

Tannic acid-modified silver nanoparticles enhance anti-*Acanthamoeba* activity without increasing cytotoxicity of three multipurpose contact lens solutions

Edyta Beata Hendiger (✉ edyta.hendiger@wum.edu.pl)

Warszawski Uniwersytet Medyczny <https://orcid.org/0000-0002-9463-2505>

Marcin Padzik

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Agnieszka Żochowska

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Wanda Baltaza

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Gabriela Olędzka

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Diana Zyskowska

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Julita Bluszcz

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Sylwia Jarzynka

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Lidia Chomicz

Medical University of Warsaw: Warszawski Uniwersytet Medyczny

Marta Grodzik

Warsaw University of Life Sciences: Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Jacek Hendiger

Warsaw University of Technology: Politechnika Warszawska

Jose E. Piñero

University of La Laguna: Universidad de la Laguna

Jarosław Grobelny

University of Lodz: Uniwersytet Lodzki

Katarzyna Ranoszek-Soliwoda

University of Lodz: Uniwersytet Lodzki

Jacob Lorenzo-Morales

University of La Laguna: Universidad de la Laguna

Short report

Keywords: Acanthamoeba keratitis, contact lens solutions, silver nanoparticles, tannic acid

Posted Date: November 6th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-68448/v4>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Parasites & Vectors on December 22nd, 2020. See the published version at <https://doi.org/10.1186/s13071-020-04453-z>.

Abstract

Background: Free living amoebae of the genus *Acanthamoeba* are cosmopolitan, widely distributed protozoans causing a severe, vision-threatening corneal infection known as *Acanthamoeba* keratitis (AK). The majority of the increasing number of AK cases are associated with contact lenses use. Appropriate eye hygiene and effective contact lenses disinfection are crucial in prevention of this infection because of the lack of effective therapies against AK. Currently available multipurpose contact lens disinfection systems are not fully effective against *Acanthamoeba* trophozoites and cysts. There is an urgent need to increase the disinfecting activity of these systems to prevent *Acanthamoeba* keratitis infections. Synthesized nanoparticles have been recently studied and proposed as a new generation of anti-microbial agents. It is also known that plant metabolites, including tannins, present anti-parasitic activity. The aim of this study was to evaluate the anti-amoebic activity and cytotoxicity of the tannic acid-modified silver nanoparticles (AgTANPs) conjugated with the selected multipurpose contact lens solutions.

Methods: The anti-amoebic activity of pure contact lens care solutions and nanoparticles conjugated with contact lens care solutions were examined *in vitro* by a colorimetric assay, based on the oxidation-reduction of AlamarBlue. The cytotoxicity assays were performed using a fibroblast HS-5 (ATCC CRL-11882) cell line. The results were statistically analyzed by ANOVA and Student-Newman-Keuls tests using the $P < 0.05$ level of a statistical significance.

Results: We show that nanoparticles enhanced anti-*Acanthamoeba* activity of the tested contact lens solutions without increasing their cytotoxicity profile. The activity is enhanced within the minimal disinfection time recommended by the manufacturer.

Conclusions: The conjugation of the selected contact lens solutions with AgTANPs might be a novel and promising approach as a part of preventive actions of *Acanthamoeba* keratitis infections among contact lens users.

Background

Amoebae of the genus *Acanthamoeba* are free-living, abundant and cosmopolitan protozoans that show various degrees of pathogenicity to humans. They are ubiquitous in both natural and man-created environment. As facultative human parasites, when transmitted from the environment into the eye surface, they may cause progressive, sight-threatening corneal infection known as *Acanthamoeba* keratitis [1-5]. Improper use and disinfection of contact lenses, corneal damages and exposure of eyes to the polluted water are the primary risk factors of the AK. Lack of specific symptoms in the early stage of the infection, and co-infections with other microorganisms cause serious diagnostic difficulties and delay treatment. The number of AK infections has been increasing worldwide. Current therapeutic approaches are limited to a prolonged application of diamidines and biguanides. However, the treatment is not specific and very toxic to the eye [6-9]. Amoebae trophozoites may attach to the surface of both contact

lenses and contact lenses storage cases. Multipurpose contact lens disinfection systems are not effective against *Acanthamoeba* and need improvement on their anti-amoebic activity [10-12]. Summarizing, the prevention, including proper eye hygiene and effective contact lenses disinfection seem to be the best approach to limit the AK incidence.

