Annual Research & Review in Biology
9(3): 1-8, 2016, Article no.ARRB.23907
ISSN: 2347-565X, NLM ID: 101632869

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Gold Nanoparticle Uptake and Distribution in the Digestive Tract of *Hermetia illucens* Stratiomyidae: *Diptera* (L.1758) Based on Transmission Electron Microscopy

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Authors' contributions

This work was carried out in collaboration between all authors. Author FRO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KD supervised and managed the study and wrote the final draft. Author RPS supervised the electron microscope study. All authors contributed at the same way, read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2016/23907
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) A. Ayeshamariam, Khadir Mohideen College, Adirampattinam, India.
(2) Antonina Yashchenko, Danylo Halytsky Lviv National Medical University, Ukraine.
Complete Peer review History: http://sciencedomain.org/review-history/13057

Original Research Article

Received 28th December 2015
Accepted 13th January 2016
Published 23rd January 2016

ABSTRACT

This study investigated the interaction of gold nano partilcles with the digestive tract of the black soldier larva, using transmission electron microscopy (TEM). For this study an overall of 30 *Hermetia illucens* was bread at 28°C to 30°C at a moisture content of 70% to 80% with a diet based on cow manure. The study showed that gold nanoparticles were absorbed into different cells and were not treated as foreign particles. A gold nanoparticle accumulation was found in some goblet like cells. Gold nanoparticles were able to pass the peritrophic membrane and enter the gut epithelial cells.

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Keywords: Hermetia illucens; gold nanoparticle black soldier fly larva; scanning electron microscopy; digestive trackt.

1. INTRODUCTION

Gold nanoparticles are resistant to oxidative dissolution and are easily detected. They have been used as stable probes for the observation within biological systems. Extensive attention has been paid to studying the ecological safety of nanoparticles on microorganisms, animals and plants regarding the application of nanoparticles [1]. Even in cases where nanoparticles do not show any acute toxicity, the question of long-term effects, bioaccumulation and the impact on food webs remains unknown. Engineered nanoparticles may also affect the toxicity of other substances, since natural nanomaterials are known to act as nanovectors for contaminants [2]. Currently, the lack of identified risks from nanomaterial exposure has given rise to their use in commercial applications. However, as the quantity and types of this nanomaterial increases, the potential for environment consequences also increases [3]. Understanding the biology of the black soldier fly (BSF) is crucial to understanding the behavior of the cells when nanoparticles are ingested. In order to enter a cell, the nanoparticle must cross the cell membrane that separates the internal components of the cell from the outside. Upon assimilation by an organism. Nanoparticles interact with extracellular biomolecules dissolved in body fluids such as protein, sugar and lipids before reaching the plasma membrane [4]. Endocytosis and penetration mechanisms involved in cellular uptake are discussed by [4,5]. Endocytosis mechanisms when activated permit the membrane to wrap up the NPs slowly until fully inside [5]. More studies have been done for a thorough understanding of how nanoparticles enter the cells and cellular responses to nanoparticle exposure.

Microscopy-based methods are available that could be used in the detection and characterization of engineered nanoparticles. These methods include optical approaches including scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In TEM, electrons are transmitted through a specimen to obtain an image whereas in a SEM scattered electrons are detected at the sample interface for imaging.

Understanding interactions between NPs and biomolecules or cells has yet to be achieved. Presented here is an in vivo method to observe interactions between nanoparticles and the digestive tract of *Hermetia illucens*, using transmission electron microscopy (TEM).

2. MATERIALS AND METHODS

2.1 Breeding BSF Larva

For the investigation sixteen BSF larvae eggs were used. BSF larva hatched after 72 hours and were bread under ideal conditions with temperatures between 28-30°C and a moisture content of 70-80%. The BSL larva were placed individually in containers of 6.0 cm x 5.0 cm containing 1.000 g of cow manure. In ten containers 1.000 mL of gold nanoparticles with a mass concentration of 0.052 mg/mL was added. The remaining containers were used as controls. Two hours and twenty four hours after feeding, five larvae fed with cow manure and gold nanoparticles and three larvae from control were collected for analyses.

