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Cyclodextrin-based nano-devices for improved antibiotic activity

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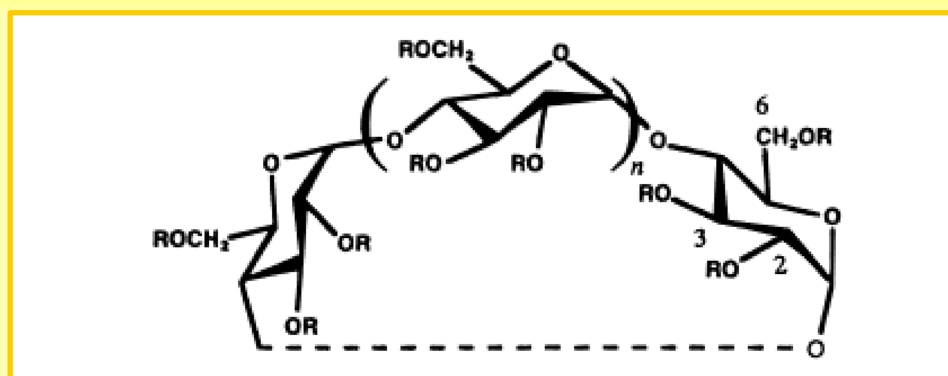
Background

Due to the rapid spread of antibiotic resistance among pathogens the discovery of more effective antibiotics is necessary.
Cyclodextrins are cyclic oligosaccharides, usefull as chelating agents; these molecules possess a hydrophobic cavity, in which they are able to bind reversibly a wide range of organic compounds; furthermore, β-ciclodextrins have almost none cytotoxicity to human cells.
All these properties makes cyclodextrins suitable for the production of innovative, smart and low cost drug carriers aimed at the improvement of antibacterial efficacy.



Cyclodextrin	Abbreviation	R	n
α-Cyclodextrin	α-CD	H	4
β-Cyclodextrin	β-CD	H	5
γ-Cyclodextrin	γ-CD	H	6
Carboxymethyl-β-cyclodextrin	CM-β-CD	CH_2CO_2H or H	5
Carboxymethyl-ethyl-β-cyclodextrin	CME-β-CD	CH_2CO_2H, CH_2CH_3 or H	5
Diethyl-β-cyclodextrin	DE-β-CD	CH_2CH_3 or H	5
Dimethyl-β-cyclodextrin	DM-β-CD	CH_3 or H	5
Methyl-β-cyclodextrin	M-β-CD	CH_3 or H	5
Random methyl-β-cyclodextrin	RM-β-CD	CH_3 or H	5
Glucosyl-β-cyclodextrin	G_1-β-CD	Glucosyl or H	5
Maltosyl-β-cyclodextrin	G_2-β-CD	Maltosyl or H	5
Hydroxyethyl-β-cyclodextrin	HE-β-CD	CH_2CH_2OH or H	5
Hydroxypropyl-β-cyclodextrin	HP-β-CD	$CH_2CHOHCH_3$ or H	5
Sulfobutylether-β-cyclodextrin	SBE-β-CD	$(CH_2)_4SO_3Na$ or H	5

Figure 1. Cyclodextrin molecule

Aim

In this study two different β-cyclodextrin-based nano-devices are developed and used for antibiotic loading and antimicrobial efficacy improvement:
i) polyaminocyclodextrin-silver nanoparticles (Pac-Ag Nps)
ii) cyclodextrin-calixarene nanosponges (Cy-Cal NSs).
Antibacterial activity is quantified, for both systems, as the minimal concentration inhibiting at least the 90% of bacterial growth (MIC_{90}), using tester Gram positive and negative strains such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively.

Results

Polyaminocyclodextrin-silver nanoparticles (Pac-Ag NPs)

- Pac-Ag NPs showed an intrinsic antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and an ampicillin resistant *Escherichia coli* (*E. coli* pUC19).

S. aureus MIC_{90}		*P. aeruginosa* MIC_{90}		*E. coli + pUC19* MIC_{90}	
Pac-Ag Nps	Amp	Pac-Ag Nps	Amp	Pac-Ag Nps	Amp
10	10	20	10	5	>>100

Table 1. MIC_{90} of Ampicillin and Pac-Ag NPs (μg/mL) against *Staphylococcus aureus, Pseudomonas aeruginosa* and an ampicillin resistant *Escherichia coli* (*E. coli* pUC19)

- Pac-Ag Nps exert a synergistic effect with ampicillin against the same tested bacteria, reducing significantly both Amp and Pac-Ag NPs MIC_{90} values, including ampicillin resistant *E. coli* strain.

