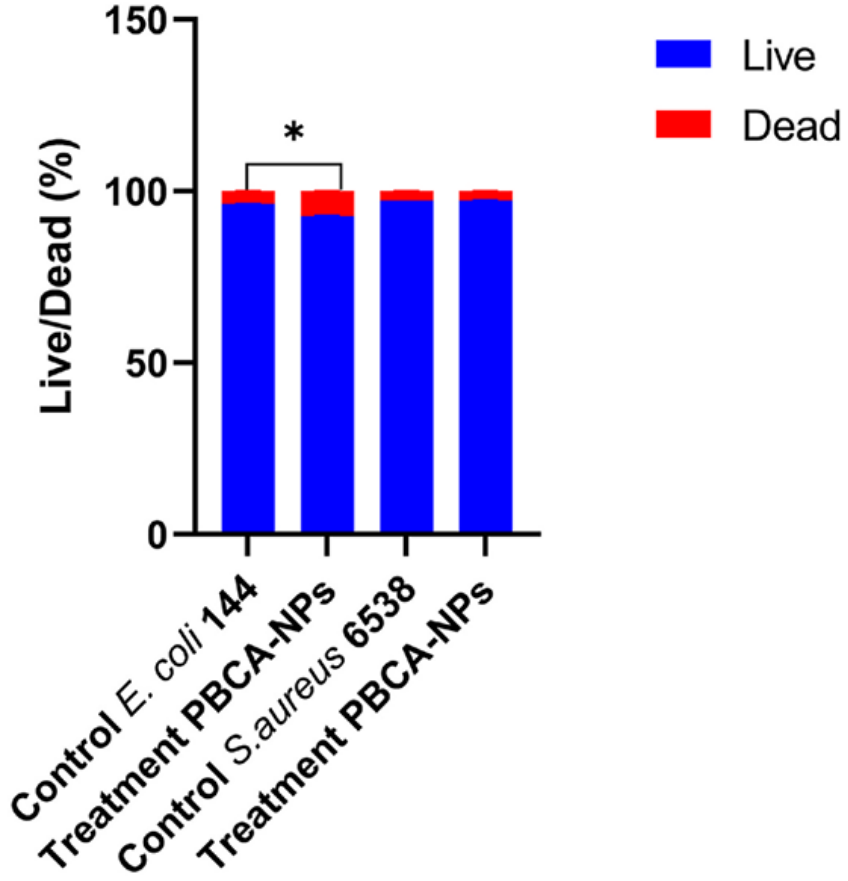
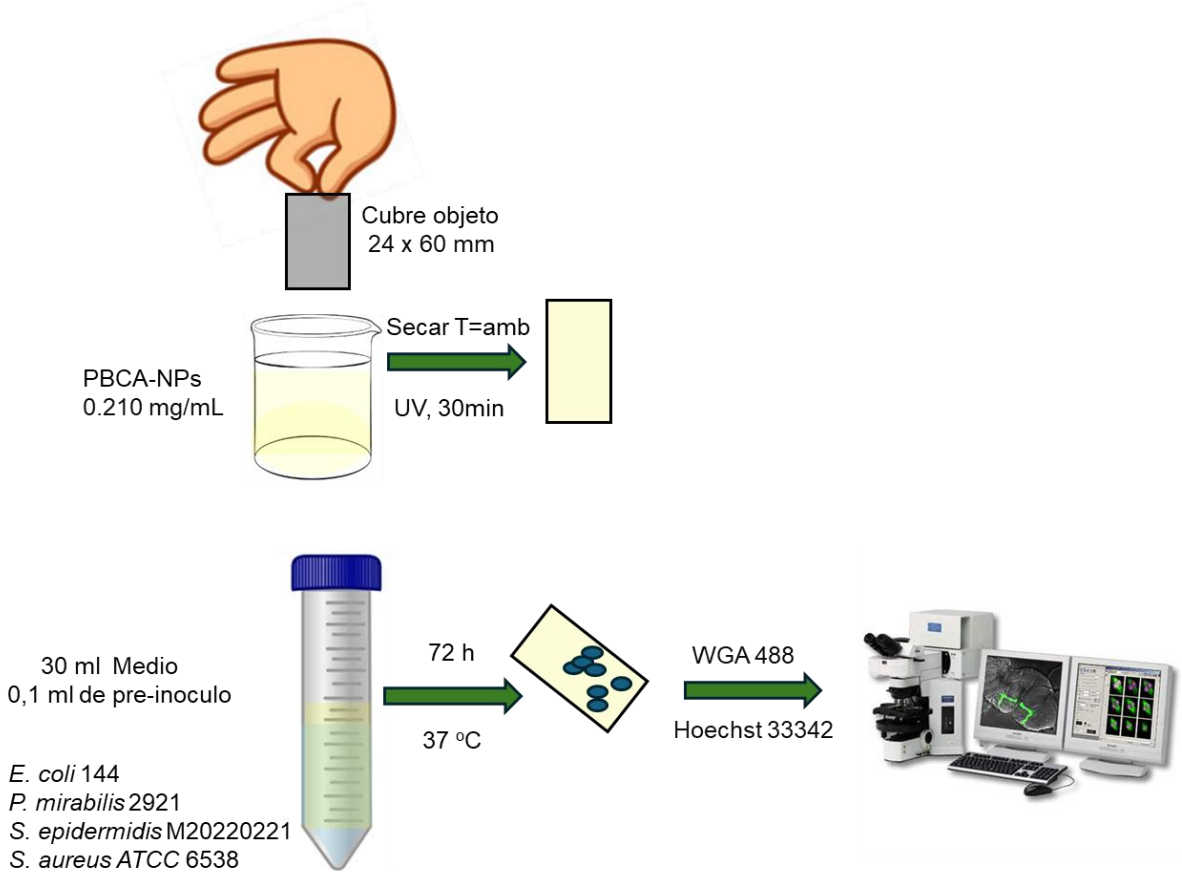
