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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

# Interaction between gold nanoparticles and *Cryptococcus* spp

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2009, 26(4):520–522

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OBJECTIVE **The effects on the human cell of nanoparticles has been documented; cells in tissue culture stained for nanoparticles were observed adhering to the bottom of the wells of the tissue culture plates. There is also evidence for the binding of nanoparticles to the micro-organism cell membrane and transport inside the cell. However, the effect of nanoparticles on fungus has been little studied. Here, the authors report the interaction between gold nanoparticles and *Cryptococcus* spp.** METHOD **A gold nanoparticle solution was mixed with clinical samples of cerebrospinal fluid with and without *Cryptococcus* spp, and the characteristic color change was observed.** RESULTS **A protein bonding effect between gold nanoparticles and *Cryptococcus* spp was detected.** CONCLUSIONS **According to this study, the clinical application for gold nanoparticles as a new therapeutic option for cryptococcosis can be justified.**

Αλληλεπίδραση νανοσωματιδίων χρυσού και ειδών *Cryptococcus*

*Περίληψη στο τέλος του άρθρου*

**Key words**

*Cryptococcus*
Gold nanoparticle

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Nanotechnology is a new widely used biotechnology. Nanoparticles differ in their chemical and physical properties from the same material at a larger scale.[1] Colloidal nanometer-sized gold suspension is red in color, while aggregation of gold nanoparticles by any precipitating factors changes the color to purple-grey; the aggregation shifts the surface plasmon band.[2,3] In situations with high protein, such as urine samples with proteinuria,[4] prevention of aggregation by protein bonding is possible. Interference from non protein chemical substances has not been documented.[2,3]

Effects on human cells of nanoparticles have been documented. Cells, stained for nanoparticle binding, were observed to adhere to the bottom of the wells of the tissue culture plates, with their morphology preserved, indicating that the cell membrane was intact.[5] However, the effects on the microbial cell have not been well established, although there is evidence for the binding of nanoparticles to the micro-organism cell membrane and transport inside the cell. Here, the authors report their findings on the interaction between gold nanoparticles and *Cryptococcus* spp in cerebrospinal fluid.

## MATERIAL AND METHOD

### Cerebrospinal fluid sample

Cerebrospinal fluid (CSF) samples from routine laboratory analysis, either with (5 samples) or without (5 samples) identification of *Cryptococcus* spp were used for this study. All the CSF samples had been collected according to standard lumbar puncture procedure and transferred according to the standard procedures of the medical laboratory. The study protocol was approved by the ethical committee of the authors' affiliation.

### Gold nanoparticle solution preparation

Following the classical Turkevich citrate reduction method,[5] 9 nanometer-sized gold nanoparticles were established and gold nanoparticle concentration was prepared specifically at 44 ppm. According to the protocol, the derived gold nanoparticles can be stored in the dark at temperatures below 4 °C for over four weeks.

### Study of the direct effect of gold nanoparticles

A mixture between equal amounts (500 mL) of gold nanoparticle solution and *Cryptococcus* spp CNS sample was prepared. After

being left for 15 minutes, the color change to red was observed in the mixture. Confirmation of the interaction was performed by light microscopic examination using the standard clinical microscopic technique.

## RESULTS

According to this study, it was revealed that after mixing a *Cryptococcus* spp CNS sample with gold nanoparticle solution, red color can be observed, while the non *Cryptococcus* spp sample showed grey color.

## DISCUSSION

Recent developments in biotechnology have made feasible the use of colloidal gold particles in a large size range for specific labeling of living cells for the purposes of electron microscopy.[6] Attachment of gold nanoparticles to the human cell was clearly shown by the study of Tanev el al.[7] Transmission electron microscopy of ultrathin sections showed the presence of nanoparticles within the cytoplasm and in the nucleus of the cells, the latter being much smaller in dimension.[5] In this study, the authors further investigated the interaction between gold nanoparticles and cells, in this case microbial cells. The interaction between gold nanoparticles and the cell membrane of the *Cryptococcus* spp yeast was confirmed.

Here, it was demonstrated that the gold nanoparticle can cause protein binding with the *Cryptococcus* spp. The experiment performed proved that the metal nanoparticles interact within the membrane of the *Cryptococcus* spp. Because the sample used in the study was an actual clinical specimen, the proteins or other molecules stabilizing the nanoparticles could not have been from another source, as that when the fungi are prepared in a culture medium. This is not a surprising finding; interaction/adsorption of metal nanoparticles on proteins and peptides is well known and therefore any organisms producing proteins, from prokaryotes to eukaryotes, should be able to bind such nanoparticles, and this therefore might not be a particularity of the *Cryptococcus* spp used in the present study. The mechanism of entry of the nanoparticles into the cell needs to be further studied. The possible mechanism might be direct bonding between nanoparticles and protein in the capsule of *Cryptococcus* spp, as in the case of the urine sample with proteinuria.[4] Indeed, tagging the nanoparticles with amphotericin B, the drug of choice for cryptococcosis, is reported to crease the pharmacological effect.[8] According to this study, the clinical applications of gold nanoparticles as a new therapeutic option for cryptococcosis can be justified.

## ΠΕΡΙΛΗΨΗ

**Αλληλεπίδραση νανοσωματιδίων χρυσού και ειδών *Cryptococcus***

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**ΣΚΟΠΟΣ** Προτείνεται η δράση των νανοσωματιδίων στα ανθρώπινα κύτταρα. Τα κύτταρα που χρωματίζονται λόγω της ανάπτυξης νανοσωματιδίων προσκολλώνται στον πυθμένα των τοιχωμάτων των δοχείων καλλιέργειας ιστών. Υπάρχουν δεδομένα με αποτελέσματα που δείχνουν αυτή την αλληλεπίδραση, όπως σύνδεση των νανοσωματιδίων με τη μεμβράνη μικροοργανισμών, μεταφορά εντός των κυττάρων, καταστροφή των νανοσωματιδίων κ.λπ. Εντούτοις, η δράση των νανοσωματιδίων σε μύκητες δεν έχει μελετηθεί ευρέως. **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Αναφέρεται η αλληλεπίδραση μεταξύ νανοσωματιδίων χρυσού και ειδών κρυπτοκόκκου. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Το αποτέλεσμα της σύνδεσης πρωτεΐνης μεταξύ νανοσωματιδίων χρυσού και ειδών κρυπτοκόκκου μπορεί να ανιχνευτεί. **ΣΥΜΠΕΡΑΣΜΑΤΑ** Σύμφωνα με τη μελέτη, η χρήση νανοσωματιδίων χρυσού μπορεί να έχει κλινική εφαρμογή στις θεραπευτικές προσπάθειες για τη λοίμωξη από κρυπτόκοκκο.

**Λέξεις ευρετηρίου:** Κρυπτόκοκκος, Νανοσωματίδια χρυσού

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