In the recent years fast development of nanotechnology has been observed. Synthesized nanoparticles (NPs) are currently proposed as a new generation of anti-bacterial, anti-viral and anti-fungal agents [13,14]. Moreover, nanoparticles activity against different protozoans such as *Giardia intestinalis*, *Entamoeba histolytica*, *Cryptosporidium parvum* and *Leishmania* spp. has been already confirmed [15-17]. Plant metabolites, including tannins, present anti-microbial activity [18,19]. They are capable to form insoluble complexes with nucleic acids, carbohydrates, proteins and to chelate metal ions. Tannic acid (penta-m-digalloyl glucose) is the simplest, polyphenolic, hydrolysable plant metabolite with confirmed anti-bacterial, anti-cancer and anti-oxidant activity [20-23]. In our previous studies we demonstrated that tannic AgTANPs were well absorbed and showed anti-amoebic activity against *Acanthamoeba* strains belonging to T4 genotype [24]. Other authors confirmed that nanoparticles enhance anti-amoebic effect of biguanides such as chlorhexidine digluconate and other therapeutic compounds [25-27]. The aim of this study was to evaluate the activity and cytotoxicity of AgTANPs conjugated with selected multipurpose contact lens solutions against the trophozoite stage of a strain of *Acanthamoeba castellanii* belonging to the T4 genotype.

Methods

Cultivation of the strain

Acanthamoeba castellanii Neff strain ATCC 30010 type was cultured axenically in 25 cm² culture tissue flasks, without shaking, at 27 °C in PYG medium [0.75% (w/v) proteose peptone, 0.75% (w/v) yeast extract and 1.5% (w/v) glucose] containing gentamicin 10 mg/ml, in the Department of Medical Biology, Medical University of Warsaw, Poland. The culture was sub-cultured twice a month and the growth was observed under direct light microscope using a Bürker chamber (haemocytometer).

Nanoparticles

AgTANPs were synthesized by a chemical reduction method using silver nitrate (AgNO₃) purity 99.999% (Sigma-Aldrich, St Louis, MO, USA). AgTANPs were prepared by mixing the heated aqueous solution of AgNO₃ (95.2 g, 0.017%) with the aqueous solution of a tannic acid (0.6 g, 5% C₇₆H₅₂O₄₆ Sigma-Aldrich). The long-term stability of the colloidal dispersions of all tested NPs (zeta potential) was measured and confirmed by the electrophoretic light-scattering method with a Zetasizer Nano ZS, model ZEN3500 (Malvern Instruments, Worcestershire, UK) [26,28]. The size and shape of AgTANPs were determined by using the high-resolution scanning transmission electron microscopy (HR-STEM) technique (Fig. 1). Measurements were taken with a scanning electron microscope (Nova NanoSEM 450, FEI) using a transmission mode (STEM II) at an accelerating voltage of 30 kV. Samples for HR-STEM investigations

were prepared as follows: a drop of colloid was deposited onto carbon-coated copper grids (300 mesh) and left for 2 h for solvent evaporation. The well-dispersed nanofluids were used as a stock solution and were appropriately diluted to various concentrations ranging between 0.25–2.5 ppm and used in a subsequent activity and cytotoxicity assays.

Contact lens solutions

The multipurpose solutions used in this study, represent the three most common types of solutions used for contact lens care in Poland, namely: Solo Care Aqua (SCA), Opti-Free (O-F) and ReNu MultiPlus (ReNu). The tested contact lens care solutions and their ingredients are included in Table 1. All multipurpose solutions used in the study were purchased from authorized agents.

Activity assays

Pure contact lens solutions and nanoparticles at concentrations of 0.25, 0.5, 1.25 and 2.5 ppm conjugated with the contact lens care solutions were examined *in vitro* and assessed for their anti-amoebic activity. To determine the anti-amoebic efficacy on trophozoites (log growth phase after 6 days following sub-culturing), the previously described colorimetric 96-well microtitre plate assay, based on the oxido-reduction of AlamarBlue was used [29]. Subsequently, the plates were analysed over a period of 6 h, 24 h, 48 h, 72 h and 96 h in the Synergy HTX Multi mode plate reader (BioTek) using the Gen5 software programme, a test wavelength of 570 nm and a reference wavelength of 630 nm in order to calculate the inhibition curves of the analysis. All experiments were performed three times, in triplicate. Amoebae growth and viability (trophozoites movement and presence of acanthopodia) in both control and tested assays were visualized by Microscope Evos fl Cell Imaging System.

Cytotoxicity

Briefly, the cytotoxicity assays were performed using a fibroblast HS-5 (ATCC CRL-11882) cell line as described in our previous studies [24]. A commercial kit for the evaluation of drug-induced cytotoxic effects based on the measurement of lactate dehydrogenase (LDH) activity released to the media (Pierce LDH cytotoxicity assay kit 88953, 88954) was used as per protocol. The fibroblasts were incubated with each of the contact lens solution separately and the contact lens solution + nanoparticles added in the same concentration as in the activity assays. To calculate the percent cytotoxicity, absorbance was measured at 490 nm and 680 nm.

