



Nanotecnología Aplicada a Biofilms

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Tabla de Contenidos



Definición de Nanotecnología

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Metodologías Síntesis Generales

3



Nanotransportadores

Ejemplos Nt

- Lipídicos
- Poliméricos
- Metálicos





"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.



Definición de Nanotecnología



INTRODUCCIÓN

"Nanotechnology is the creation of functional materials, devices, and systems through control and manipulation of matter on the nanometer length scale (1-100 nanometers). At this scale, engineers have the ability to exploit novel phenomena and material properties, be they physical, chemical, biological, mechanical, or electrical"







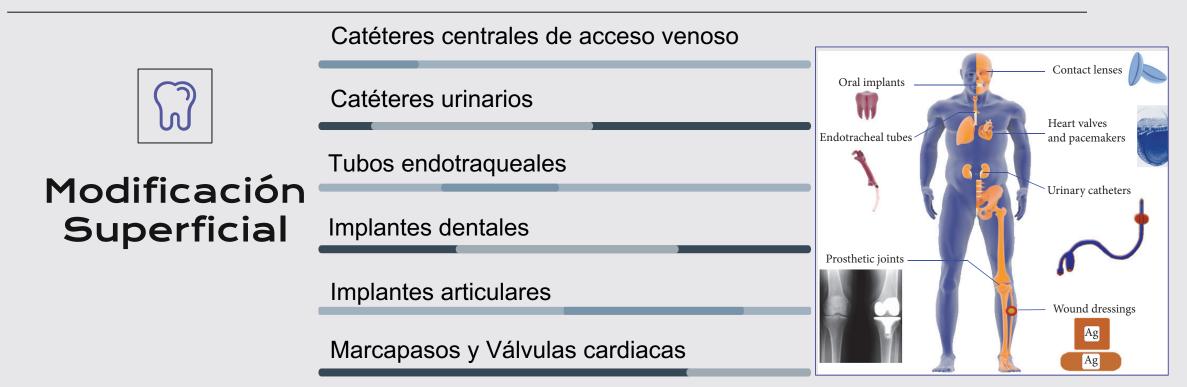


Modificación Superficial



Nanocarriers ATB/ATM

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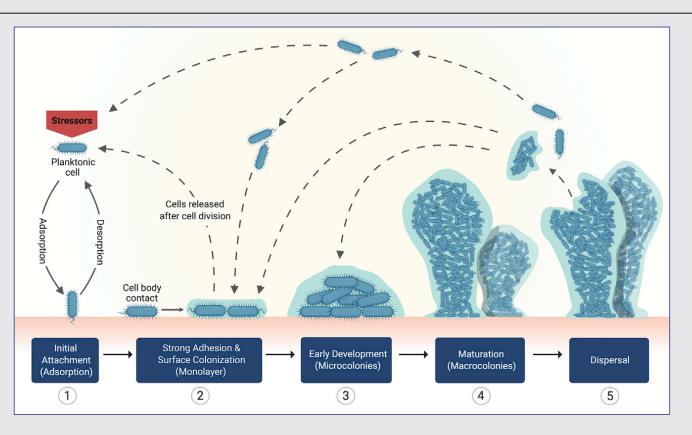


Ramasamy M, Lee J. Recent Nanotechnology Approaches for Prevention and Treatment of Biofilm-Associated Infections on Medical Devices. Biomed Res Int. 2016.





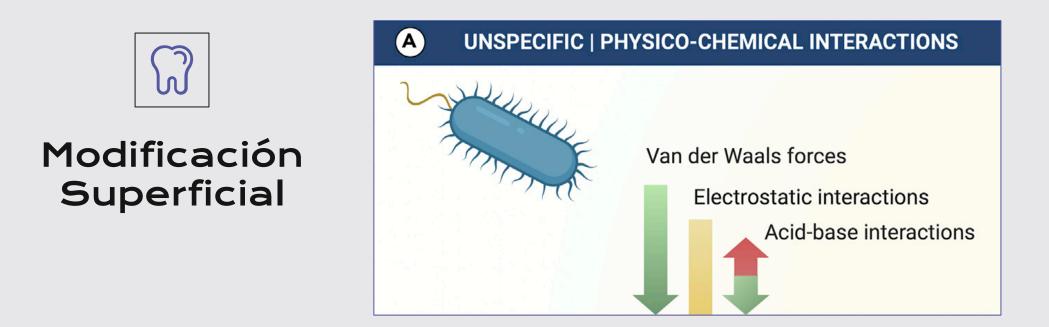
Modificación Superficial



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





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





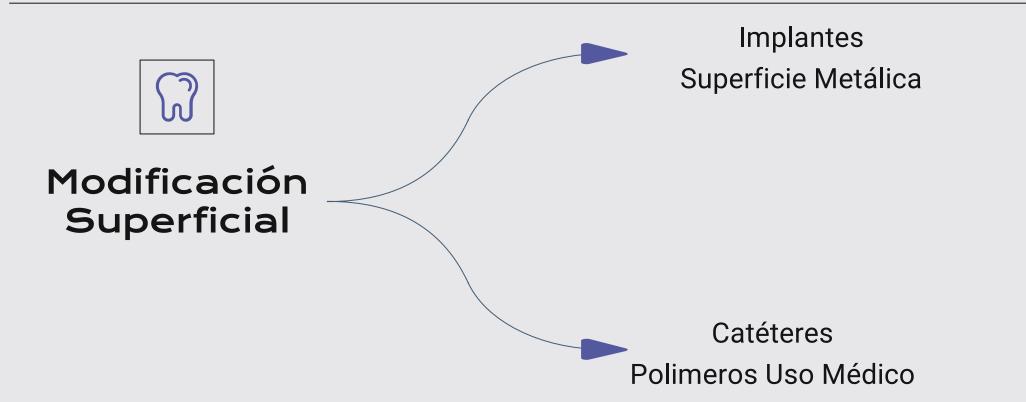
Modificación Superficial

- 1) Antifouling coatings (Dureza, carga, hidrofobicidad)
- 2) Modificaciones antiadhesivas (polímeros)
- 3) Adición de antimicrobianos.
- 4) Modificación físico-química.
- 5) Adición de moléculas bioactivas (QSi).

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











Modificación Superficies Metálicas

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.



Modificación Superficies Metálicas

Plasma Spraying

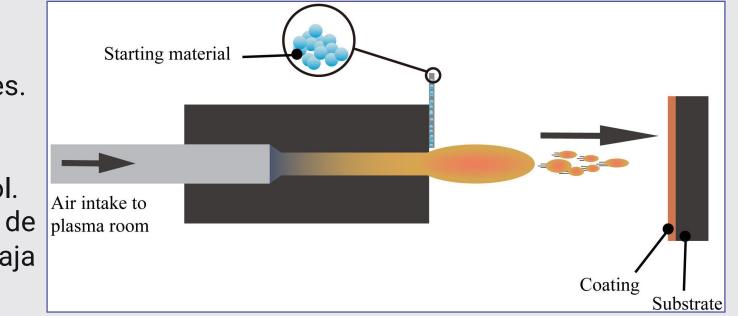
Electrochemical Anodization

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



Plasma Spraying

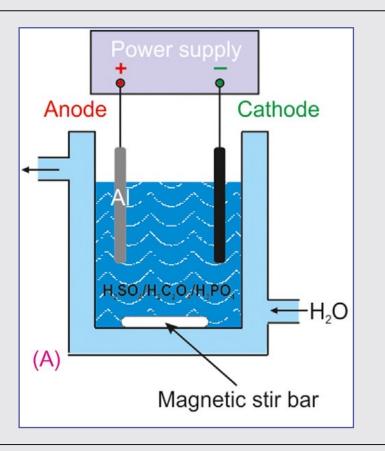
- Gold Estándar.
- Cerámicas (HA) y Metales.
- Recubrimiento Nanométrico.
- Gran estabilidad y control.
- Alta eficiencia de deposición y baja porosidad.