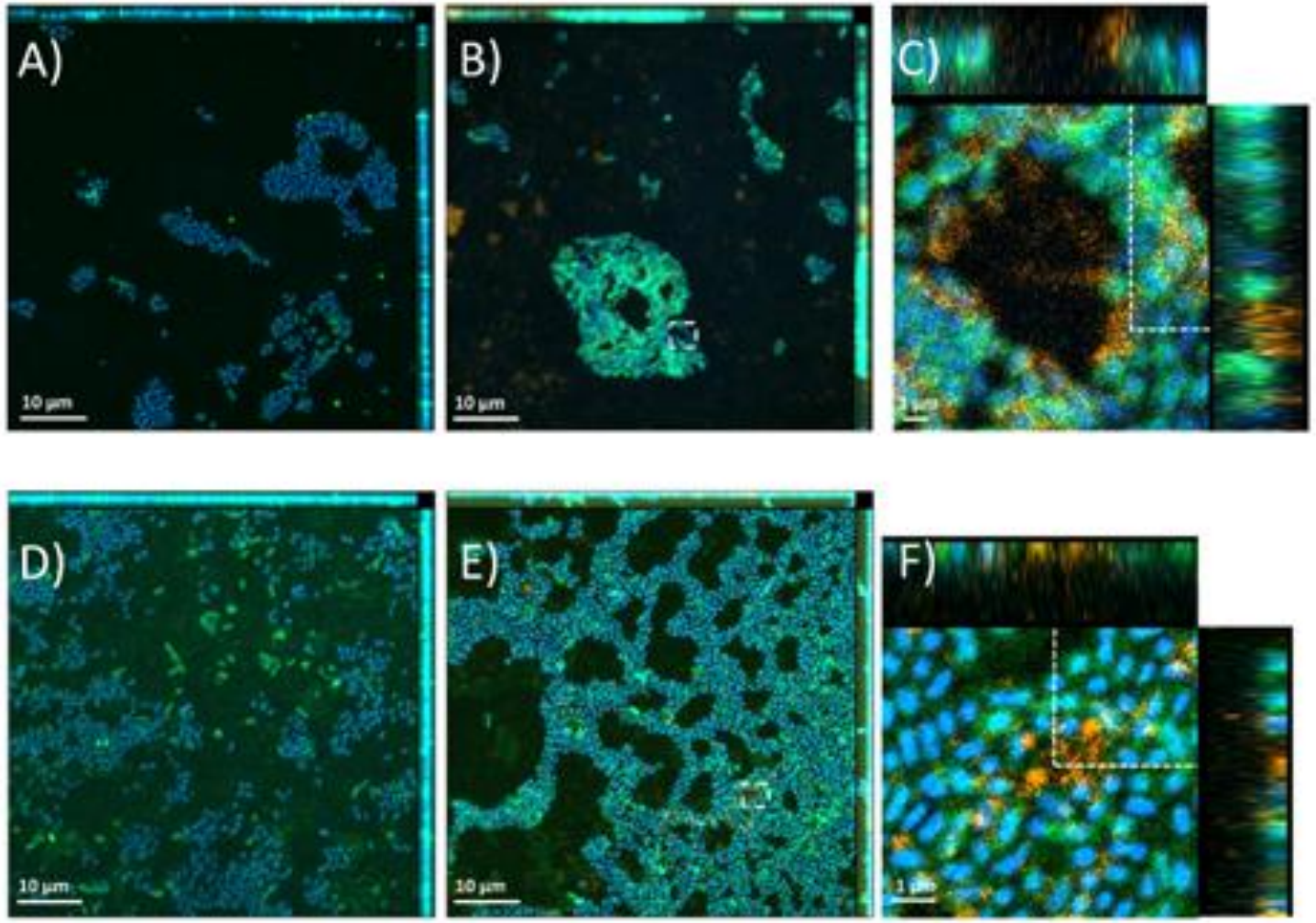