Statistical analysis

All experiments were performed three times in triplicate. For all activity and cytotoxicity detailed results standard deviation (SD) and mean values were calculated. The results were statistically analyzed by ANOVA and Student-Newman-Keuls tests using the $P < 0.05$ level of a statistical significance. For statistically insignificant results, “no activity” comment was added in Table 2.

Results

Activity

Our initial results confirmed the insufficient anti-amoebic effect of the tested contact lens solutions against *Acanthamoeba* trophozoites. Anti-amoebic activity was revealed for SCA and reached 32 % of inhibition after 6h of incubation. ReNu and O-F did not show anti-amoebic effect on the tested *Acanthamoeba* strain within the first 24 h of incubation. The detailed data are shown in Table 2.

AgTANPs significantly enhanced anti-*Acanthamoeba* activity of the tested contact lens solutions. Specifically, AgTANPs conjugated with SCA, after the minimal disinfection time recommended by the manufacturers (6 h), showed the most promising dose-dependent increase of the amoebae inhibition (Fig. 2). Similar anti-amoebic effect was achieved for AgTANPs conjugated with ReNu (Fig. 3). The enhanced anti-amoebic effect of both conjugates lasted up to 96 h of incubation. O-F conjugated with the nanoparticles did not show any enhanced effect during the first 24 h of incubation (Fig. 4). The anti-amoebic effect was revealed just after 48 h of incubation. The detailed results are shown in Table 2.

Compared to the control culture (Fig. 5a), 6 h of incubation with AgTANPs did not influence the morphology and the viability of the amoebae at this level of microscopic observation. Incubation with SCA caused decreased mobility of the trophozoites. Observed acanthopodia were less extensive compared to the control cultures. Fragments of the disrupted cells were visualized between viable trophozoites (Fig. 5a). After 6 h of incubation with AgTANPs conjugated with SCA, morphological degeneration of the trophozoites developed (Fig. 5a). The size of the cells and the number of visible acanthopodia were lower compared to the assays illustrated in Fig. 5a, b and c. There were more disrupted cell fragments visualized. Some trophozoites started developing into rounded forms.

Cytotoxicity

The overall cytotoxicity measured for SCA and O-F was similar and reached 36%. The cytotoxicity of ReNu reached 26%. Cytotoxicity values of nanoparticles conjugated with the contact lens solutions comparing to the pure contact lens solutions were not statistically significant. The cytotoxicity results are listed in Table 3.

Discussion

In the recent years cases of AK have been increasingly diagnosed worldwide. The available anti-amoebic therapies are not fully effective and often result in damaging cytotoxicity to the human eye. The main key predisposing factor for AK is contact lens use. Effective contact lens disinfection is the best approach to minimize the number of AK incidences. In this study, we tested multipurpose contact lens disinfecting systems containing different active ingredients but characterized by similar mode of action, resulting in cell membrane perturbation (Table 1). Our results confirmed the lack of amoebicidal activity for all tested

multipurpose contact lens solutions against *Acanthamoeba* strain in accordance with other publications revealing that the disinfecting capabilities of current contact lens solutions are insufficient [11,12,30-33].

Rapid developments in nanotechnology have significantly improved the anti-microbial potential of nanoparticles, especially silver nanoparticles (AgNPs) [14,34,35]. The specific mechanism of action of AgNPs is still not entirely understood; however, recent studies conducted on bacteria, shed more light on this process. We know that nanoparticles cause damage to the cell membrane. Adhesion of the nanoparticles based on the electrostatic attraction of the negatively charged cell membrane and positively or less negatively charged nanoparticles. The interaction decreases Zeta potential and depolarizes the cell membrane. The process leads to a disruption of membrane permeability and an alteration of the respiratory functions of the cell, eventually leading to a disruption of the cell integrity [36]. After crossing the cell membrane, nanoparticles can interact with DNA, RNA and proteins altering both transcription and translation processes. The presence of nanoparticles in the cell causes oxidative stress and disruption of enzymatic pathways result from the free radicals. Altogether, nanoparticles cause cytotoxic effects and finally lead to cell death. Cytotoxicity of AgNPs depends on their physico-chemical properties such as size and density. Typically, smaller nanoparticles have a relatively increased stability and enhanced anti-microbial activity. Similarly, higher concentrations of nanoparticles show increased anti-microbial activity. However, this property is strictly correlated to the tested microbial species and the type of nanoparticles used. The shape of the nanoparticles has not been proved to be a crucial factor influencing the anti-microbial activity. Some authors showed that truncated triangular or similar geometries such as hexagonal and octahedral shape of the AgNPs are more effective against bacteria while other authors reported that the shape of AgNPs does not have any influence on their activity [36-38]. Recent publications showed that nanoparticles can prolong the ocular retention of some topical drugs, thus enabling treatment of eye diseases using reduced drug dosages [39,40]. It was confirmed that nanoparticles coated on the contact lenses caused significant reduction in microbial colonization on the contact lens surface [41]. Contact lenses impregnated with AgNPs, after 6 h of incubation, did not exhibit desirable anti-bacterial activity against *Staphylococcus aureus* while demonstrated excellent anti-bacterial effects against *Pseudomonas aeruginosa* [42]. Silver-impregnated lens cases showed lower proportion of microbial contamination compared to the control cases. Most microorganisms isolated from silver-impregnated cases were members of the normal skin flora [43].