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



- Películas de óxido anódicas.
- Morfología compacta, nanoporosa o nanotubular.
- Dopaje metales (Zn, Ag, Au, Cu, etc).
- Modificación estructural.
- Bajo costo.
- Escalabilidad técnica.



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

Formación de nanotubos en superficie de implantes de titanio.

- Bajo costo
- Control de características superficiales
 - El diámetro del nanotubo se puede controlar mediante el voltaje de oxidación.
 - La longitud del nanotubo se controla mediante la combinación del tiempo de oxidación, la temperatura de oxidación y el valor de pH del electrolito.

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

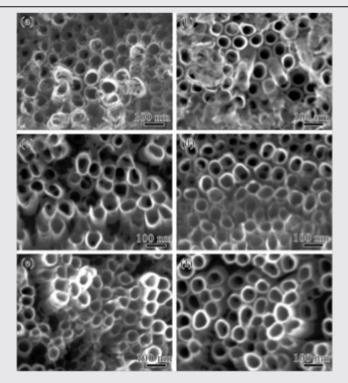


Figure 4. FE-SEM images representing the top view of ZnO-TNTs. (a) Undoped TNTs (spray-gold), (b) 1 mM · L⁻¹ ZnO-TNTs, (c) 2 mM · L⁻¹ ZnO-TNTs, (d) 3 mM · L⁻¹ ZnO-TNTs, (e) 4 mM · L⁻¹ ZnO-TNTs, (f) 5 mM · L⁻¹ ZnO-TNTs.

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

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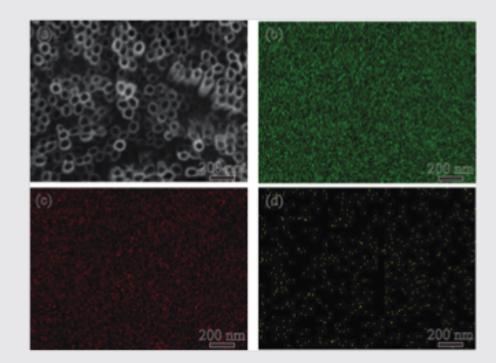
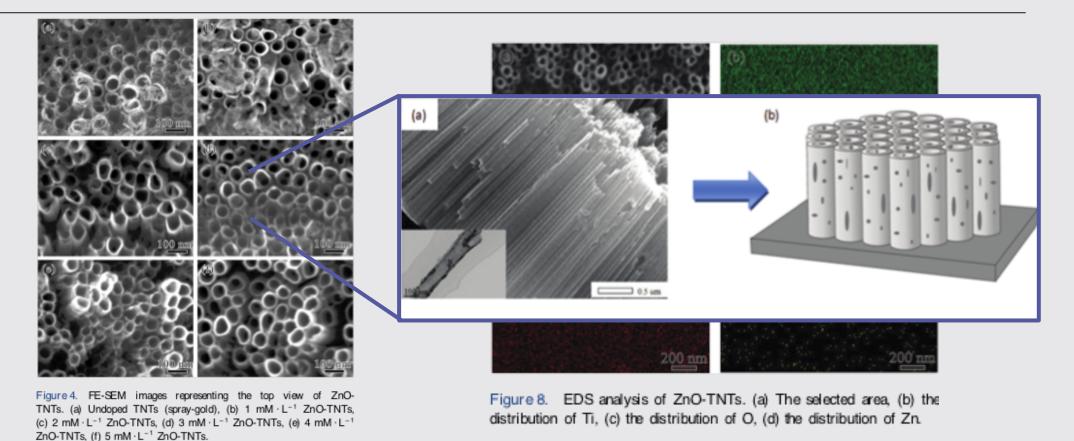


Figure 8. EDS analysis of ZnO-TNTs. (a) The selected area, (b) the distribution of Ti, (c) the distribution of O, (d) the distribution of Zn.



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

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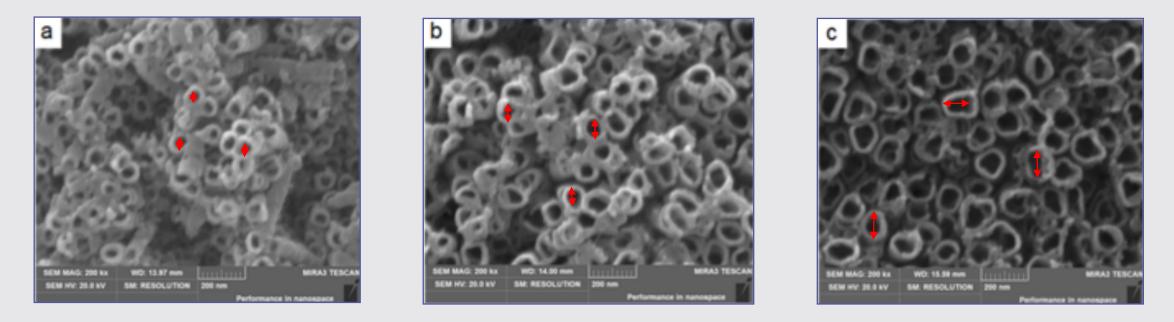
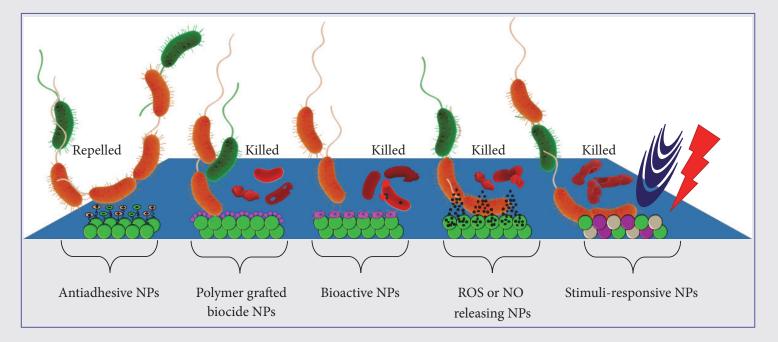


Figure 3. SEM images of nanotube arrays anodized at the following times: (a) 3 h, (b) 6 h and (c) 12 h, with inner diameters of approximately 44.5, 71.2 and 136.8 nm, respectively.

Zhang X, et al. Materials (Basel). 2019 Jun 20;12(12):1979.

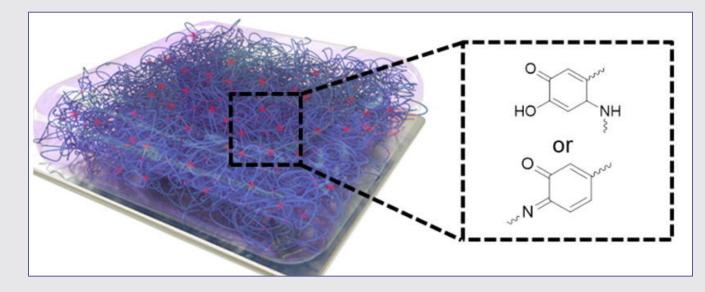
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- Utilización polímeros con propiedades bactericidas.
- Bajo costo.
- Utilización de materiales "aprobados" por FDA.
- En conjunto con moléculas activas (Nps/ATB).