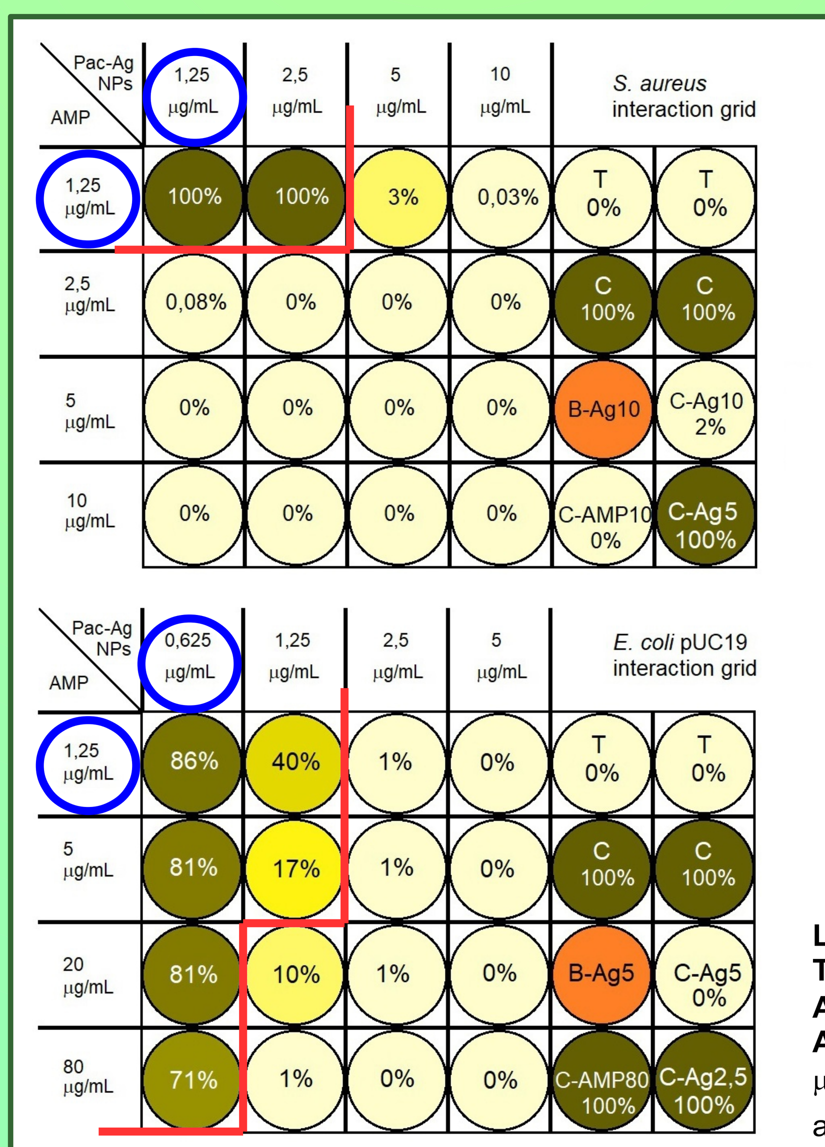


Figure 2. Synergistic effect between Pac-Ag NPs and Ampicillin against *S. aureus, P. aeruginosa* and *E. coli.* MIC_{90} values are highlighted by blue cycles. Growth is reported as percentage value in respect of untreated control condition.

Legend:
T= colture broth; **C**= untreated colture used as control; **B-AgN**=colture broth and Pac-Ag NPs (10-20-5 μg/mL); **C-AgN**= bacterial colture treated with Pac-Ag NPs (10-20-5 μg/mL); **C-AMPN**= bacterial colture treated with MIC_{90} ampicillin value (10-80 μg/mL)

- Polarimeter and microcalorimeter assays revealed that Pac-Ag NPs do not incorporate Ampicillin molecules.

- A synergistic effect with other antibiotics is under investigation.

Cyclodextrin-calixarene nanosponges (Cy-Cal NSs)

- 3 different Cy-Cal NSs (2OT, 2OTR, 4OT) were tested for pH dependent antibiotic incorporation.
- All Cy-Cal NSs types adsorb a higher amount of each tested antibiotics in a pH 6.7 solution than a pH 4.4 solution.

	Ampicillin			Tetracycline		
	2OT	2OTR	4OT	2OT	2OTR	4OT
pH 6,7	98%	89%	76%	71%	86%	73%
pH 4,4	88%	62%	29%	36%	15%	65%

Table 2. Percentage of Ampicillin and Tetracycline amounts incorporated inside 2OT, 2OTR and 4OT Cy-Cal Nss at two different pH.

- 2OT Cy-Cal NSs did not show any intrinsic antibacterial activity against *E. coli*.
- 2OT Cy-Cal NSs/tetracycline composites don't exert a synergistic effect against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.*

	MIC_{90} Tetracycline (EtOH 50%)	MIC_{90} TNP (water)	MIC_{90} TP (water)	MIC_{90} Cy-Cal Nss/Tetracycline
B. subtilis	5	2.5	2.5	3.3
E. coli	1	1	1	3.3
P. aeruginosa	5	2.5	5	4.9
S. aureus	5	2.5	5	9.7

Table 3. MIC_{90} values of Tetracycline (μg/mL) against *B. subtilis, E. coli, P. aeruginosa, and S. aureus*; **EtOH 50%:** Tetracyclin dissolved in 50% ethanol; **water**: Tetracyclin dissolved in water; **TNP**: not pastorized tetracycline; **TP**: tetracycline pastorized.