There are just a few studies that examined nanoparticles influence against *Acanthamoeba* spp. Cobalt nanoparticles have been studied for their anti-amoebic potential and confirmed that hexagonal microflakes showed the most promising anti-*Acanthamoeba* effects compared to nanoflakes and granular cobalt nanoparticles. Apart from the concentration and size, the composition and morphology of the tested noncompounds also determine their anti-amoebic activity [44,45]. AgNPs are well absorbed by the *Acanthamoeba* trophozoites and integrated in the cell matrix. The nanoparticles decrease trophozoites viability and alter their metabolic activity on the dose dependent manner [46]. In our previous studies we confirmed that AgNPs conjugated with the contact lens solutions showed dose dependent enhanced anti-amoebic activity [47]. Recently published studies confirmed enhanced anti-microbial effect of silver and gold (AuNPs) nanoparticles conjugated with commonly used drugs like chlorhexidine,

fluconazole or amphotericin B as well as with some disinfectants [27,48]. Guanabenz, a drug already approved for hypertension that crosses the blood-brain barrier, conjugated with AuNPs and AgNPs showed significant anti-amoebic activity against both *A. castellanii* and *Naegleria fowleri*. Significant reduction in the host cell cytopathogenicity, especially for silver nanoconjugates, was revealed and associated with negligible cytotoxicity against human cells [49].

In the 21st century, eco-friendly and cost-effective bio-nanotechnology techniques are used to prepare anti-microbial active conjugates as potential candidates to eradicate infections and reduce microbial contaminations of a healthcare equipment including contact lenses. The integration and conjugation of bioactive agents into nanomaterials were tested mainly for their anti-bacterial activities. Green synthesis of AgNPs, AuNPs and platinum (PtNPs) nanoparticles showed enhanced anti-bacterial activity after combining with different classes of antibiotics [50]. Biosynthesis of AgNPs with the plant extract of *Salvia spinosa* resulted in increased bactericidal activity against Gram-positive and Gram-negative bacteria [51]. Novel conjugates using biogenic AgNPs from *Convolvulus arvensis* extract and chitosan showed anti-microbial, anti-biofilm, and anti-cancer potentialities [52]. Extract of *Oscillatoria limnetica* conjugated with silver nanoparticles exhibited strong anti-bacterial activity against multidrug-resistant bacteria as well as cytotoxic effects against both human breast cancer cell line and human colon cancer cell line [53]. Synthesis of silver chloride nanoparticles (AgCl-NPs), using walnut green husk extract as well as silver nanoparticles with *Peganum harmala* L. leaf extract resulted in significant inhibitory effects against *Escherichia coli* and *S. aureus* clinical isolates [54,55]. Bio-nanotechnology has been not studied on protozoan species extensively. There are just a few published studies focusing on the influence of nanoparticles conjugated with plants extracts on amoebae. Studies performed on *Jatropha curcas*, *Jatropha gossypifolia* and *Euphorbia milii* extracts combined with nanoparticles exhibited significant reduction of the *Acanthamoeba* trophozoites with low cytotoxic effect to the human cells [25]. In our previous studies we confirmed that tannic acid-modified silver nanoparticles showed increased anti-amoebic activity and less cytotoxicity to the human cells in comparison to the pure silver nanoparticles [24]. In this study we revealed that tannic acid-modified silver nanoparticles conjugated with the contact lens solutions exhibited even better anti-amoebic activity in relation to the cytotoxicity than in the results obtained in our previous studies where we tested pure silver nanoparticles conjugates [47]. We conclude that differences in the anti-amoebic activity of the tested conjugates may be mainly driven by the anti-amoebic activity of the pure contact lens solutions. Nanoparticles in the tested concentration seem to enhance the already existing anti-amoebic potential of the selected contact lens solution.