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

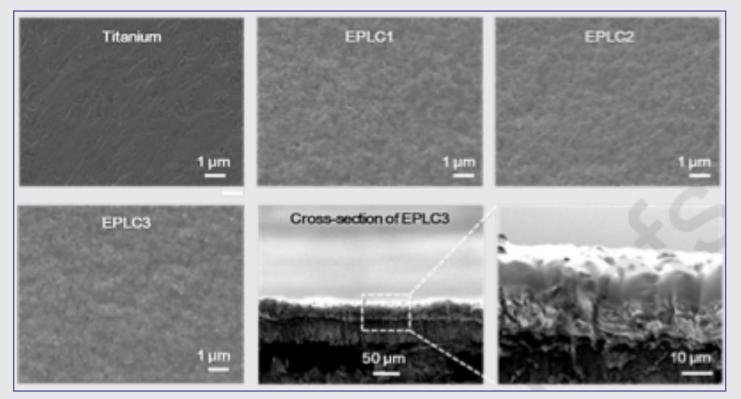




Characterization of musselinspired EPLC coatings. (a) SEM images of top surfaces of pristine Ti, and EPLC1/EPLC2/EPLC3 painted Ti substrate(Scale bar = 1 μ m), and cross-section of EPLC3 coating on Ti substrate.

Miao Xu, et al. Chemical Engineering Journal, Volume 396, 2020.

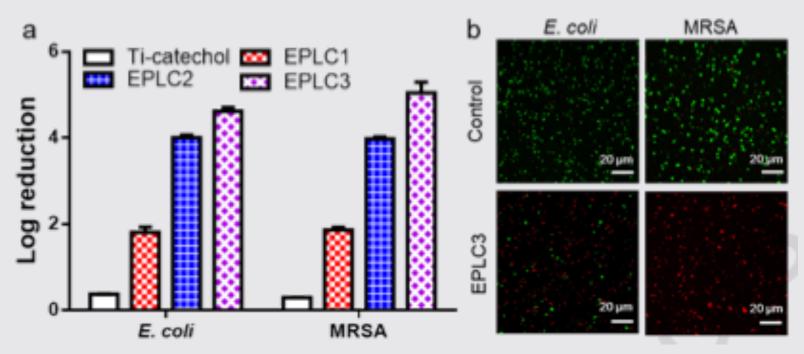




Characterization of musselinspired EPLC coatings. (a) SEM images of top surfaces of pristine Ti, and EPLC1/EPLC2/EPLC3 painted Ti substrate(Scale bar = 1 μ m), and cross-section of EPLC3 coating on Ti substrate.

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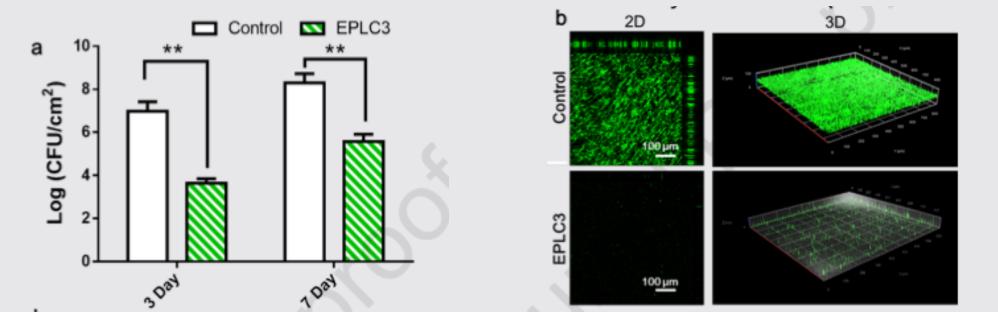
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In vitro antimicrobial activities of EPLC coatings. (a) Bacterial reduction of EPLC coatings against E.coli and MRSA. Each data point represents the mean \pm standard deviation for three separate samples (n = 3). (b) Live/Dead bacterial viability assay of E. coli (Left) and MRSA (Right) on the control and EPLC3 coating.

Miao Xu, et al. Chemical Engineering Journal, Volume 396, 2020.

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In vitro anti-biofilm activity. (a) Bacterial (MRSA) count from biofilm at day 3 and day 7 (n = 5, **p < 0.01). Confocal microscopy images of MRSA growing on the surfaces of (b) control and EPLC3 painted quartz slides after 3 days. MRSA was stained using SYTOTM 9 green fluorescent nucleic acid stain. (Scale bar = 100 µm)

Miao Xu, et al. Chemical Engineering Journal, Volume 396, 2020.

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FDA Guidelines

FDA **NO aprueba** el uso individual de materiales o recubrimientos, si no que el dispositivo médico terminado en su conjunto.

FDA evalúa:

- Análisis metalúrgico.
- Microestrutura de la superficie modificada (grosor, forma, tamaño, diámetro o área, diámetro de poro, volumen).
- Propiedades físicas (dureza).
- Propiedades mecánicas (Resistencia/Fatiga).
- Biocompatibilidad.
- Información clínica.

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

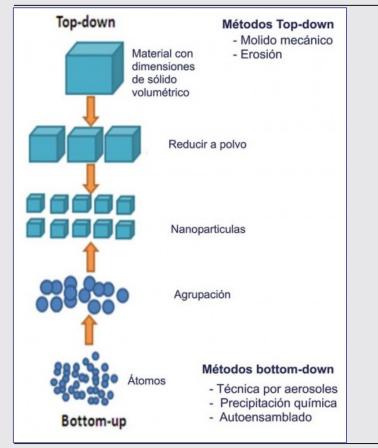


Nanotransportadores

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Metodologías de Síntesis Generales

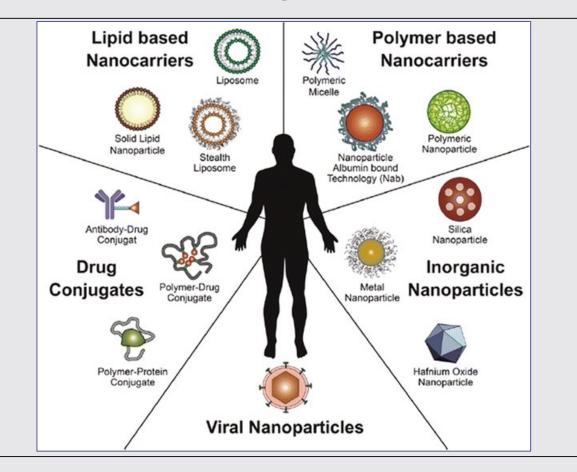
Nanotransportadores



Son sistemas coloidales en la escala nanométrica (1-100 nm), capaces de transportar drogas u otras sustancias de distinta naturaleza.

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.