2.2 Sample Preparation for Transmission Electron Microscopy

Specimens were dissected using 2.5% glutaraldehyde and the midgut was removed for analysis. Then they were fixed with 2.5% glutaraldehyde in Phosphate Buffered Saline (PBS) buffer for one hour followed by osmium tetroxide 1% (in water). The samples were dehydrated in a series of alcohol solutions (30%, 50%, 70%, 95%) for 10 minutes each and finally 100% for 5 minutes, 3 times. After, they were air dried and embedded with epoxy resin and placed in 60C during 24 hours. An Ultramicotome was used to get the sections on cleaned 300 lines/mesh hexagonal Cu grids. The thick sections obtained were first analyzed with light microscope for tissue structure location. After finding the membranes and organelles, the grids were taken to TEM for higher magnification and nanoparticles observation.

2.3 Transmission Electron Microscopy

JEOL JSM-2000EX with an accelerating voltage of 80-200kV was used for the BSF larva nanoparticle analyses. The instrument has a lattice image resolution of 0.14 nm and a point image resolution of 0.28 nm and can be operated

at a magnification of 1,000,000 X. low vacuum scanning electron microscope equipped with an EDAX energy dispersive x-ray spectrometer. The JSM 5800 LV has superior resolution in high vacuum mode, and it was used for nanoparticle observation in the BSF larva tissue.

2.4 Gold Nanoparticles Characterization

Gold nanoparticles characterization was provided by the manufacturer (NanoComposix) and it is detailed in Table 1. The diameter of gold nanoparticles was measured as part of the control and found in the manufacturer's specification.

The Manufacturer also provided the absorbance (cm-1) x wavelength (nm) (Fig. 1) which confirms the size of the nanoparticles according the color absorbed.

3. RESULTS AND DISCUSSION

3.1 Nanoparticles Characterization

20 nm gold nanoparticles were obtained from a calibrated TEM and measured at different magnifications. The diameter of the gold nanoparticles is showed in Fig. 2 at various magnifications. The measurement varies from 17.0 to 20.4 nm.

3.2 Light Microscopy of BSF Larva Identification

Light microscopy was used to identify the tissues in the midgut of the larvae examined. Fig. 3 identifies the membrane and food bolus inside the gut and gives a broad image of tissue observed under TEM. The tissue shows the epithelial, goblet cells the muscles and basement membrane that are protected from coarse food viruses and bacteria by a peritrophic membrane (PM). This membrane is present in most arthopods and is composed of chitin and proteins, of which peritrophins are the most important. The main purpose is protection against abrasion and microorganism invasion [6]. Oddly the goblet cells secrete muscin into the gut for digestion and protection too. It is odd that insects would have two systems doing the same function. The basement membrane is at the base of the gut epithelium and attached on the inside there is a smooth muscule coat with longitudinal and circularly arranged muscles [7]. It serves to constrict the food bolus and as a backing for the epithelial cells and separates the hemocoel from the gut.

Table 1. Gold nanoparticles characterization

20 nm gold nanoparticles			
Diameter (TEM)	18.7±1.7 nm	Hydrodynamic diameter	21.7 nm
Coefficient of variation	9%	Zeta potential	-40.0 mV
Surface area (TEM)	16.3 m²/g	pH of solution	7.6
Mass concentration	0.052 mg/mL	Particle surface	Sodium citrate
Particle concentration	7.9E+11 particles/mL	Solvent	Aqueous 2 mM citrate

