# Antimicrobial Peptide - Selenium Nanoparticles to Combat Antimicrobial Resistant Infections in Burn Wounds

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## INTRODUCTION

Infection is the leading cause of death after burn injury and the rise of multidrug resistant pathogens is an increasing challenge in wound management. Silver-based antimicrobial dressings are widely used, however resistance to silver has been found clinically. Inorganic nanoparticles (NPs) are a promising alternative to conventional drugs as they can kill microbes by multiple mechanisms at doses not toxic to human cells.

**AIM**:
To develop NPs suitable for wound dressings:
- killing the pathogens found clinically in wounds;
- cytocompatible with human cells;
- limiting development of new antimicrobial resistance to the NPs.

## METHODS

- Selenium NPs of ~80 nm were synthesised by simple aqueous phase redox chemistry via batch and microfluidic processes (Fig.1).
- Se NPs were coated with the cationic antimicrobial peptide (AMP), ε-poly-L-lysine
- Characterizations included: zeta potential, light scattering, TEM (Fig.2) and cytocompatibility.
- Antimicrobial tests and assessed their ability to inhibit the growth and kill Gram-positive and -negative bacteria, including multidrug-resistant and clinically isolated strains.
- The ability of bacteria to develop resistance to the NPs in culture was compared to their response to a conventional antibiotic (kanamycin) via the minimum bactericidal concentration (MBC) over several weeks' exposure.

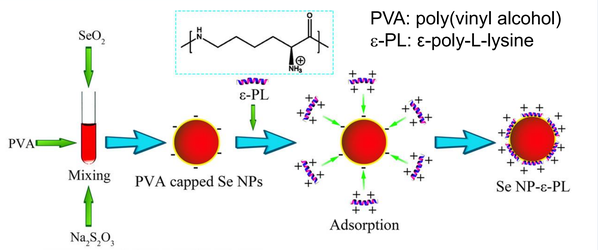



**Fig. 1: Synthesis of AMP-Se nanoparticles**

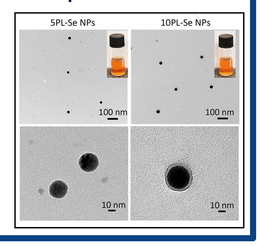
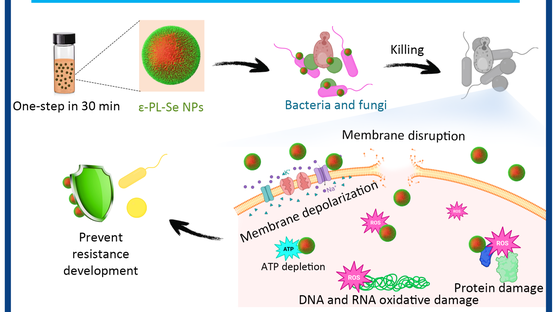



**Fig. 2: TEM images of AMP-Se nanoparticles with different amounts of ε-poly-L-lysine coating**

## AMP-Se NPs




**AMP-selenium nanoparticles:**
- ✓ **Simple synthesis**
- ✓ **Low toxicity**
- ✓ **Broad spectrum**
- ✓ **Multiple attack mechanisms**

## RESULTS

- AMP-selenium NPs inhibited growth (Fig.3, Table1) of:
  - ✓ **laboratory strains**, including: *S. aureus,* MRSA *(*Methicillin resistant *S. aureus), E. faecalis, E. faecalis (*MDR - multidrug resistant*), E. coli, E. coli (*MDR*), P. aeruginosa, P. aeruginosa (*MDR*), C.albicans*
  - ✓ **clinical isolates** from burn wounds, including *S. aureus,* MRSA*, P. aeruginosa, P. aeruginosa (*MDR*), S. lugdunesis, E. clocae* complex, and *S. agalactiae*
- **Mechanisms** of action of the NPs on bacteria included membrane disruption, ATP depletion, membrane depolarisation and increased reactive oxygen species production
- **Resistance was delayed or avoided** in longer term culture at sub-lethal doses (Fig. 5).