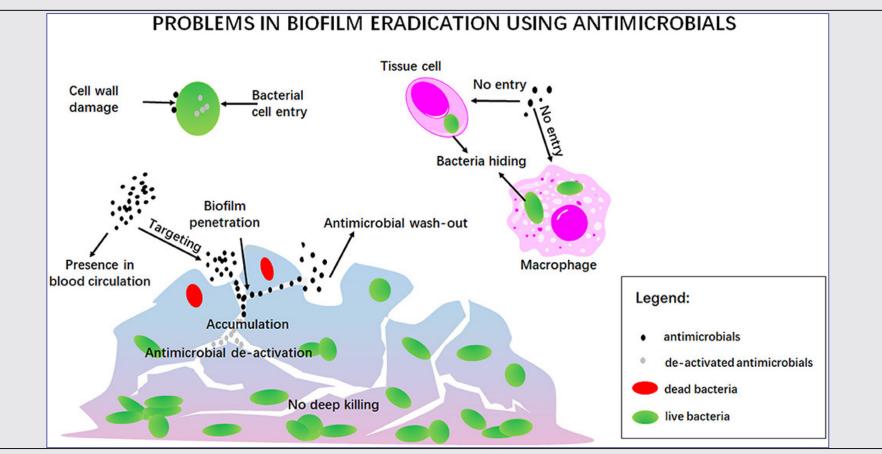
Nanotransportadores



A. Wicki et al., Journal of Controlled Release 200, 138 (2015).

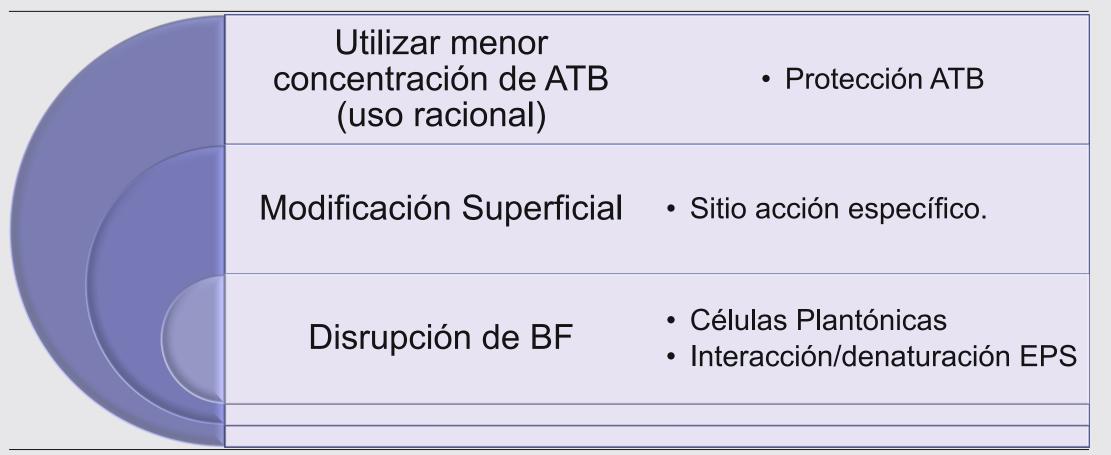
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Wang DY, van der Mei HC, Ren Y, Busscher HJ, Shi L. Lipid-Based Antimicrobial Delivery-Systems for the Treatment of Bacterial Infections. Front Chem. 2020

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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.

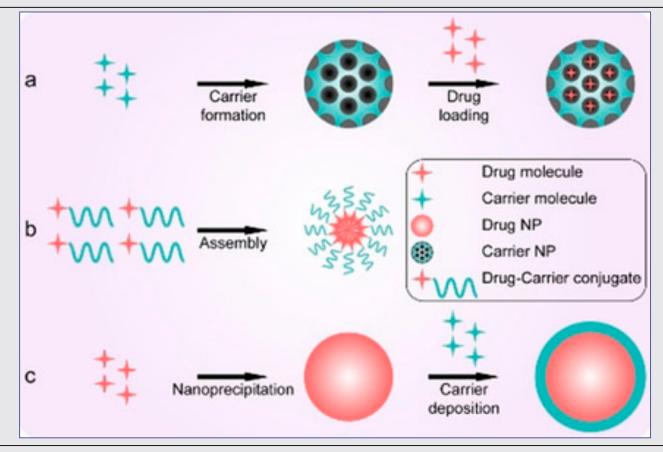




C	Strain	MIC against free antibiotics (mg/L)	MIC against liposomal encapsulated antibiotics (mg/L)	References	otección ATB
v	/ancomycin				
	. coli	512	6-25	Nicolosi et al., 2010	
M		512	10.5		ón específico.
ĸ	Klebsiella	512	25–50		
F	? aeruginosa	512	50		
		512	83.7		
	Acinetobacter Daumanii	512	6–125		lantónicas
	5. aureus MRSA)	1	0.5	Bhise et al., 2018	n/denaturación EPS
A	Amikacin				
F	? aeruginosa	8	4	Mugabe et al., 2006	

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

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



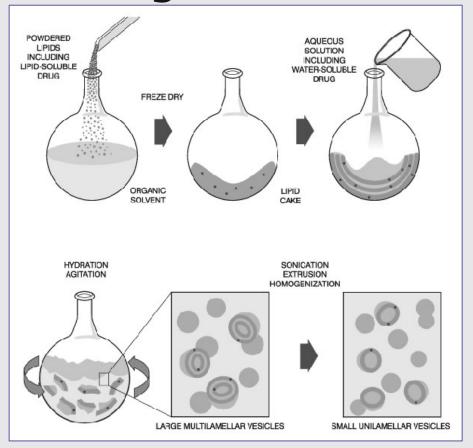


Algunos Ejemplos



liposomal surface. The surface zeta potential chang confirmed the association of positively-charged by

Actividad antibiofil^{through} fi<mark>posoffias asociados a</mark> lisozima/gentamicina contra *P. aeruginosa* u *S. aureus*



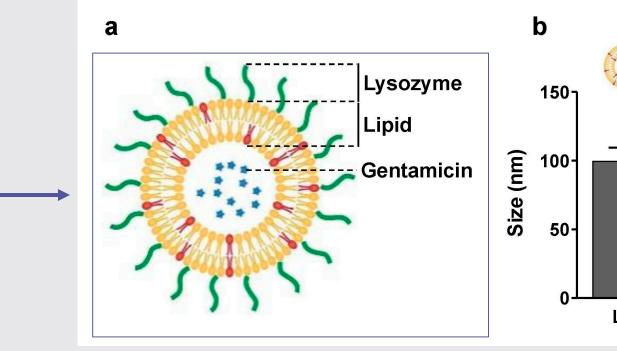


Figure 1. (a)
Schematic
structure
of
lysozyme-a

Hou Y, Wang Z, Zhang P, Bai H, Sun Y, Duan J, Mu H. Lysozyme Associated Liposomal Gentamicin Inhibits Bacterial Biofilm. Int J Mol Sci. 2017 Apr 9;18(4):784.
Figure 1. (a)
Schematic
structure
figure 1. (b)
structure
figure 1. (c)
structure
structure
structure
figur

2.2. Stability of Lysozyme-Associated Liposomal Gentami

on of positively-charged lysozyme to the negatively-charged liposomes action. Actividad antibiofilm de liposomas asociados a lisozima/dentamicina contra P. aerudinosa y S. aureus D Lysozyme 40-150-Zeta potential (mV) 20 Lipid Size (nm) 100-Gentamicin -20--40-50--60--80-

atic structure^{v, w}of ^{zha}ly sodzy me-associated ^{Lipo}hipotroinhial acteriated they solve of the solution o

LLG

LG

biofilms after 24 h treatment compared to blank control (Figure 3a,b). LLG treatment marked reduced both biofilm mass and viable cell counts. To see whether LLG was able to eliminate bacteria biofilms built han so for the and a second provide the second provide the second of th ysanythelatoned a mildefildet subtaha enavide

Actividad antibiofilm den nosomes and a costa Int. J. Hisozima/gentamicina contra

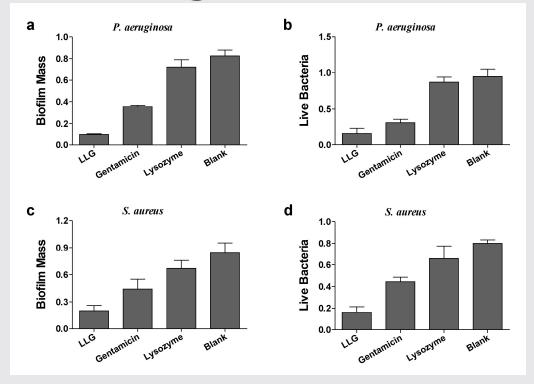


Figure 7. The inhibitory effects of LLG on P. aerusinosa (a,b) and S. aureus (c,d) biofilm formation.