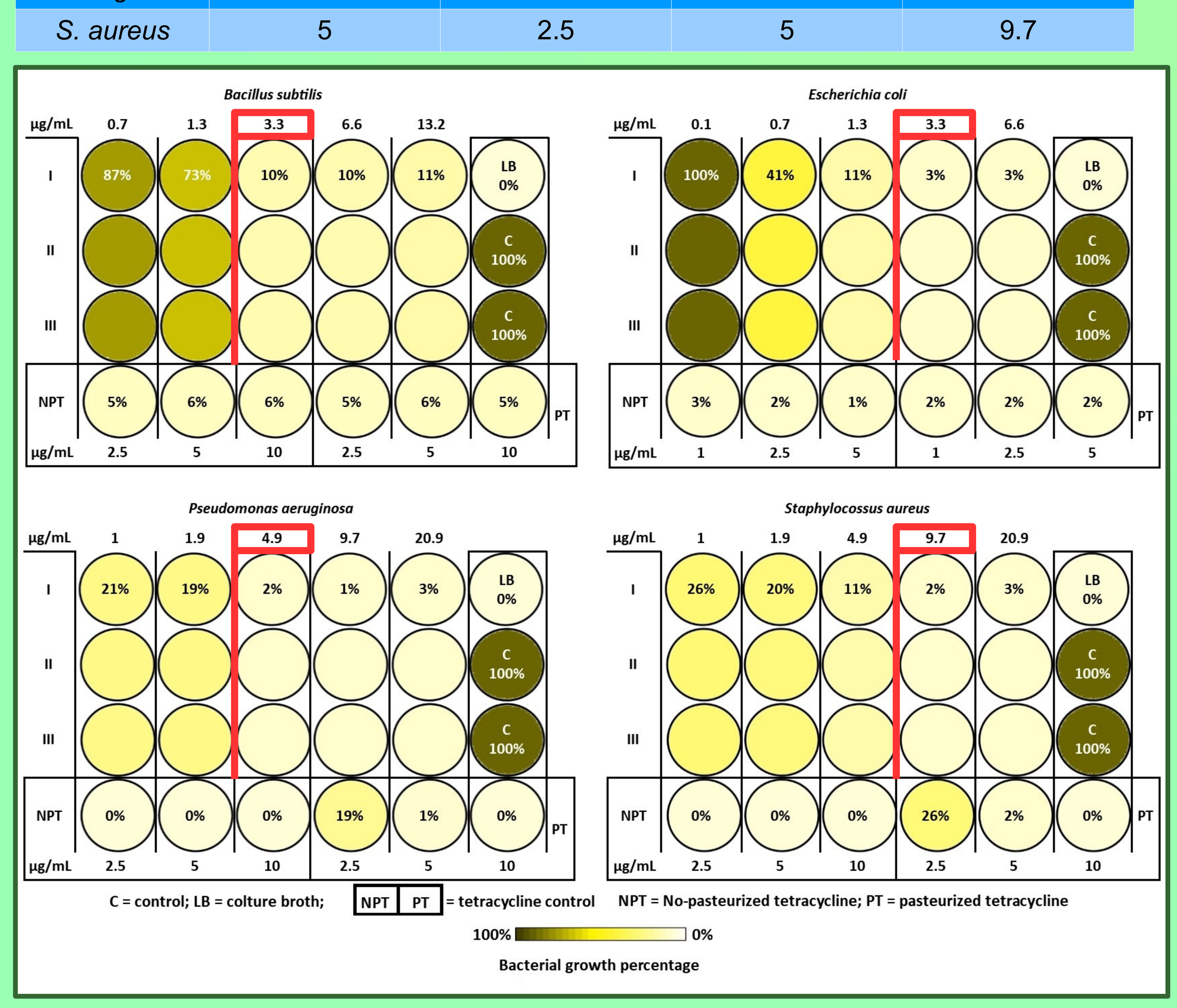


Figure 3. MIC_{90} values of 2OT Cy-Cal NSs/ tetracycline composites against *B. subtilis, E. coli, P. aeruginosa, and S. aureus.* Pasteurized tetracycline and not was used as positive control in each plate. MIC_{90} values are highlighted by red rectangles. Growth is reported as percentage value in respect of untreated control condition.

- The complex formed by the selected antibiotics and 2OTR or 4OT Cy-Cal NSs are under investigation for their antimicrobial capability.

Conclusion

Pac-Ag NPs show synergistic effect, although it is not able to incorporate ampicillin; in this way, a lower amount both antibiotic and nanoparticles is necessary to cause a 90% bacterial growth reduction. The effect of Pac-Ag NPs/Amp is significantly relevant, and suggests investigating about the action mechanism.
Cy-Cal NSs haven't a synergistic effect with tetracycline, but its ability to incorporate and release antibiotics in a pH dependent manner; making them valuable as antibiotic carriers.