# BACTERIA MEET NANOPARTICLES - ELECTRON MICROSCOPY INSIGHTS

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## Introduction

Nanoparticles (NPs) application in medicine has great potential for future advances not only in innovative therapies but also as a diagnostic tool [1]. Because of their biocompatibility and unique properties, such as surface plasmon resonance or superparamagnetism in some cases, NPs are widely investigated for cancer therapies, pharmacology, advanced diagnostics, treating bacterial infections, and antiseptics [2,3]. The last two are of special scientific interest, aiming at targeting pathogenic bacteria with the use of antibiotics alternatives [4]. However, understanding of NPs antibacterial interaction remains a great challenge. There are several possible mechanisms including mechanical damages of cells, oxidative stress, photo-killing, ions homeostasis disturbance, and proteins dysfunctions [5,6]. However, there is no doubt that independently on the mechanism involved, NPs need to be in direct contact with bacterial cells to achieve their antibacterial properties. Therefore, the initial stages of NPs bactericidal mechanisms are needed to be investigated to identify the principal parameters governing the NPs interaction with the bacteria [2]. This study addresses this issue via the application of advanced *in-situ* TEM observations.

## Materials and Methods

Electron microscopy techniques allow for high-resolution observations but cannot be directly applied to biological samples. Typically, for TEM observations biological moieties have to be fixed during multi-step protocols. In this study a state-of-the-art, *in situ* liquid-phase transmission electron microscopy (LP-TEM) was applied to observe the interaction between bacteria and gold nanoparticles (15 nm) in real-time. The specialized Poseidon TEM holder and Protochips systems dedicated to in situ observations in liquids were used (FIG. 1). The reference strain used was *Staphylococcus carnosus* DSM 20501 (Deutsche Sammlung von Mikroorganismen und Zellkulturen), a typical gram-positive coccus, spherical in shape and forming grape-like clusters. The average size of a single cell ranges between 0.5 and 1.5 µm. The static observations were performed with an e-chip with a 5 µm spacer. The bacteria in water solution (~$6 \cdot 10^8$ /ml) were dropped on the large e-chip and subsequently dried. Next 0.1 µm of the AuNPs solution (~25 mg/l) was dropped in the small chip loaded into the holder. After the *in situ* observations the small e-chip was dried, loaded into a single tilt holder, and observed in BF and HAADF STEM mode.

## Results and Discussion

For the in-depth studies of bacteria-NPs interactions, a dedicated in situ Poseidon TEM holder was successfully applied. The micrograph (FIG. 1) shows *ex post facto* observations of bacteria with adhered gold NPs. The investigations revealed that bacteria retain their properties despite being coated with gold nanoparticles. The bacterial cell life processes like cell division with the formation of the bacterial septum, intercellular nanotubes creation, and outer membrane vesicles formation were observed. In opposite to standard TEM observations, LP-TEM allows for direct investigations of fully hydrated and native cells in their natural liquid conditions. This approach provides new opportunities in life science microscopic investigations to study dynamic processes in real-time at a near-atomic scale.

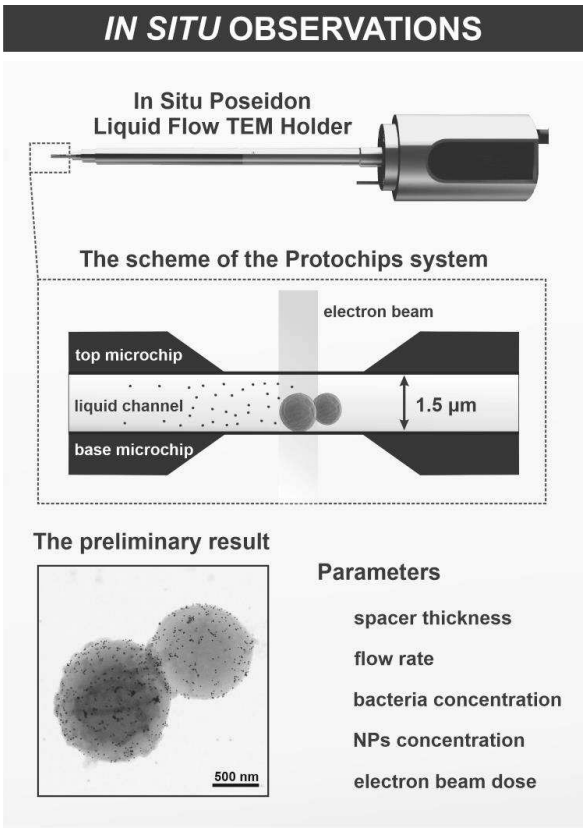


FIG. 1 Graphical representation of the approach of the *in situ* observations of bacteria-NPs interface showing dedicated Poseidon TEM holder, equipped with special Protochips system for electron microscopy observations in liquids and the obtain representative image of Au-coated *S. carnosus*.

## Conclusions

Taking into account the novelty of application LP-TEM for biointerface studies allows for a unique insight into the bacteria-NPs interface. The studies also reveal that for effective observations, the methodology should be carefully optimized considering the size of a bacterial cell and chip size to provide suitable space within the liquid channel of the Protochips system as well as the incubation conditions.

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## References

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