Clàudia Lliso Pascual
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Literature review
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Biosynthesis, optimization and toxicity of metallic nanoparticles with antimicrobial and antibiofilm activity for bacterial infections treatment

UAB Universitat Autònoma de Barcelona

1. Introduction

Antibiotic-resistant bacterial infections arising from acquired resistance mechanisms (AMR) and/or through biofilm formation necessitate the development of innovative "outside of the box" therapeutics. Metallic nanomaterial-based therapies are promising tools featuring the capacity to evade existing mechanisms associated with acquired drug resistance. In particular, metallic nanoparticles (MNP) have been demonstrated to be active in treating several multidrug-resistant bacterial infections and eradicate biofilm formation[1,2,3].

Goals

- ✓ Give an overview about the MNP biosynthesis.
- ✓ Highlight the ultimate optimization procedures to enhance MNP antibacterial capabilities.
- ✓ Discuss the hazard potential of MNP for the environment and humans.

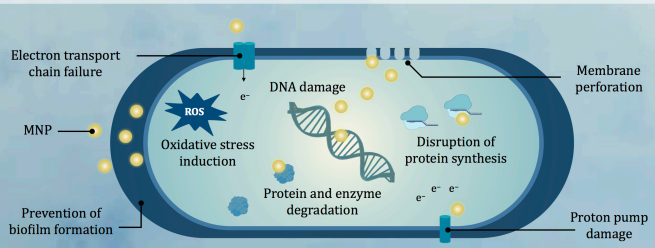


Figure 1. Main antimicrobial mechanisms of MNP on bacteria.

2. Mechanisms of action

MNP display a variety of bactericidal mechanisms that not only hinder the development of bacterial resistance, but also broadens the spectrum of their therapeutic activity[3,2,4]. Non-specific electrostatic interactions with the negatively charged groups present on bacterial surfaces mainly result in:

- Cell wall and membrane disruption → Membrane damage and cytoplasmatic leakage[4].
- Reactive oxygen species (ROS) generation → Lethal oxidative stress[4,5].
- Damage to intracellular components → Binding and disruption of ribosomes, proteins and DNA[4].

3. Bioproduction

Synthetic MNP synthesis typically requires physical and/or chemical methods that involve harsh and environmentally hazardous conditions. Recently, the use of microorganisms as potencial biofactories for MNP production has been received global attention due to the many offered benefits as to production under mild and environmentally friendly conditions[5].

✘ Synthetic methods
✓ Green synthesis

- Non-toxic solvents/products
- Lower energy input
- Time saving
- More economical
- Naturally product stabilization
- Biocompatibility enhancement

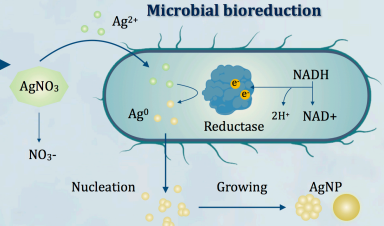


Figure 2. Bacterial bioreduction of silver nanoparticles.

- Through exposure to metal salts, microbial cells have developed means to quickly remove these toxic ions, such as the reduction of the metallic cations to their elemental forms.
- Enzymes like NADH-dependent reductase and some non-enzyme proteins or peptides are known as the mainly involved in bioreduction to afford intracellular NPM.
- Extracellular biosynthesis is also possible and makes use of secreted enzymes and or enzymes located on the outer membrane.

4. Biocatalytic refinement and characterization

Although wild-type microbial cells have been widely used for MNP synthesis, the biosynthetic efficiency is rather low. Thus, researchers have sought to genetically engineer microbial cells to optimize MNP biosynthesis, wether increase metallic-ion-binding affinity, enhance reduction capacity and attenuate toxicity. The heterologous coexpression of genes that code for peptides and proteins involved in those processes have proven useful in making a more efficient synthesis. In particular, *Escherichia coli* has become one of the most commonly engineered hosts owing to its rapid growth and ease of manipulation[4,5].