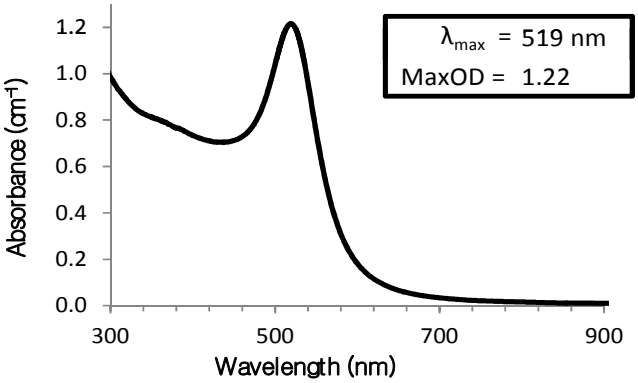

Fig. 1. Optical properties of gold nanoparticles

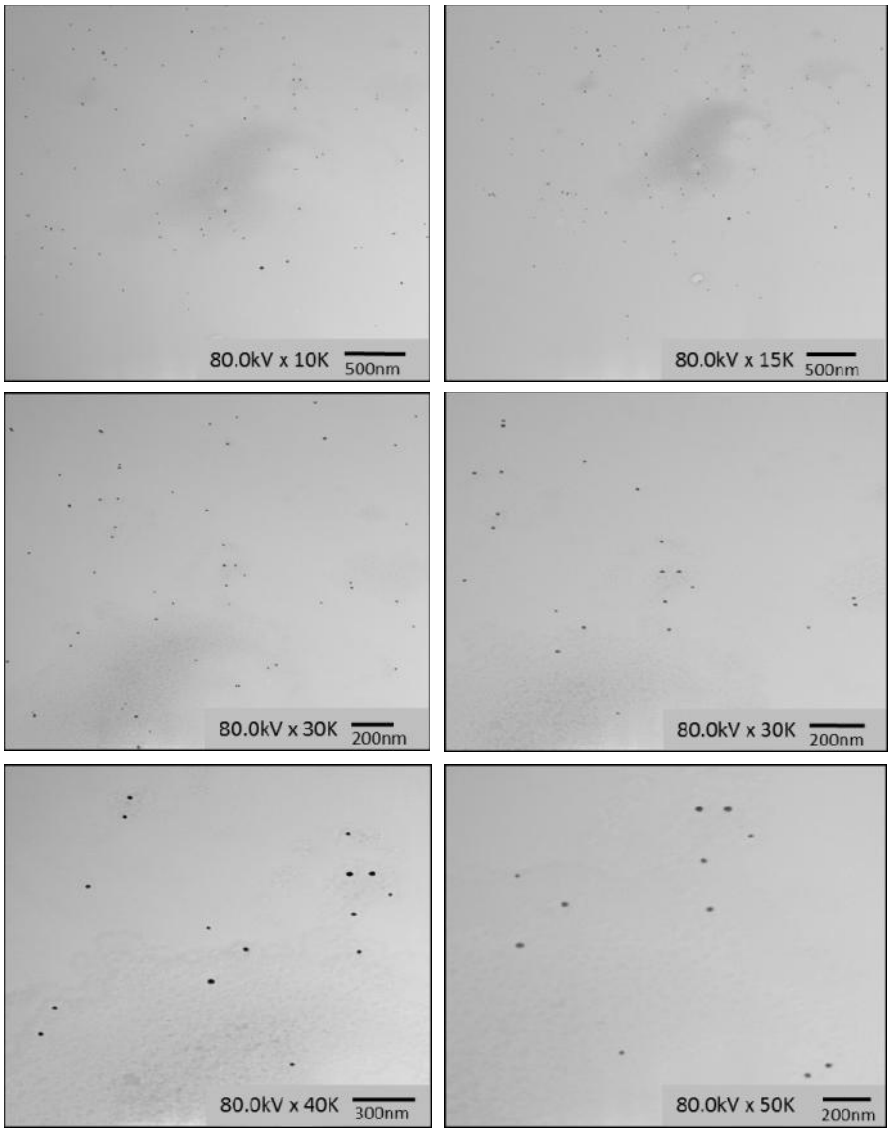

Fig. 2. 20 nm gold nanoparticles in different magnifications. x10K, x15k, x20k, x30k, x40k and x50k used as control

Fig. 4 shows the slender Malpighian tubules in the posterior regions of the alimentary canal. They have a single layer of cells attached to a BM joining the alimentary canal at the junction between the midgut and hindgut. Malpighian tubules are the insect's kidney and serve to excrete toxic compounds from the hemolymph to the hindgut.

3.3 Nanoparticle Distribution

Fig. 5 shows that 20 nm gold nanoparticles were able to pass the peritrophic membrane and enter the gut epithelial cells. The nanoparticles invaded the epithelial cell by an unobserved mechanism. It must be noted that the particles are not isolated by the cell as invaders as they would have had open space around them where membranes containing enzymes would form, instead they are shown closely adhering to existing cell parts. Moreover, the nanoparticles are agglomerated probably due to endocytosis of several particles at once.

Gold nanoparticles were absorbed into different cells and they were not treated as foreign particles by the cells. In contrast we can observe the spores as much larger in size and are

isolated from the cell organelles by an open space. Some species of bacteria and yeast can develop these resistant structures called spores. The cell that develops the spore is dehydrated, forms a thick wall and has its metabolic activity reduced [8]. Spores are able to remain unexpressed for decades until they find a suitable environment to rehydrate and become active (Fig. 6), and reproduce by binary fission [9]. Since the BSF larva in this investigation were fed with cow manure, it can be assumed that bacteria and yeast are present in the food contained in the gut.

