ANTIMICROBIAL AND ANTI-BIOFILM ACTIVITIES OF BIOLOGICALLY SINTHESIZED SELENIUM NANOPARTICLES

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BACKGROUND

Tailored nanoparticles (NPs) with desired physico-chemical properties have been proposed as a new line in the battle against antibiotic-resistant microorganisms.

In this study, SeNPs generated by two bacterial isolates of environmental origin have been tested and compared with chemically synthesized NPs for their antibacterial, antifungal and cytotoxic activity. **Antimicrobial and anti-biofilm characteristics of SeNPs** were tested toward clinical strains of ***Pseudomonas aeruginosa*** and clinical isolates of ***Candida*** **species**. The antifungal activity was tested against clinical isolates of ***Aspergillus spp.***

METHODS

1. The gram-positive strain *Bacillus mycoides* SeITE01 and the gram-negative strain *Stenotrophomonas maltophilia* SeITE02 were used to produce biogenic SeNPs [(Bm-SeNPs(+) and Sm-SeNPs(-) respectively]. Chemically synthesized SeNPs (Ch-SeNPs) were produced using a standard protocol.

2. *Pseudomonas* and *Candida* strains were treated for 48 hours at 37° C with different concentrations of Bm-SeNPs(+), Sm-SeNPs(-) and Ch-SeNPs to test their **inhibition activity on biofilm formation**. The **biofilm degradation activity** was evaluated by analyzing the quantity of the biofilm remaining after treatment of the synthesized exopolymer with different concentrations of biogenic and chemically synthesized SeNPs. The biofilm was quantified after methylene blue staining.

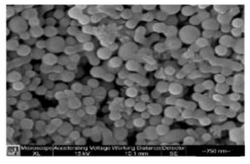

Figure 1. Sm-SeNPs (-).

3. The zone of inhibition (ZOI) technique was used to observe **the activity of SeNPs on fungal species**. The effect on mycelia growth was evaluated after filtering suspensions of *A. fumigatus* and *A. flauvus* inoculated for 8 days with 64µg/ml (MIC) of Sm-SeNPs(-).

4. Evaluation of the Se-NPs **cytotoxic activity and their effect on cell viability** was determined by ELISA method, AlamarBlue® assay and cytochrome C reduction in human dendritic cells and fibroblast.

RESULTS

Table 1. Inhibition of biofilm synthesis by Se-NPs. **In bold**, percentages of biofilm formation inhibition higher than 80%. (*) Quantity of biofilm in arbitrary unit.

Bacterial/ Yeast strain (*)	*S. maltophilia* - SeNP (µg/ml)				*Bacillus mycoides* - Se NPs (µg/ml)				Chemically synthesized Se NPs (µg/ml)			
	50	100	250	500	50	100	250	500	50	100	250	500
P. aeruginosa PAO1 (1.10)	40±2.5	45±3	70±2.5	**96±1**	33±3	47±6	63±4.5	**95±1.2**	9±0.7	21±2.1	**94±0.7**	**96±1.4**
P. aeruginosa Pyo 27853 (1.00)	15±0.7	30±0.7	41±0.7	66±2.1	15±4.2	17±2	44±2.8	64±1	4±1.4	10±0.7	35±2.1	44±2
P. aeruginosa INT (1.00)	23±1	34±1	59±3.5	**95±1**	25±4.5	29±3.5	49±2.5	**94±1.5**	2±3.5	2±1.4	20±0.7	30±0.7
P. aeruginosa CFC21 (1.20)	66±5	**86±0.5**	**95±2**	**96±1.5**	37±5.5	66±3.5	**93±1.7**	**95±2**	10±3.5	33±3.5	71±1.4	65±1.4
P. aeruginosa CFC20 (1.10)	75±0.5	**82±1**	**86±1**	**93±1.5**	72±2	76±0.5	**85±1**	**91±2**	53±0.7	**90±0.7**	**95±1**	**95±1**
P. aeruginosa CFCA (2.75)	31±1	39±3	39±2	**88±4**	28±1.5	34±5	**81±4.5**	**85±5**	1±0.5	1±0.5	**97±0.7**	**98±1**
P. aeruginosa CFCB (3.00)	39±1	**94±1**	**94±3.5**	**96±1**	25±5	25±1.5	**94±0.5**	**96±1**	5±0.7	6±0.7	**96±1**	**97±1.5**
C. albicans (0.9)	61± 0.5	60±3	60±1	**94±1**	60±6.5	69±2	74±2.5	**93±0.5**	0	0	0	9±0.7
C. parapsilosis (0.85)	72±1.5	79±0.5	73±1	**95±1**	75±1.5	73±5	72±3	**94±0.5**	0	0	0	5±0.7

Table 2. Percentages of biofilm disgregation caused by Se-NPs. **In bold**, percentages of disaggregation higher than 80% of the biofilm initial quantity. (*) Quantity of biofilm in arbitrary unit.

