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Nanotechnology as a promising strategy for controlling oral polymicrobial biofilms

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INTRODUCTION & AIM

The oral polymicrobial biofilm formation occurred by the synergistic interaction of fungal (e.g., *Candida albicans*) and bacterial pathogens (e.g., *Staphylococcus aureus*) and has been reported to cause severe dental caries and periodontal diseases. The application of nanoparticles synthesized using the green chemistry approach has been recognized as one of the promising strategies to combat biofilms. The present study aimed to synthesize gold nanoparticles (NPs) using natural molecules such as fucoidan, β-caryophyllene, and phloroglucinol to control the oral polymicrobial biofilm caused by *S. aureus* and *C. albicans*.

METHOD

1. Microbial Strains and Growth Media:

- *S. aureus* (KCTC 1916) and *C. albicans* (KCCM 11282). Growth media: TSB for bacteria; PDB with 5% glucose for *C. albicans*; growth at 37°C.

2. Synthesis of Gold Nanoparticles:

- 1 mM HAuCl4·3H2O dissolved in 200 mL deionized water at 60°C, pH adjusted to 8.5 using NaOH. 5 mg fucoidan or beta-caryophyllene or PG added dropwise and stirred for 2 h.
- NPs were monitored via UV-Vis absorption spectra (200–700 nm).

3. Characterization of AuNPs:

- Shape and size determined using FETEM (JEM-F200). FTIR (JASCO FT-4100) for functional group analysis (400–4000 cm−1). Zeta potential and size were measured using Litesizer 500. Crystalline structure analyzed via XRD (Rigaku Ultima IV).

4. MIC Determination:

- MIC determined by microbroth dilution from 64 to 2048 µg/mL.
- After incubation at 37°C for 24 h, OD_{600} measured.

5. Biofilm Assays:

- Biofilm formation in 96-well plates using sub-MIC level of AuNPs.
- Planktonic cells removed, biofilm scraped and plated for CFU count.

6. Eradication of Mature Biofilms:

- Mature biofilms treated with MIC and >MIC and sub-MIC of AuNPs after 24 h incubation. Biofilm viability assessed by CFU count.

7. SEM analysis of biofilm Architecture:

- Biofilms treated with AuNPs, fixed with formaldehyde/glutaraldehyde, and dehydrated in ethanol and analyzed using TESCAN SEM at 10 kV with magnification 3.5k.