Goblet like cells as shown in Fig. 7 are found scattered in the epithelial tissue of the intestinal tract. A goblet cell is a glandular, modified simple columnar epithelial cell that excretes mucus. A major function of this mucus is to digest food, protect the cells against abrasion and guard against infectious agents such as fungi, bacteria and viruses [10]. An accumulation of gold nanoparticles were found in some GLC, which may be a natural pathway to excrete the nanoparticles through intestinal secretions. Elimination methods of gold nanoparticles used in biomedical applications still remains unknown. However, the above findings might be used to investigate but not limited to the following: a) nanoparticles as model compounds to understand how viruses can cross cell membranes, b) investigate mechanisms of cellular uptake of nanoparticles, c) how a increasing amount of nanoparticles in human bodies may implicate future problems of blocking pathways in the blood stream, d) investigate bioaccumulation of nanoparticles in the nervous system and e) uptake of nanoparticles and its impacts in the food chain.

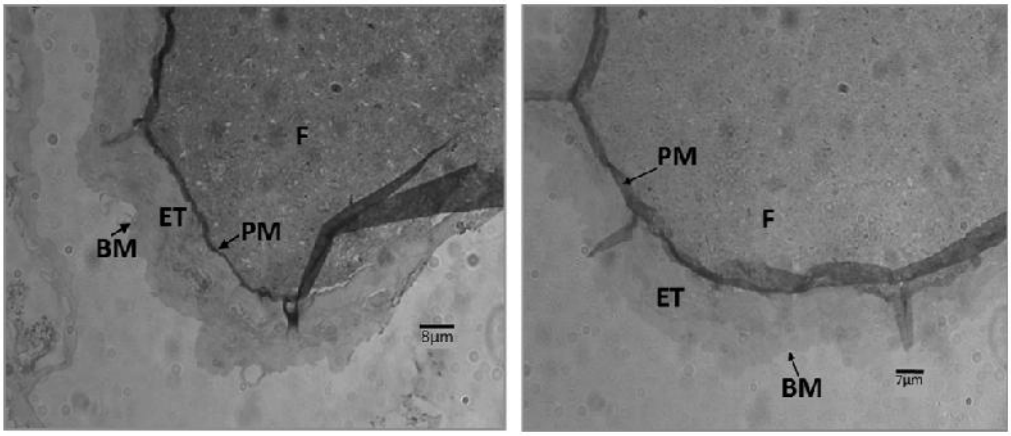

Fig. 3. Light microscope picture at 600x. Detail of the peritrophic membrane (PM), Basement membrane (BM), Epithelial tissue (ET) and the food (F) inside the gut

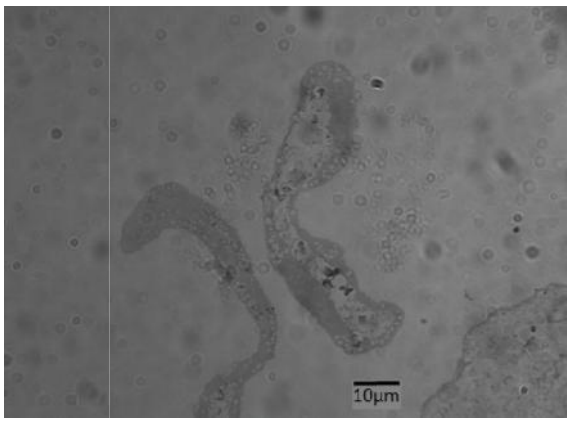

Fig. 4. Light microscope picture at 600x. Cross section of the Malphigian tubules