Conclusions

In this study we showed dose dependent enhanced anti-amoebic effect of the tannic acid-modified silver nanoparticles (AgTANPs) conjugated with SCA and ReNu solutions against *Acanthamoeba* T4 strain. The promising results were obtained within the minimal disinfection time recommended by the manufacturers (6 h) and without increased toxicity to the human cells. Summarizing, conjugation of the selected contact lens solutions with AgTANPs might be a promising approach to prevent *Acanthamoeba* keratitis

infections among the contact lens users. Nevertheless, further studies should be conducted to elucidate the stability of the conjugation and activity against *Acanthamoeba* spp. cysts.

Abbreviations

AK: *Acanthamoeba* keratitis, AgTANPs: tannic acid-modified silver nanoparticles, NPs: nanoparticles, SCA: Solo Care Aqua, O-F: Opti-Free, ReNu: ReNu MultiPlus, SD: standard deviation, AgNPs: silver nanoparticles, AuNPs: gold nanoparticles, PtNPs: platinum nanoparticles, AgCl-NPs: silver chloride nanoparticles

Declarations

Acknowledgements

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the grant NZI/PM1/18 of the Medical University of Warsaw. JLM and JEP were funded by PI18/01380 from Instituto de Salud Carlos III, Spain and RICET [RD16/0027/0001 project, from Programa Redes Temáticas de Investigación Cooperativa, FIS (Ministerio Español de Salud, Madrid, Spain).

Authors' contributions

Data curation: EBH, MP and AZ. Formal analysis: EBH, MP, AZ and J.H. Funding acquisition: MP, JEP and JLM. Investigation: EBH and AZ. Methodology: EBH, MP, AZ, MG, JH, JG, KRS and JLM. Project administration: MP, GO and JLM. Resources: all authors. Supervision: MP, and JLM. Writing (original

draft): MP, EBH, JEP and JLM. Writing (all authors). Review and editing: MP, EBH and JLM. All authors read and approved the final manuscript.

References

1. Martinez AJ, Visvesvara GS. Free-living, amphizoic and opportunistic amebas. *Brain Pathol.* 1997;1:583-98.
2. Chomicz L, Padzik M, Graczyk Z, Starosciak B, Graczyk TK, Naprawska A, et al. *Acanthamoeba castellanii*: *in vitro* effects of selected biological, physical and chemical factors. *Exp Parasitol.* 2010;1:103-5.
3. Lorenzo-Morales J, Khan NA, Walochnik J. An update on *Acanthamoeba* keratitis: diagnosis, pathogenesis and treatment. *Parasite.* 2015;22:10.
4. Walochnik J, Scheikl U, Haller-Schober EM. Twenty years of *Acanthamoeba* diagnostics in Austria. *J Eukaryot Microbiol.* 2015;1:3-11.
5. Padzik M, Hendiger E, Szaflik J, Chomicz L. Amoebae of the genus *Acanthamoeba* – pathological agents in humans. *Postepy Mikrobiologii.* 2017;56:429-39.
6. Clarke B, Sinha A, Parmar DN, Sykakis E. Advances in the diagnosis and treatment of *Acanthamoeba* keratitis. *J Ophthalmol.* 2012;2012:484892.
7. Trabelsi H, Dendana F, Sellami A, Sellami H, Cheikhrouhou F, Neji S, et al. Pathogenic free-living amoebae: epidemiology and clinical review. *Pathol Biol (Paris).* 2012;6:399-405.
8. Lorenzo-Morales J, Martin-Navarro CM, Lopez-Arencibia A, Arnalich-Montiel F, Pinero JE, Valladares B. *Acanthamoeba* keratitis: an emerging disease gathering importance worldwide?. *Trends Parasitol.* 2013;4:181-87.
9. Chomicz L, Conn DB, Padzik M, Szaflik JP, Walochnik J, Zawadzki PJ, et al. Emerging threats for human health in Poland: pathogenic isolates from drug resistant *Acanthamoeba* keratitis monitored in terms of their *in vitro* dynamics and temperature adaptability. *Biomed Res Int.* 2015;2015:231285.
10. Lonnen J, Kilvington S, Lam A, Heaselgrave W. 58 Biocidal efficacy of multipurpose contact lens disinfectant solutions against *Acanthamoeba* species. *Cont Lens Anterior Eye.* 2011;34:S30.
11. Padzik M, Chomicz L, Szaflik JP, Chruscikowska A, Perkowski K, Szaflik J. *In vitro* effects of selected contact lens care solutions on *Acanthamoeba castellanii* strains in Poland. *Exp Parasitol.* 2014;145 Suppl:S98-S101.
12. Niyyati M, Sasani R, Mohebbali M, Ghazikhansari M, Kargar F, Hajjalilo E, et al. Anti-*Acanthamoeba* effects of silver and gold nanoparticles and contact lenses disinfection solutions. *Iran J Parasitol.* 2018;2:180-5.
13. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci.* 2004;1:177-82.
14. Rzeszutek J, Matysiak - Kucharek M, Czajka M, Sawicki K, Rachubik P, Kruszewski M, et al. Application of nanoparticles and nanomaterials in medicine. *Hygeia Public Health.* 2014;49:449-57.