Fig. 8. Percentage of live and dead bacteria after treatment with PBCA nano particles. Viability was assessed using a dual staining assay with Syto 9 (green, live cells) and propidium iodide (PI, red, dead cells). E. coli (Gram-negative) and S. aureus (Gram-positive) were used as representative models. Error bars represent standard error. *$p \leq 0.05$.

PREVENTION OF BF FORMATION: COATED GLASS COVERSLIPS

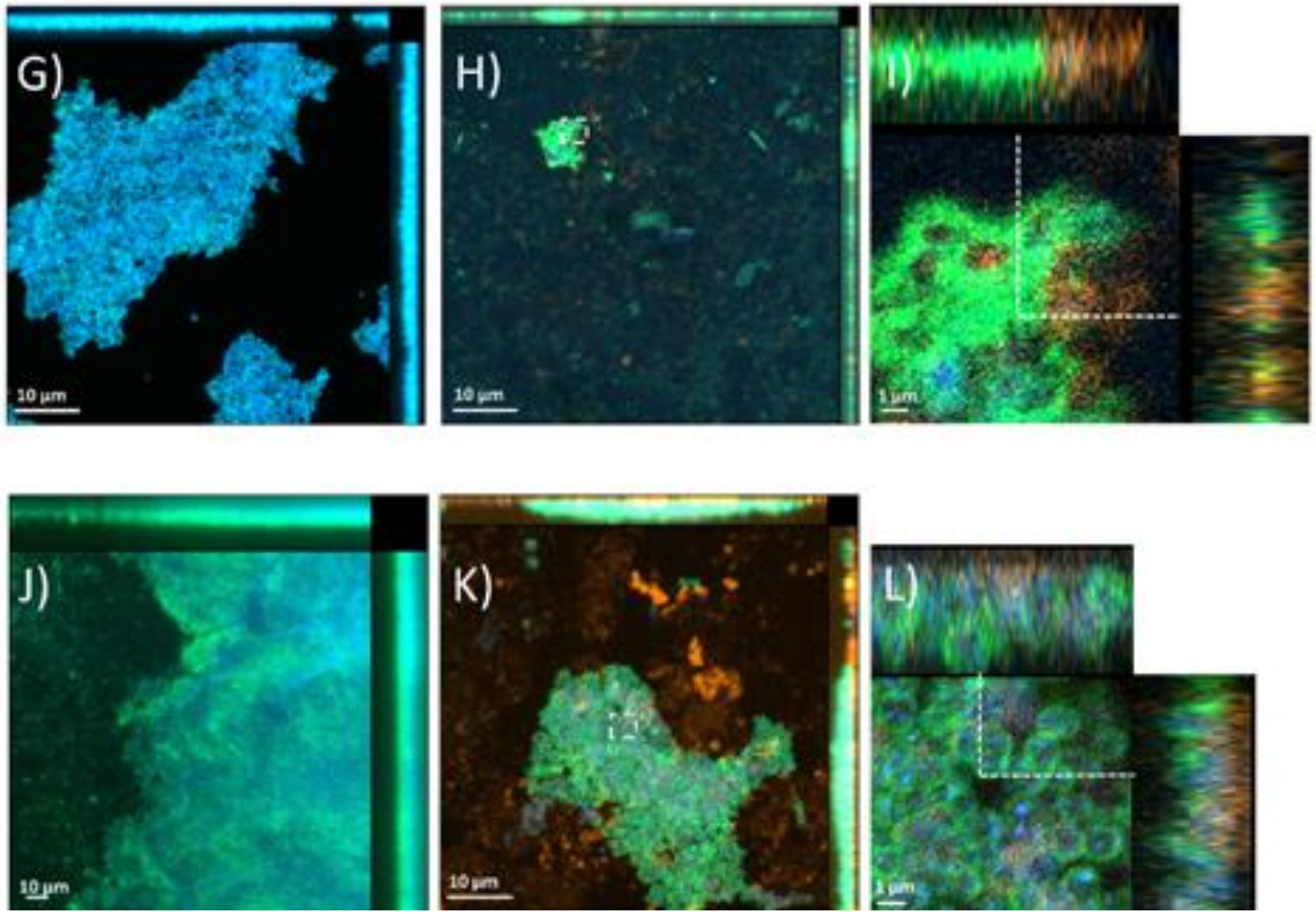


PREVENTION OF BF FORMATION: COATED GLASS COVERSLIPS



A - C) E. coli 144, D - F) P. mirabilis 2921

PREVENTION OF BF FORMATION: COATED GLASS COVERSLIPS



G - I) *S. aureus* 6538, and J - L) *S. epidermidis* M20220221 biofilms.

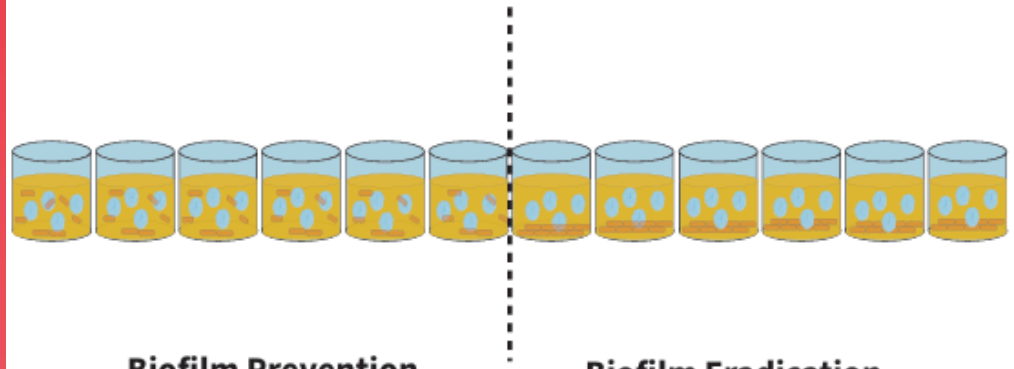
PBCA Nanoparticles