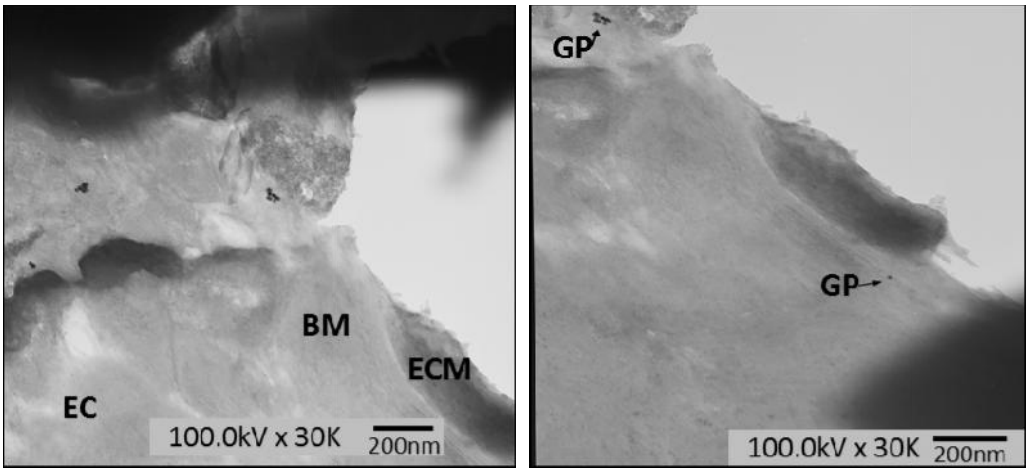

Fig. 5. Detail of the epithelial cell (EC) basement membrane with extracellular matrix (ECM) and gold nanoparticles in the gut. Dark spheres (GP)

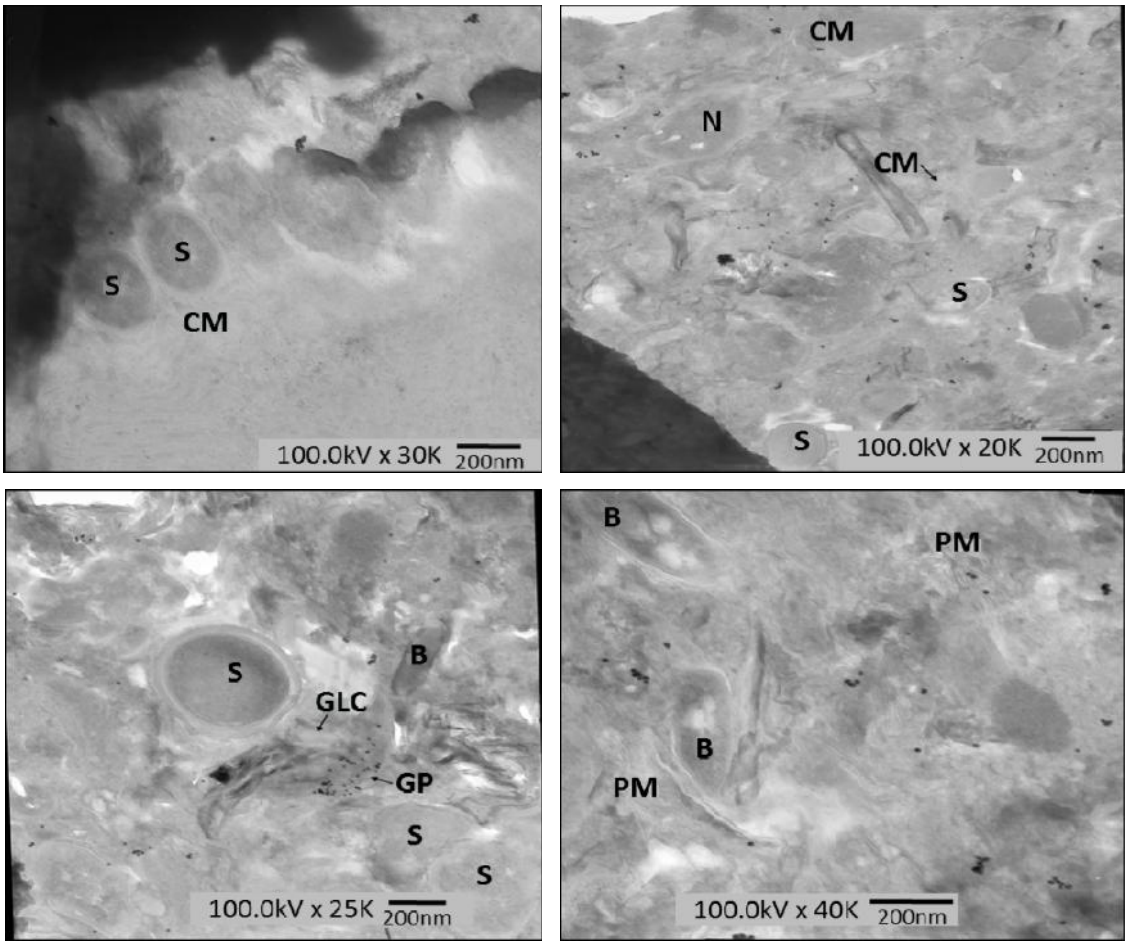

Fig. 6. Detail of gold particles distribution (GP) in an epithelial cell, spores (S), bacteria (B), goblet-like cell (GLC) and cell membrane (CM)