15. Said DE, Elsamad LM, Gohar YM. Validity of silver, chitosan, and curcumin nanoparticles as anti-*Giardia* agents. *Parasitol Res.* 2012;2:545-54.
16. Saad HA, Soliman MI, Azzam AM, Mostafa B. Antiparasitic activity of silver and copper oxide nanoparticles against *Entamoeba histolytica* and *Cryptosporidium parvum* cysts. *J Egypt Soc Parasitol.* 2015;3:593-602.
17. Ullah I, Cosar G, Abamor ES, Bagirova M, Shinwari ZK, Allahverdiyev AM. Comparative study on the antileishmanial activities of chemically and biologically synthesized silver nanoparticles (AgNPs). *3 Biotech.* 2018;2:98.
18. Sieniawska E. Activities of tannins - from *in vitro* studies to clinical trials. *Nat Prod Commun.* 2015;10:1877-84.
19. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. *Crit Rev Food Sci Nutr.* 1998;38:421-64.
20. Athar M, Khan WA, Mukhtar H. Effect of dietary tannic acid on epidermal, lung, and forestomach polycyclic aromatic hydrocarbon metabolism and tumorigenicity in Sencar mice. *Cancer Res.* 1989;21:5784-88.
21. Scalbert A, Monties B, Janin G. Tannins in wood: comparison of different estimation methods. *J Agric Food Chem.* 1989;5:1324-29.
22. Khan NS, Ahmad A, Hadi SM. Anti-oxidant, pro-oxidant properties of tannic acid and its binding to DNA. *Chemico-Biological Interactions.* 2000;3:177-89.
23. Haslam E. Vegetable tannins - lessons of a phytochemical lifetime. *Phytochemistry.* 2007;22-24:2713-21.
24. Padzik M, Hendiger EB, Chomicz L, Grodzik M, Szmidt M, Grobelny J, et al. Tannic acid-modified silver nanoparticles as a novel therapeutic agent against *Acanthamoeba*. *Parasitol Res.* 2018;11:3519-25.
25. Borase HP, Patil CD, Sauter IP, Rott MB, Patil SV. Amoebicidal activity of phytosynthesized silver nanoparticles and their *in vitro* cytotoxicity to human cells. *FEMS Microbiol Lett.* 2013;2:127-31.
26. Orłowski P, Tomaszewska E, Gniadek M, Baska P, Nowakowska J, Sokolowska J, et al. Tannic acid modified silver nanoparticles show antiviral activity in herpes simplex virus type 2 infection. *PLoS One.* 2014;9 8:e104113.
27. Aqeel Y, Siddiqui R, Anwar A, Shah MR, Khan NA. Gold nanoparticle conjugation enhances the antiacanthamoebic effects of chlorhexidine. *Antimicrob Agents Chemother.* 2015;3:1283-88.
28. Zielinska M, Sawosz E, Grodzik M, Wierzbicki M, Gromadka M, Hotowy A, et al. Effect of heparan sulfate and gold nanoparticles on muscle development during embryogenesis. *Int J Nanomedicine.* 2011;6:3163-72.
29. McBride J, Ingram PR, Henriquez FL, Roberts CW. Development of colorimetric microtiter plate assay for assessment of antimicrobials against *Acanthamoeba*. *J Clin Microbiol.* 2005;2:629-34.