Controlled drug delivery by lipid nanoparticles have attracted much attention. More recently, Harker et al. utilized an electrohydrzetyaamic technique to prepare porestelligiscoarea partielles reaction and the state of the state with a tunable size and high active ingredient loading capacity, encapsulation efficiency and suders controlled release [19]. Many nano materials, for example, the metal-loaded nanofibers [20-32], have shown high antibacterial activity against planktonic or biofilm bacteria [23,24]. In the current st we developed a platform to deliver antibiotic to treat bacterial biofilms through lysosome assoc liposomes. This approach made liposomes more stable and easier to attach to biofilms; a universal

demonstrated that LLG had a more pronounced

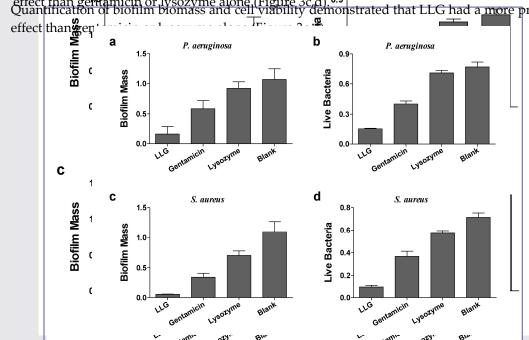
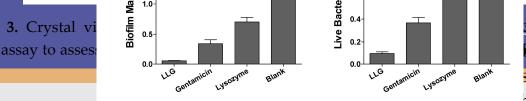


Figure 3. Figure 3. Crystal violet assay and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazoliumitermideomide (MTT) assay to assess the antibiofilm activity of LLG against *P* acruginose biofilm (**a**, **b**) and *S*. average (MTT) ass**ay to assess the antibiofilm activity of LLG against** *P* **acruginose biofilm (a**, **b**) and *S*. average (MTT) assay to assess the antibiofilm activity of the biggina set of th 1C.a). ASSAV tasssests thesastilbio filmilaictiility activity of a loss againsg iProceedig films (ajbijimd & by und s (cad) eus $(\mathbf{c},\mathbf{d}).$

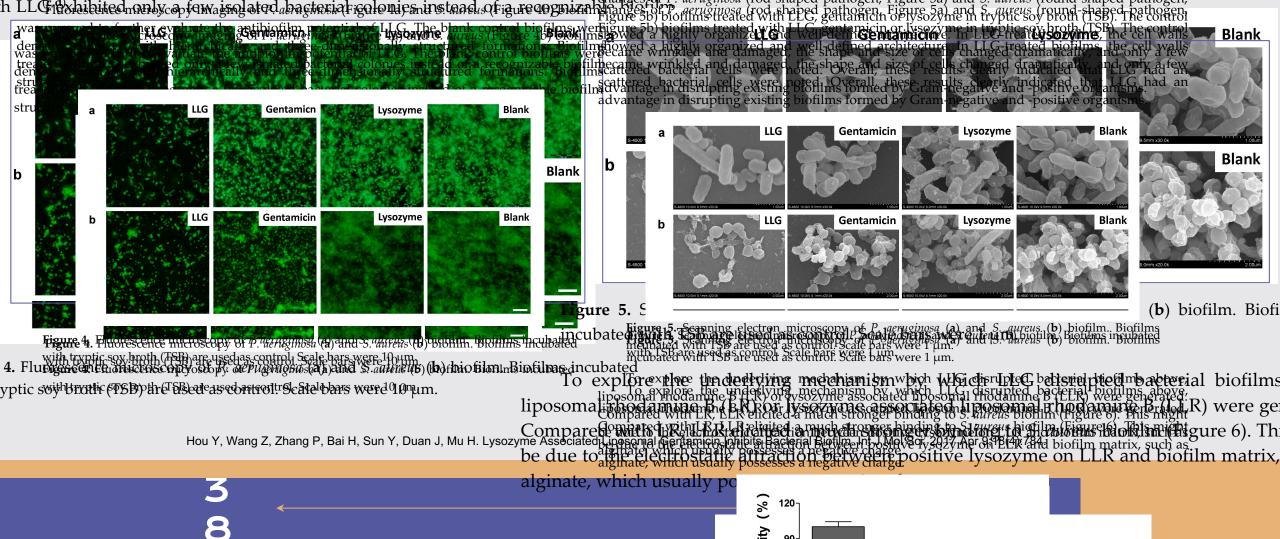
(c,d). Fluorescence microscopy imaging of *P. aeruginosa* (Figure 4a) and *S. aureus* (Figure 4b) biofilms structure. Gentamicn Lysozyme Blank

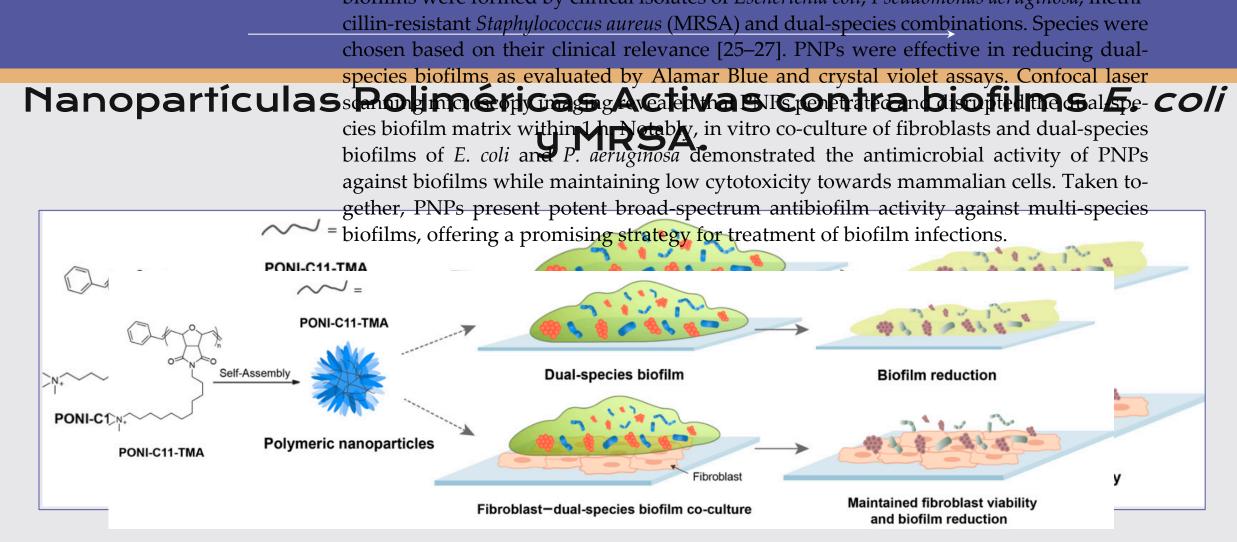


Scaling election incroscopy (SEW) was also applied to evaluate the sufface mor zhängebrofnReaeruginosa (rod shaped pathogen, Figure 5a) and S. aureus (round-shaped pa Ebgand 5b)/biofilms treated with LLG, gentamicin or lysozyme in tryptic soy broth (TSB). The showed a highly organized and well-defined architecture. In LLG-treated biofilms, the co