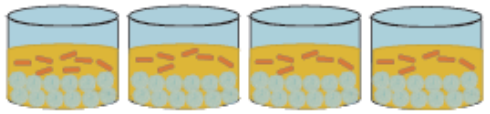
Biofilm Prevention and Eradication

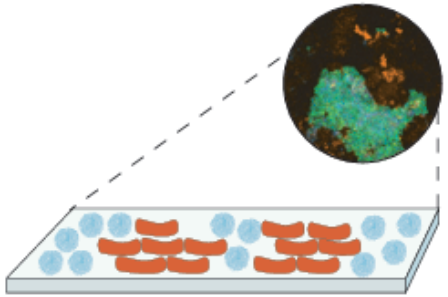
MIC > 0.85 mg/mL

Biofilm Prevention on Coated Surfaces

MBC > 0.85 mg/mL







Biofilm Prevention

- *S. aureus* ⊘
- *A. baumannii* ⊘
- *P. aeruginosa* ↑
- *S. epidermidis* ⊘
- *E. coli* ⊘
- *P. mirabilis* ⊘

Biofilm Eradication

- *S. aureus* ↑
- *A. baumannii* ↑
- *P. aeruginosa* ↑
- *S. epidermidis* ↑
- *E. coli* ↑
- *P. mirabilis* ↑

Coated 96 well plates

- *S. aureus* ⊘
- *A. baumannii* ⊘
- *P. aeruginosa* ↑
- *S. epidermidis* ⊘
- *E. coli* ⊘
- *P. mirabilis* ⊘

Coated Coverslips

- *S. aureus* ⊘
- *S. epidermidis* ⊘
- *E. coli* ⊘
- *P. mirabilis* ⊘

⊘ Prevention of Biofilm Formation

↑ Increase of Biofilm Biomass

GRACIAS!