30. Radford CF, Minassian DC, Dart JK. *Acanthamoeba* keratitis in England and Wales: incidence, outcome, and risk factors. *Br J Ophthalmol*. 2002;5:536-42.
31. Codling CE, Maillard JY, Russell AD. Aspects of the antimicrobial mechanisms of action of a polyquaternium and an amidoamine. *J Antimicrob Chemother*. 2003;5:1153-58.
32. Santodomingo-Rubido J, Mori O, Kawaminami S. Cytotoxicity and antimicrobial activity of six multipurpose soft contact lens disinfecting solutions. *Ophthalmic and Physiological Optics*. 2006;5:476-82.
33. Kal A, Toker MI, Kaya S. The comparison of antimicrobial effectiveness of contact lens solutions. *Int Ophthalmol*. 2017;5:1103-14.
34. Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. *Int J Nanomedicine*. 2007;2:129-41.
35. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*. 2007;22:225103.
36. Roy A, Bulut O, Some S, Mandal AK, Yilmaz MD. Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. *RSC Adv*. 2019;5:2673-702.
37. Yan X, He B, Liu L, Qu G, Shi J, Hu L, et al. Antibacterial mechanism of silver nanoparticles in *Pseudomonas aeruginosa*: proteomics approach. *Metallomics*. 2018;4:557-64.
38. Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A. Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells *in vitro*: a critical review. *Arch Toxicol*. 2013;7:1181-1200.
39. Behl G, Iqbal J, O'Reilly NJ, McLoughlin P, Fitzhenry L. Synthesis and characterization of Poly(2-hydroxyethylmethacrylate) contact lenses containing chitosan nanoparticles as an ocular delivery system for dexamethasone sodium phosphate. *Pharm Res*. 2016;7:1638-48.
40. Liu S, Dozois MD, Chang CN, Ahmad A, Ng DL, Hileeto D, et al. Prolonged ocular retention of mucoadhesive nanoparticle eye drop formulation enables treatment of eye diseases using significantly reduced dosage. *Mol Pharm*. 2016;9:2897-905.
41. Willcox MDP, Hume EBH, Vijay AK, Petcavich R. Ability of silver-impregnated contact lenses to control microbial growth and colonisation. *J Optometry*. 2010;3:143-48.
42. Fazly Bazzaz BS, Khameneh B, Jalili-Behabadi MM, Malaekheh-Nikouei B, Mohajeri SA. Preparation, characterization and antimicrobial study of a hydrogel (soft contact lens) material impregnated with silver nanoparticles. *Cont Lens Anterior Eye*. 2014;3:149-52.
43. Amos CF, George MD. Clinical and laboratory testing of a silver-impregnated lens case. *Cont Lens Anterior Eye*. 2006;5:247-55.
44. Anwar A, Chi Fung L, Anwar A, Jagadish P, Numan A, Khalid M, et al. Effects of shape and size of cobalt phosphate nanoparticles against *Acanthamoeba castellanii*. *Pathogens*. 2019;4:10.
45. Anwar A, Mungroo MR, Anwar A, Sullivan WJ, Khan NA, Siddiqui R. Repositioning of guanabenz in conjugation with gold and silver nanoparticles against pathogenic amoebae *Acanthamoeba*

- castellanii* and *Naegleria fowleri*. ACS Infect Dis. 2019;12:2039-46.
46. Grün A, Scheid P, Hauröder B, Emmerling C, Manz W. Assessment of the effect of silver nanoparticles on the relevant soil protozoan genus *Acanthamoeba*. J Plant Nutr Soil Sci. 2017;5:602-13.
47. Padzik M, Hendiger EB, Zochowska A, Szczepaniak J, Baltaza W, Pietruczuk-Padzik A, et al. Evaluation of *in vitro* effect of selected contact lens solutions conjugated with nanoparticles in terms of preventive approach to public health risk generated by *Acanthamoeba* strains. Ann Agric Environ Med. 2019;1:198-202.
48. Anwar A, Siddiqui R, Hussain MA, Ahmed D, Shah MR, Khan NA. Silver nanoparticle conjugation affects antiacanthamoebic activities of amphotericin B, nystatin, and fluconazole. Parasitol Res. 2018;1:265-71.
49. Anwar A, Numan A, Siddiqui R, Khalid M, Khan NA. Cobalt nanoparticles as novel nanotherapeutics against *Acanthamoeba castellanii*. Parasit Vectors. 2019;1:280.
50. Nishanthi R, Malathi S, John Paul S, Palani P. Green synthesis and characterization of bioinspired silver, gold and platinum nanoparticles and evaluation of their synergistic antibacterial activity after combining with different classes of antibiotics. Mater Sci Eng. 2019;96:693-707.
51. Pirtarighat S, Ghannadnia M, Baghshahi S. Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown *in vitro* and their antibacterial activity assessment. J Nanostructre Chem. 2019;1:1-9.
52. Bilal M, Zhao Y, Rasheed T, Ahmed I, Hassan STS, Nawaz MZ, et al. Biogenic nanoparticle chitosan conjugates with antimicrobial, antibiofilm, and anticancer potentialities: Development and Characterization. Int J Environ Res Public Health. 2019;4:10.
53. Hamouda RA, Hussein MH, Abo-Elmagd RA, Bawazir SS. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. Sci Rep. 2019;1:13071-y.
54. Kohan Baghkheirati E, Bagherieh-Najjar MB, Khandan Fadafan H, Abdolzadeh A. Synthesis and antibacterial activity of stable bio-conjugated nanoparticles mediated by walnut (*Juglans regia*) green husk extract. J Exp Nanosci. 2016;7:512-517.
55. Alomari AA, Kloub Fares KE, Moustafa NE. Green synthesis of assembled silver nanoparticles in nano capsules of *Peganum harmala* L. leaf extract. Antibacterial activity and conjugate investigation. Cogent Chemistry. 2018;4:1.