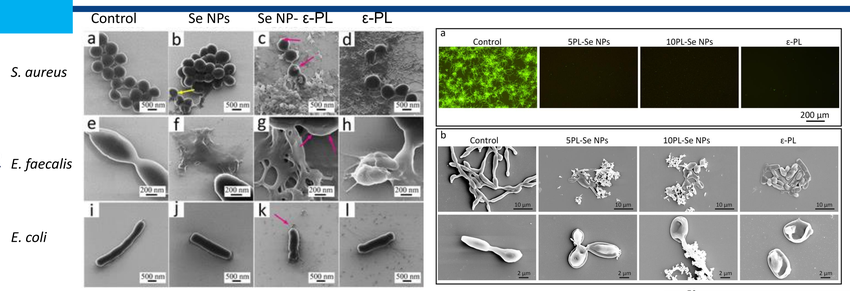



**Fig. 3: Microscopy shows damage to bacteria (left) and *C.albicans* (right) after treatment with Se NP-ε-PL compared to media control or ε-PL alone.**

**Table 1: Minimum inhibitory concentrations (MIC) of Se NPs, Se NP-ε-PL and ε-PL alone against laboratory bacterial strains.**

| strains | gram | MIC (μg/mL) Se NPs | ε-PL | Se NP-ε-PL |
|---|---|---|---|---|
| *S. aureus* | + | 10.1 ± 6.6 | 7.5 ± 1.0 | 6.0 ± 0.3 |
| MRSA | + | 11.5 ± 4.8 | 11.6 ± 2.0 | 8.6 ± 4.2 |
| *E. faecalis* | + | 10.4 ± 5.8 | 27.1 ± 2.2 | 9.4 ± 3.8 |
| *E. coli* | – | 340 ± 200[b] | 13.2 ± 0.5 | 19.5 ± 9.2 |
| *A. baumannii* | – | 229 ± 10[b] | 21.6 ± 7.0 | 13.8 ± 1.2 |
| *P. aeruginosa* | – | 290 ± 66[b] | 9.5 ± 3.6 | 12.5 ± 0.5 |
| *K. pneumoniae* | – | 255 ± 29[b] | 12.0 ± 0.6 | 12.3 ± 0.2 |
| *K. pneumoniae* (MDR) | – | 363 ± 46[b] | 26.5 ± 0.5 | 26.2 ± 0.4 |

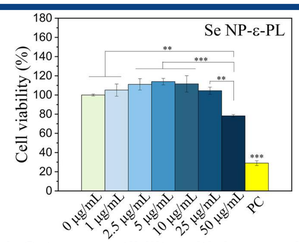



**Fig. 4: Cytocompatibility with human dermal fibroblasts, 24 h; ** p<0.01, *** p<0.001.**

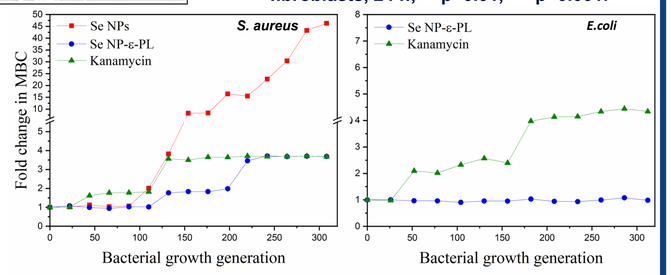



**Fig. 5: Resistance development of *S. aureus* and *E. coli* treated with Se NPs with and without ε-PL coating compared to the antibiotic, kanamycin, at 50% of the MBC.**

## CONCLUSIONS

This study demonstrates the potential of ε-poly-L-lysine coated selenium nanoparticles as an alternative antimicrobial agent that could be used in burn wound dressings. The NPs inhibit and kill a broad range of laboratory and clinically isolated Gram-positive and -negative bacteria, including drug resistant strains, as well as *C.albicans*. They function via multiple mechanisms of action and can delay or limit the onset of resistance.

REFERENCES

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