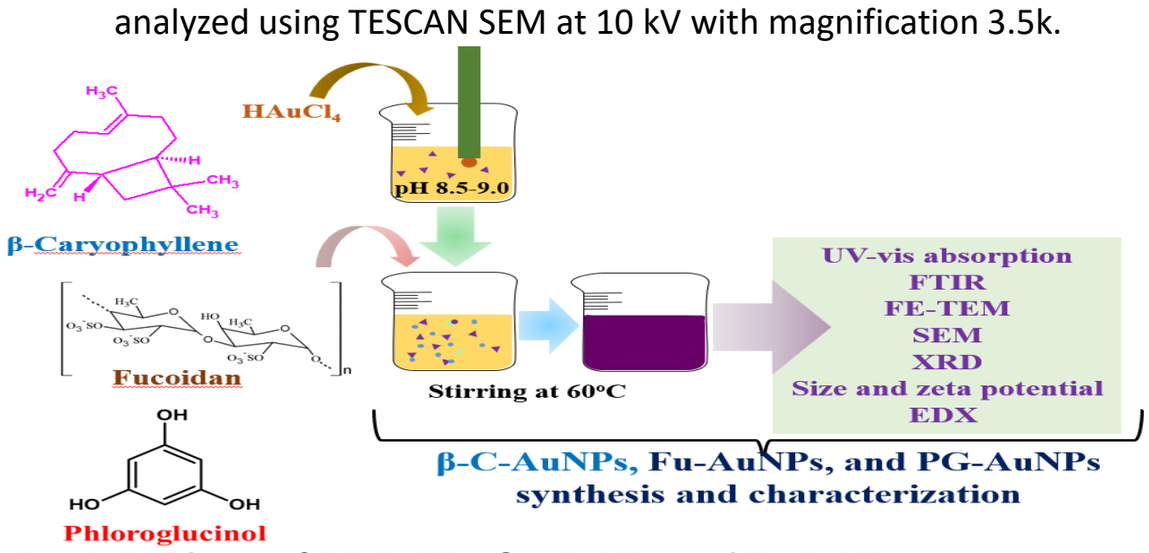

Fig. 1. Synthesis of PG-AuNPs, β-c-AuNPs, and Fu-AuNPs

RESULTS & DISCUSSION

Table 1. MIC values of PG-AuNPs, β-c-AuNPs, and Fu-AuNPs

Nanoparticles	MIC (µg/mL)	
	S. Aureus	*C. albicans*
Fu-AuNPs	1024	2048
β-c-AuNPs	521	512
PG-AuNPs	2048	2048

Table 2. Comparative Physiochemical Characterization of β-c-AuNPs, Fu-AuNPs, and PG-AuNPs

Techniques	β-c-AuNPs	Fu–AuNPs	PG-AuNPs
Synthesis Observation	Color change from yellow to wine-red	Color change from yellow to wine-red	Color change from yellow to red wine
UV-Vis Absorption	534 nm	570 nm	510 nm
Average Size (DLS)	17.6 ± 1.2 nm	75.66 ± 9.28 nm	46.71 ± 6.40 nm (PDI = 0.73)
Zeta Potential	−31.76 ± 0.73 mV	−36.09 ± 0.63 mV	Not reported
FE-TEM Morphology	Non-uniform shape	Spherical and non-spherical shapes	Spherical shape

Table 3. Biofilm Inhibition in Standard Media by Gold Nanoparticles

Nanoparticle	Species	Biofilm Type	Concentration (µg/mL)	Log CFU Reduction
PG-AuNPs	*C. albicans*	Mixed (with *S. aureus*)	1024	2.79
	S. aureus	Mixed (with *C. albicans*)	1024	2.67
Fu–AuNPs	*C. albicans*	Mixed (with *S. aureus*)	512	Complete
	S. aureus	Mixed (with *C. albicans*)	512	3.2
β-c-AuNPs	*S. aureus*	Mixed (with *C. albicans*)	256	4.4
	C. albicans	Mixed (with *S. aureus*)	256	3.0

Table 4. Disinfection of Mature Biofilms by PG-AuNPs, β-c-AuNPs, and Fu-AuNPs

Nanoparticle	Species	Biofilm Type	Concentration (µg/mL)	Log CFU Reduction
PG-AuNPs	*C. albicans* + *S. aureus*	Mixed	2048 (MIC)	*C. albicans*: 8.1, *S. aureus*: 6.0
β-c-AuNPs	*C. albicans* + *S. aureus*	Mixed	1024 (>MIC)	*C. albicans*: 2.11, *S. aureus*: 1.43
		Mixed	512 (MIC)	Not provided
Fu-AuNPs	*C. albicans* + *S. aureus*	Mixed	2048 (>MIC)	*C. albicans*: 2.4, *S. aureus*: 2.9

CONCLUSION

The present study demonstrated that Fu-AuNPs, β-c-AuNPs, and PG-AuNPs act as an alternative strategy to control the oral polymicrobial biofilms by inhibiting the initial stage and eradicating the mature polymicrobial biofilm of *S. aureus* and *C. albicans.*

FUTURE WORK / REFERENCES

1. Conduct *in vivo* studies using animal models to evaluate the efficacy of PG-AuNPs, β-c-AuNPs, and Fu-AuNPs.
2. Investigate gene expression and molecular mechanisms associated with biofilm inhibition and virulence regulation.
3. Khan, F.; Tabassum, N.; Jeong, G.-J.; Jung, W.-K.; Kim, Y.-M. Inhibition of Mixed Biofilms of Candida albicans and Staphylococcus aureus by β-Caryophyllene-Gold Nanoparticles. Antibiotics 2023, 12, 726.