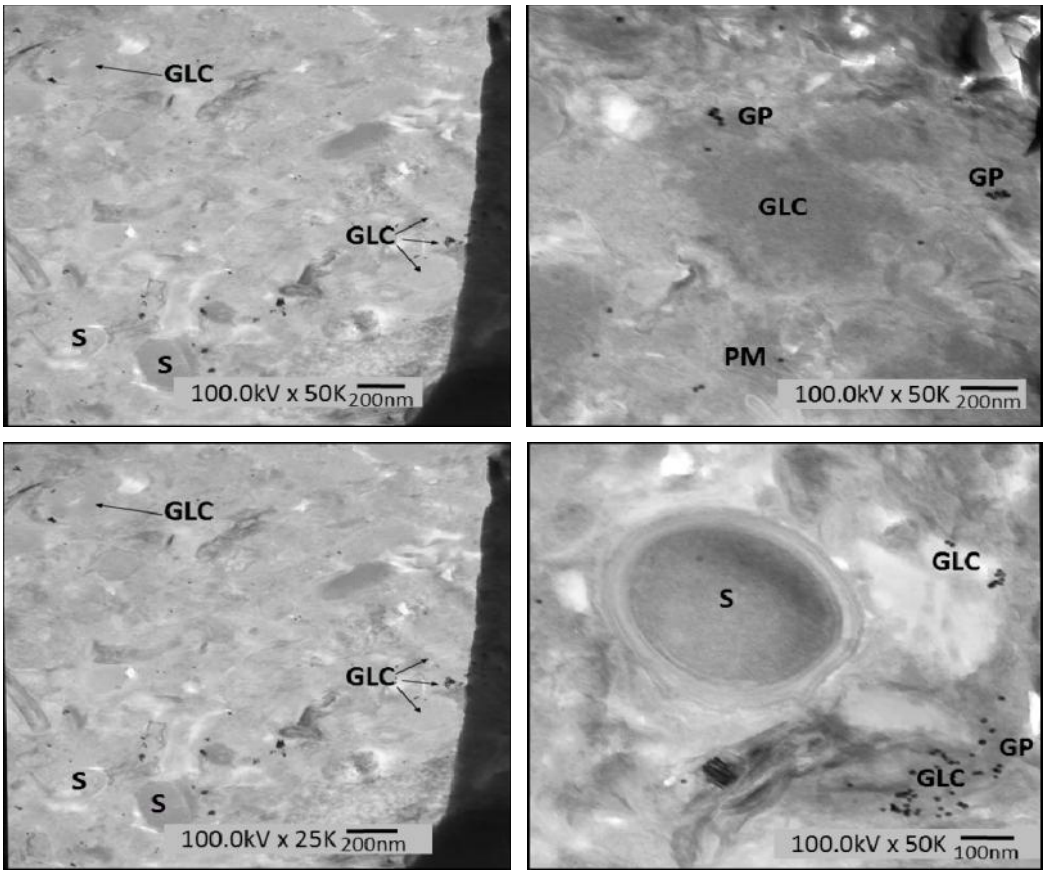

Fig. 7. Detail showing gold nanoparticles in GLC

4. CONCLUSION

Gold nanoparticles were able to pass the peritrophic membrane and enter the gut epithelial cells. They were absorbed into different cells and were not treated as foreign particles. In contrast it can be observe that spores are much larger in size and isolated from the cell organelles by an open space. An accumulation of gold nanoparticles was found in some GLC, which may be a natural pathway to excrete the nanoparticles through intestinal secretions and might be used as a model to understand how viruses might cross membranes. Elimination methods of gold nanoparticles used in biomedical applications still remain unknown.

ACKNOWLEDGEMENTS

Thanks to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Ministério da Educação (Capes/MEC) for funding, TRINITY Institute at ESF and N.C. Brown Center for Ultrastructure at ESF for providing the electron microscope.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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