escence mich Crystal viole assay and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium thouse the content of the same winkled and damaged the shape and size of fells chaped dramatically, and on Scattered the content of the same winkled assay and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium thouse the content of the same winkled assay and the same winkled as a solution of the same winkled as a solut ed to full assay to assay the set of the set

h LLGM&Aibitednonlycopfawajsolatadabacteriaficaloniesninstead.afigureaognizzhfange





Scheme 1. Prepartitionand dativity tyfof ONING + CTMAMANPANPAN Be Ferenting I PAPPA Signifiy and your destablished specific bio finite international fibro blactoriatility bility.

Makabenta JMV, Park J, Li C-H, Chattopadhyay AN, Nabawa A, bandis RE, Gupta A, Schmidt-Malan S, Patel R, Rotello VM. Polymeric Nanoparticles Active against Dual-Species Bacterial Biofilms. *Molecules*. 2021; 26(16):4958.

2.1. Dual-Species Biofilm Penetration Profile of PNPs

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The ability of the polymeric nanoparticles to penetrate the EPS of a dual-species biofilm was visualized using confocal laser scapping microscopy (CLSM). A 4-day-old dual-

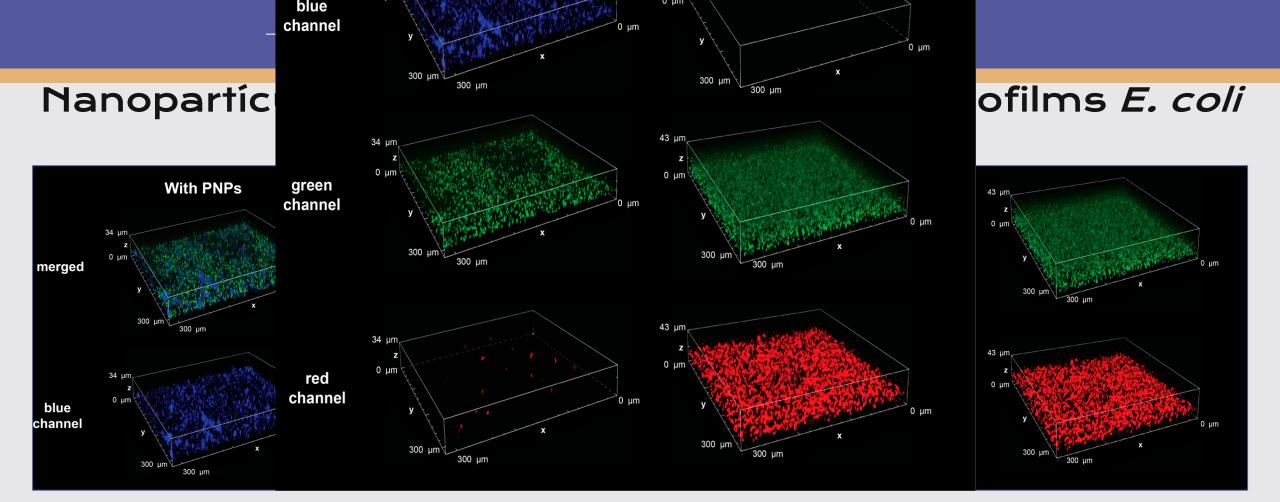


Figure 1. Representative 3D views of confocal image stacks of 4-day-old dual-species biofilm of DsRed-expressing *E. coli* (red channel) and GFP-expressing MRSA (green channel), coumarin blue-tagged PNPs (blue channel) and their overlay (red channel) and GFP-expressing MRSA (green channel), coumarin blue-tagged PNPs (blue channel) and their overlay after treating the biofilms for 1 h with 1 µM coumarin blue-tagged PNPs in M9 media.

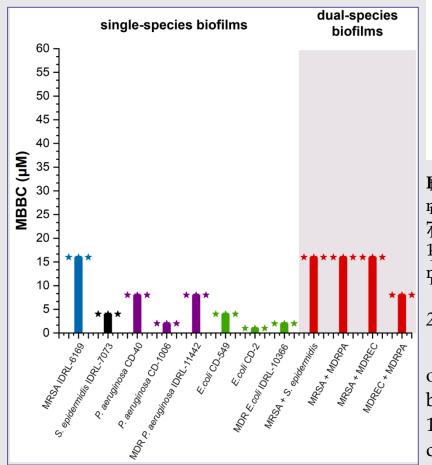
2.2. Minimum Biofilm Bactericidal Concentrations of PNPs

Makabenta JMV, Park J, Li C-H, Chattopadhyay AN, Nabawy A, Landis RF, Gupta A, Schimut Malan S, Pater R, Kotelio M. Polymetrationsa (MBBA) of PNPs against Digespecies Bacterial Biofilms. *Molecules*. 2021; 26(16):4958. and dual-species biofilms were evaluated using an established MBBC protocol [28]. Bio-

films were formed by clinical isolates of E. coli, P. aeruginosa, MRSA and Staphylococcus

epidermidis, and their combinations. PNPs eradicated the dual-species biofilms (Figure 2).