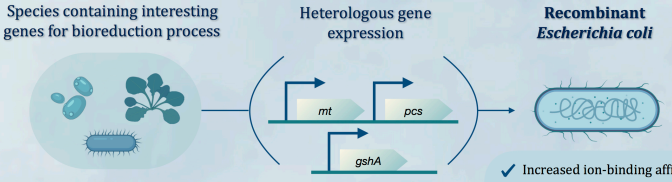
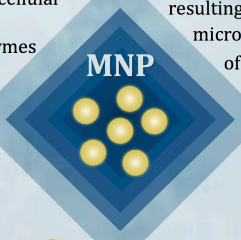


Figure 3. Optimization via heterologous expression of genes involved in bioreduction.

- ✓ Increased ion-binding affinity
- ✓ Enhanced reduction capacity
- ✓ Increased resistance

Moreover, by adjusting reaction parameters, the physicochemical properties of the resulting MNP can be tuned. Their characterization through spectroscopic and microscopic techniques will be crucial to determine the effectiveness and safety of the product.



5. Product optimization

Additionally, many strategies have been performed in order to increase the antibacterial efficacy of the synthesized MNP:

- **Size modification**

  Smaller MNP with higher surface-to-volume ratio have shown greater ease to penetrate the cells, inducing enhanced activities against several pathogens[4].

Figure 4. MNP size reduction.

- **Light activation therapies**

  Irradiation of MNP with a laser source produces local heat energy (APTT) and ROS generation (APDT) resulting in irreversible cell damage[7].

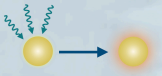


Figure 5. Light activation of MNP.

- **Bioconjugation**

  The ease of modifying MNP surface can led to the biofunctionalization of different molecules for the improvement of their intrinsic properties[6,7].

Synergistic effect: Conjugation to antibiotics or AMP	Increased specificity: Conjugation to phages	Targeting biofilms: Conjugation to enzymes

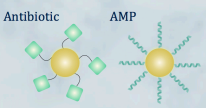


Figure 6. MNP conjugated to antimicrobials.

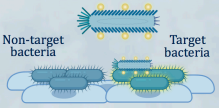


Figure 7. Selective action of MNP conjugated to phages.

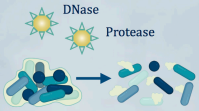


Figure 8. MNP conjugated to enzymes for biofilm eradication.

6. Toxicity

Since the therapeutic potential of MNP is being reviewed, their safety is a major factor that needs to be considered[3,4].

Ecosystem interference

Most of MNP are dispersed readily in contact with water. However, leakage of non-dispersing MNP into the environment could imply:

- Death of several bacterial communities and disruption of element cycling, bioremediation and nitrogen fixation.
- Growth inhibition of surrounding vegetation.
- Accumulation and toxicity (depending on the species).

Human cytotoxicity

In vivo and *in vitro* studies show:

- Toxicity seems to be multifaceted and hard to predict, although can be mitigated through dose titration.
- Pharmacokinetics/dynamics and biodistribution are governed by MNP physicochemical properties, dose, and route of administration.
- MNP show a size-dependent metabolic fate, accumulation and excretion route.
- Apparently, MNP cause insufficient impact to trigger an inflammatory response and tissue damage.

7. Conclusions

- ✓ The versatility of action of MNP-based therapies allows them to **overcome AMR selection**.
- ✓ Green MNP synthesis may prevail over physicochemical methods, especially **extracellular** bioreductions mediated by **recombinant bacteria**.
- ✓ MNP effectiveness and targeting capacity can be enhanced particularly through the combination of **light activation therapies** and surface **biofunctionalization**.
- ✓ Hybrid **AuNP-based nanocomposites** have emerged as a powerful tool due to their outstanding biocompatibility, ease of surface modification and optical properties.

What is next?

- Delve deeper into the **effects of morphology and surface chemistry** on MNP final activity.
- Focus on the establishment of **ecotoxicity tests** and new **regulations**.
- Gain further knowledge of MNP pharmacology and cytotoxicity to boost **human clinical trials** → valuable information on long-term safety and efficacy to guide de development of MNP-based therapies.
- Set up **standardization protocols** for the study of similar MNP so as to reach specific conclusions regarding their safety.

8. Main references

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