Bacterial/ Yeast strain (*)	*S. maltophilia* - SeNP (µg/ml)				*Bacillus mycoides* - Se NPs (µg/ml)				Chemically synthesized Se NPs (µg/ml)			
	50	100	250	500	50	100	250	500	50	100	250	500
P. aeruginosa PAO1 (1.10)	73±5	72 ±2	76 ±2	73±5	52±1	62±4.5	57±1	73±6	16±0.7	18±4.2	37±0.7	53±1
P. aeruginosa Pyo 27853 (1.00)	49±1.4	53±1.4	53±1.4	43±0.7	31±4.9	43±1.4	51±7	45±7	23±1.4	35±1	40±2.5	46±0.7
P. aeruginosa INT (1.00)	63±5.5	53±0.5	61±1.5	41±4.5	65±4.5	44±0.5	58.2±3	32±1.5	15±6.3	15±6.3	8±2.8	8±4.5
P. aeruginosa CFC21 (1.20)	21±1	45±3.5	53±5.5	63±2	44±4.5	16±3	33±1.5	61±0.5	8±1.4	5±0.7	16±1.4	1±0.7
P. aeruginosa CFC20 (1.10)	**87±3**	**84±0.5**	**85±1**	**85±0.5**	**80±1**	**86±1**	**86±1.7**	**82±2**	17±3.5	13±2.8	29±4.2	50±2.8
P. aeruginosa CFCA (2.75)	53±4	58±3	56±3	64±2	25±1	27±5	51±2.5	47±3	0	4±1.4	57±1.4	72±0.7
P. aeruginosa CFCB (3.00)	44±2.5	44±2.5	39±1	53±2	28±4	20±2.5	40±2.5	53±1	0	2±0.7	66±0.7	73±0.7
P. aeruginosa Pyo 27853	49±1.4	53±1.4	53±1.4	43±0.7	31±4.9	43±1.4	51±7	45±7	23±1.4	35±1	40±2.5	46±0.7
C. albicans (0.9)	26±2.5	43±2.5	47±3.5	60±2	11±2.5	32±2	48±1.5	60±3.5	0	0	2	0
C. parapsilosis (0.85)	52±2	48±1.5	48±2.5	64±2	48±3	38±2	47±2	42±2.5	0	1	0	0

Table 3. MIC values for biogenic and chemically sinthesized Se-NPs on different microbial strains.

Bacterial / Yeast strain	MIC (µg/ml)		
	S. maltophilia - SeNP	*Bacillus mycoides* - Se NPs	Chemically synthesized Se NPs
P.aeruginosa PAO1	128	128	128
P.aeruginosa Pyo ATCC27853	512	512	>512
P.aeruginosa INT	256	512	>512
P.aeruginosa FCF21	16	64	128
P.aeruginosa FCF20	8	32	128
P.aeruginosa CFCA	16	32	>128
P.aeruginosa CFCB	16	64	>128
C. Albicans	256	512	>512
C.parapsilosis	512	512	>512

Table 4. Mycelia quantity (in mg) after treatment with MIC doses of Sm- NPs (-) and *Itraconazole* compared with control.

Aspergillus strain	Mycelia growth (mg)		
	Control	Itraconazole 4µg/ml	*S. maltophilia* – SeNP 64µg/ml
A. flavus	25±0.1	8±0.7	5±1
A. fumigatus (A)	10±0.2	2±0.1	1±0.7
A. fumigatus (B)	10±0.1	2±0.7	2±0.7

CONCLUSIONS

➢**Biogenic SeNPs are able to inhibit biofilm synthesis** induced by both *Pseudomonas* and *Candida* as well as to **disaggregate the mature exopolysaccaride matrix** produced by these microorganisms.

➢Some *P. aeruginosa* clinical strains (*CFC20*, *CFC21* and *CFCB*) are particularly sensitive to the biogenic SeNPs: the **lowest concentrations** tested (50 and 100 µg/ml) caused a **70-90% inhibition** of the biofilm synthesis (Table 1).

➢The percentage of **biofilm degradation** (Table 2) upon treatment with **50-100 µg/ml** NPs was about **50-70%**, and did not increase with higher NP concentrations.

➢As concerns the ***Candida*** **strains**, the lowest SeNP dose used (**50 µg/ml**) was capable of causing an inhibition of the biofilm formation or the disaggregation of the biofilm of about **50-60%**.

➢**SeNPs** produced **by *S. maltophilia*** show an **antimicrobial and antifungal activity higher** than that observed with *B. mycoides* SeNPs and chemically synthesized NPs.

➢MIC values of Se-NPs varied widely with the different *Pseudomonas* clinical strains analysed: MIC values resulting from the treatments with Bm-SeNPs were significantly higher and those obtained with Ch-SeNPs indicated that these NPs had no effect on *Pseudomonas* growth (Table 3).

➢**SeNPs** produced **by *S. maltophilia*** **are able to interfere with the *Aspergillus* mycelia growth,** causing a partial inhibition of mycelia growth (Table 4).

➢**Both** biogenic and chemically synthesized **SeNPs** do not show cytotoxic activity as **do not affect the viability of human dendritic cells and fibroblasts** and do not elicit the substantial secretion of pro-inflammatory and immune-stimulatory cytokines (data not showed).

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