Nanopartículas Polimé



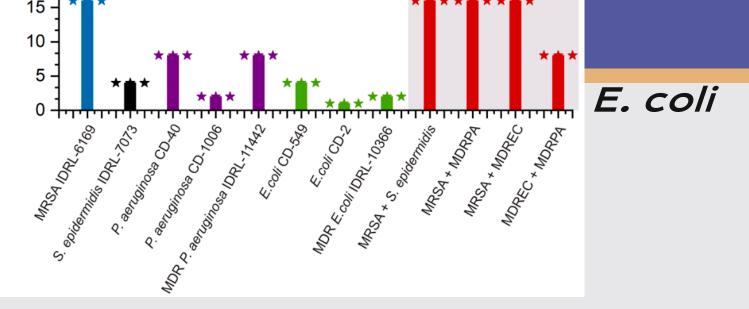


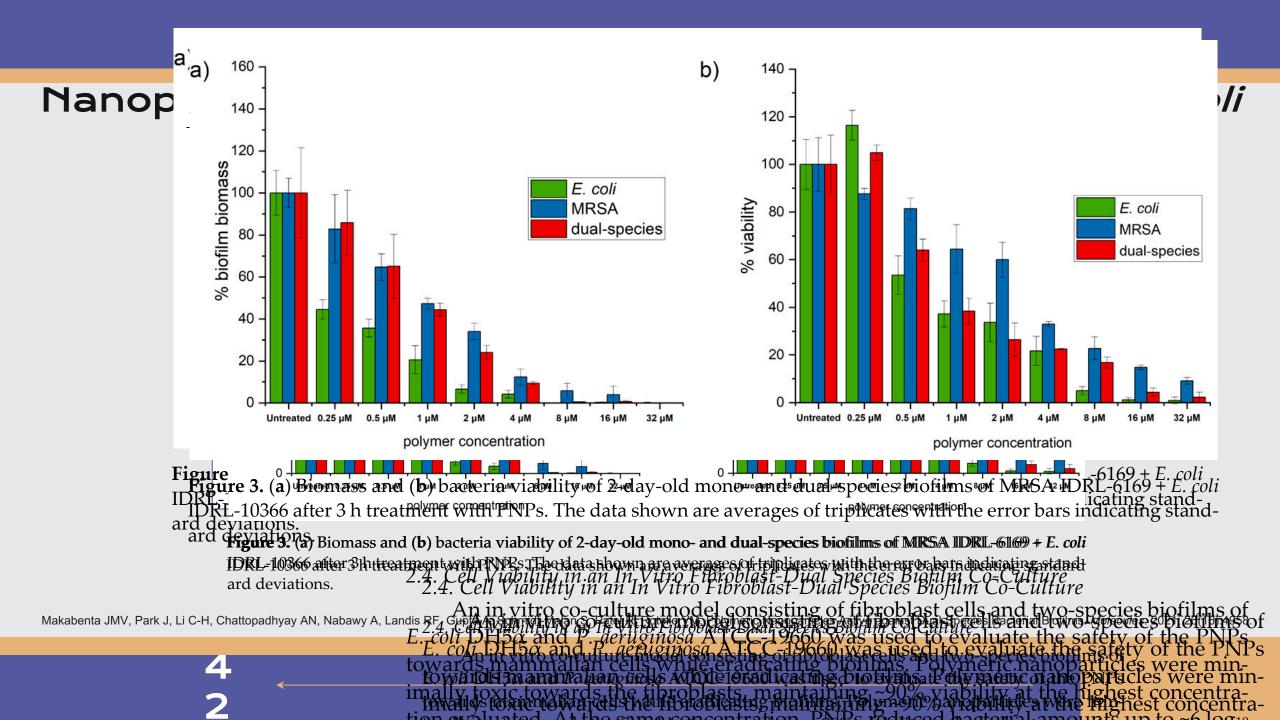
Figure 2. MBBC values of PNPB a genistation on specific tempospositive RSARS Acps depidetation G and R appropriate the second depidence of the se

2.3. Quantifying Biofilm Biomass and Bacteria Viability

Crystal violet and Alamar Blue assays were performed to evaluate the effect of PNPs on biomass and bacterial viability, respectively, of the two-species biofilms. Two-day-old biofilms of MRSA IDRL-6169 + *E. coli* IDRL-10366, *P. aeruginosa* IDRL-11442 + *E. coli* IDRL-10366 and their single-species counterparts were treated with PNPs for 3 h. Results indicated that the ability of the PNPs to kill bacteria and disrupt biofilms was retained in dual-species biofilms (Figure 3, Figure S6).

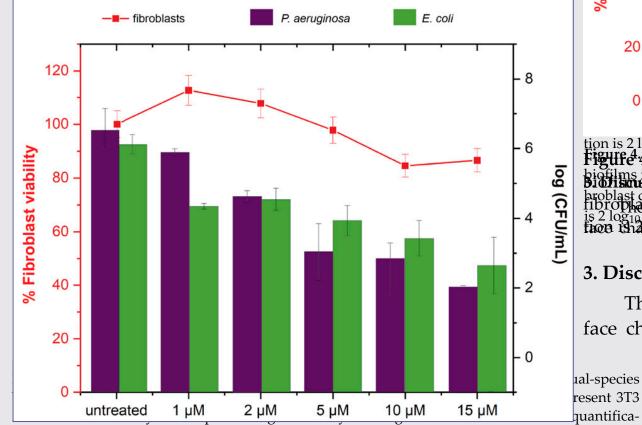
Makabenta JMV, Park J, Li C-H, Chattopadhyay AN, Nabawy A, Landis RF, Gupta A, Schmidt-Malan S, Patel R, Rotellow M. Polymeric Nanoparticles Active against Dual-Species Bacterial Biofilms. *Molecules*. 2021; 26(16):4958.

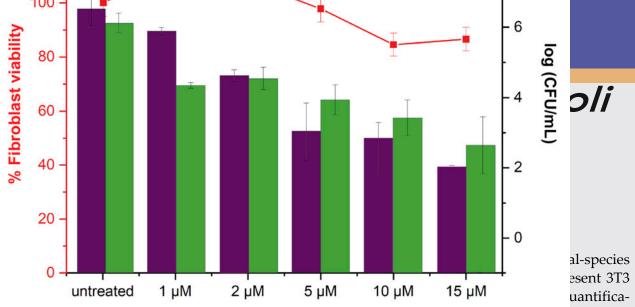




2.4. Cell Viability in an In Vitro Fibroblast-Dual Species Biofilm Co-Culture An in vitro co-culture model consisting of fibroblast cells and two-species bic *E. coli* DH5 α and *P. aeruginosa* ATCC-19660 was used to evaluate the safety of the towards mammalian cells while eradicating biofilms. Polymeric nanoparticles we They to Come the flool at a Sain ing Other field i Call the Big Son

tion evaluated. At the same concentration, PNPs reduced bacterial amounts up to colony-forming units (Figure 4, Figure S7).





tion is 2 log₁₀, Data are averages of triplicates, with error bars indicating standard deviations. **Figure 4.4.** Viability of 513 flast of last defis and E. coll of 1971 800 and 1970 and 19 CPC-1966 odel atter 3 h treatment with PNPs. Scatters and lines re The model after 3 h treatment with PNPs problast cell viapility, Bars represent log 10 of colony-forming units in biofilms. Limit of quantification in TID rophest cells viapavisive data in the properties of of other vision provided the standard deviations. is 2 log 10. Data are averages of triplicates, with error bars indicating standard deviations. two change and an another averages allow parcets to at hange of aratindicabiag madalatids deviations.

3. Discussion

The tunable physicochemical properties of polymeric nanoparticles, such face charge and hydrophobicity, allow access to a range of antimicrobia

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3. Discussion

The tunable physicochemical properties of polymeric nanoparticles, such as size, surface charge and hydrophobicity, allow access to a range of antimicrobial modalities,

Actividad antibiofilm de Silver Ultra-NanoClusters (SUNCs) contra *Helicobacter pylori*

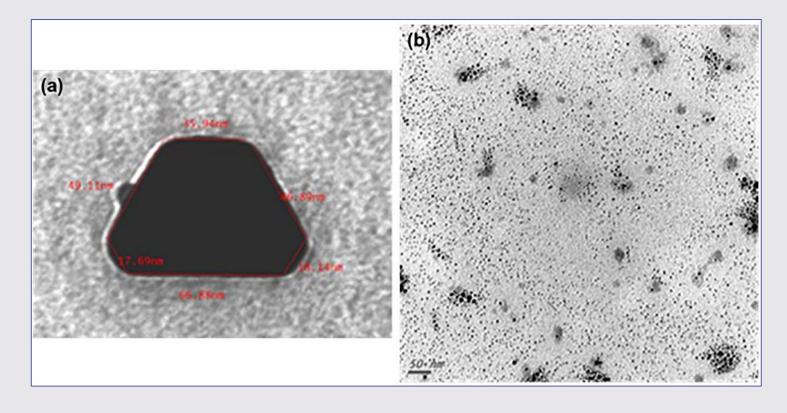
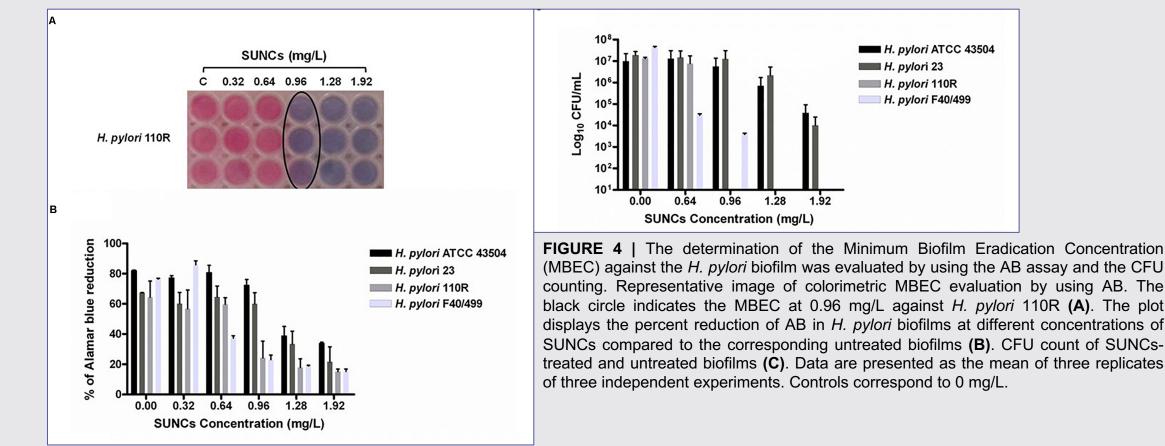


FIGURE 1: Transmission electron microscopy of SUNCs. SUNCs were electrochemically synthesized in ultrapure water. Large non-spherical nanocluster before filtration. Magnification: 250,000× (a); ultrananoclusters after filtration. A drop of 1:5 diluted stock solution of SUNCs was allowed to evaporate onto 300 mesh formvar-coated nickel grids, and then TEM image was taken at 75 kV by a ZEISS 109 microscope. Scale bar: 50 nm. Magnification: 85,000× (b).

Grande R, Sisto F, Puca V, Carradori S, Ronci M, Aceto A, Muraro R, Mincione G, Scotti L. Antimicrobial and Antibiofilm Activities of New Synthesized Silver Ultra-NanoClusters (SUNCs) Against Helicobacter pylori. Front Microbiol. 2020

Actividad antibiofilm de Silver Ultra-NanoClusters (SUNCs) contra *Helicobacter pylori*



Grande R, Sisto F, Puca V, Carradori S, Ronci M, Aceto A, Muraro R, Mincione G, Scotti L. Antimicrobial and Antibiofilm Activities of New Synthesized Silver Ultra-NanoClusters (SUNCs) Against Helicobacter pylori. Front Microbiol. 2020

Actividad antibiofilm de Silver Ultra-NanoClusters (SUNCs) contra *Helicobacter pylori*

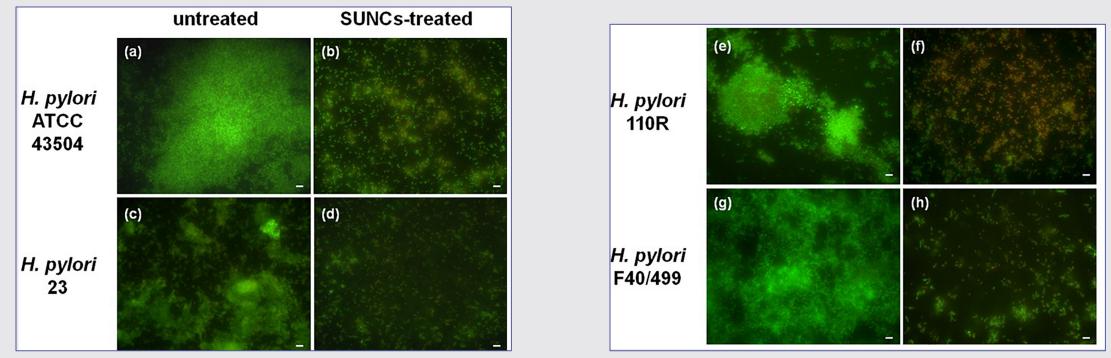


FIGURE 5 | Representative *H. pylori* biofilms stained with Live/Dead kit and analyzed using fluorescence microscopy. The green fluorescence indicates the live cells, whereas the red fluorescence indicates the dead cells or cells with a damaged cell wall. Panels (a,c,e,g) show the untreated *H. pylori* biofilms, while panels (b,d,f,h) show *H. pylori* biofilms treated with SUNCs at MBEC concentrations of 1.28 mg/L for *H. pylori* strains ATCC 43504 and 23, 0.96 mg/L for 110 R, and 0.64 mg/L for F40/499. Scale bar: 5 µm.

Grande R, Sisto F, Puca V, Carradori S, Ronci M, Aceto A, Muraro R, Mincione G, Scotti L. Antimicrobial and Antibiofilm Activities of New Synthesized Silver Ultra-NanoClusters (SUNCs) Against Helicobacter pylori. Front Microbiol. 2020

Desafíos

Biocompatiblidad

Citotoxicidad a largo plazo

Acumulación Tejidos

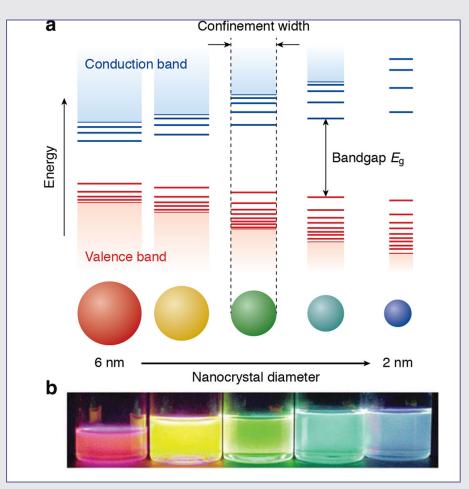
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Metabolización/Excreción

Quantum Dots

Son nanocristales de materiales semiconductores (grupos III–V y II–VI). Poseen propiedades ópticas particulares dadas por el confinamiento espacial de pares electrón-hueco (excitones) en una o más dimensiones (Efecto de confinamiento cuántico).

Este efecto es observado cuando el tamaño de estas partículas es cercano a la longitud de onda de Broglie para estos electrones, existiendo una transición de niveles de energía continuos a discretos.



Manu Sharma et al., 2 - Nanomaterials in biomedical diagnosis, Editor(s): Suvardhan Kanchi, Deepali Sharma, Nanomaterials in Diagnostic Tools and Devices, Elsevier, 2020, Pages 57-83.

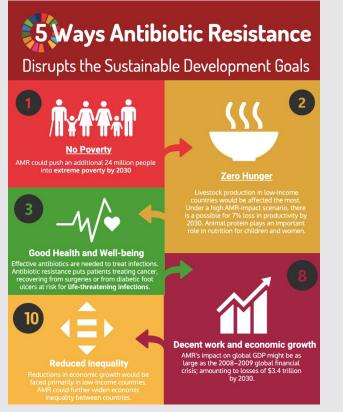
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Gracias!



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