Tables

Table 1 Multipurpose contact lens solutions ingredients and minimum disinfection time recommended by the manufacturers

Manufacturer	Solution	Ingredients	Minimum disinfection time (h)
Bausch & Lomb	ReNu MultiPlus	HYDRANATE® (hydroxyalkylphosphonate) 0,03%, boric acid, edetate disodium, poloxamine 1%, sodium borate, sodium chloride preserved with DYMED™ (polyaminopropyl biguanide 0,0001%)	4
Alcon	Opti-Free (repleniSH)	TEARGLYDE® (TETRONIC® 1304, nonannoyl ethylenediaminetriacetic acid), POLYQUAD (Polyquaternium-1) 0,001%, ALDOX (Myristamidopropyl Dimethylamine) 0.0005%	6
Menicon	Solo Care Aqua	Polyhexanide 0,0001%, Hydrolock® (deksapenthenol, sorbitol), sodium phosphate, tromethamine, poloxamer 407, disodium edetate	4

Table 2 Anti-amoebic activity of the pure contact lenses solutions and tannic acid-modified silver nanoparticles conjugated with the contact lens care solutions after 6–96 h of incubation (% of inhibition).

	6 h	24 h	48 h	72 h	96 h
Solo Care Aqua (SCA)	31.95 ± 1.70	23.15 ± 3.93	37.68 ± 1.16	51.65 ± 2.75	47.15 ± 3.25
SCA + 2.5 ppm AGTANPs	61.18 ± 1.34	51.60 ± 8.50	59.79 ± 11.19	66.02 ± 5.42	61.9 ± 2.41
SCA + 1.25 ppm AGTANPs	61.67 ± 4.63	42.79 ± 19.26	52.03 ± 11.57	60.42 ± 4.25	54.50 ± 0.21
SCA + 0.5 ppm AGTANPs	54.91 ± 3.89	36.88 ± 20.08	47.33 ± 10.25	58.21 ± 3.07	72.77 ± 1.71
SCA + 0.25 ppm AGTANPs	52.49 ± 6.35	35.45 ± 10.65	45.35 ± 6.56	55.95 ± 1.15	51.31 ± 1.13
Opti Free (O-F)	No activity	No activity	No activity	35.74 ± 0.95	47.35 ± 2.75
O-F 2.5 ppm AGTANPs	31.11 ± 3.09	21.83 ± 4.85	44.79 ± 4.92	59.58 ± 1.14	57.72 ± 0.55
O-F + 1.25 ppm AGTANPs	No activity	4.50 ± 11.51	33.32 ± 4.38	52.78 ± 0.98	52.29 ± 0.24
O-F + 0.5 ppm AGTANPs	No activity	No activity	16.63 ± 3.70	41.62 ± 3.08	41.15 ± 2.46
O-F + 0.25 ppm AGTANPs	No activity	No activity	No activity	36.82 ± 1.69	36.74 ± 1.77
ReNu Multiplus (ReNu)	No activity	No activity	24.23 ± 4.88	43.99 ± 2.10	46.76 ± 0.64
ReNu 2.5 ppm AGTANPs	41.59 ± 2.18	36.63 ± 4.69	56.72 ± 3.58	67.45 ± 2.68	64.59 ± 2.98
ReNu + 1.25 ppm AGTANPs	39.58 ± 2.66	31.17 ± 5.28	50.07 ± 3.57	62.77 ± 2.79	58.85 ± 3.63
ReNu + 0.5 ppm AGTANPs	39.18 ± 0.87	22.60 ± 7.05	45.22 ± 5.33	61.90 ± 2.21	58.56 ± 3.39
ReNu + 0.25 ppm AGTANPs	32.99 ± 4.42	13.47 ± 0.14	43.52 ± 4.20	62.12 ± 7.68	57.55 ± 0.11

Abbreviations: SCA, Sole Care Aqua; O-F, Opti Free; ReNu, ReNu Multiplus

Table 3 Cytotoxicity of the contact lens solutions and the contact lens solutions conjugated with the tannic acid-modified silver nanoparticles - AgTANPs (%)

	Contact lens solution	+ 0.25 ppm AgTANPs	+ 0.5 ppm AgTANPs	+ 1.25 ppm AgTANPs	+ 2.5 ppm AgTANPs
Solo Care Aqua (SCA)	35.3	24.2	20.7	19.2	23.0
Opti Free (O-F)	36.0	32.5	24.3	26.9	33.0
ReNu Multiplus (ReNu)	26.2	20.1	18.5	15	25.5

Figures

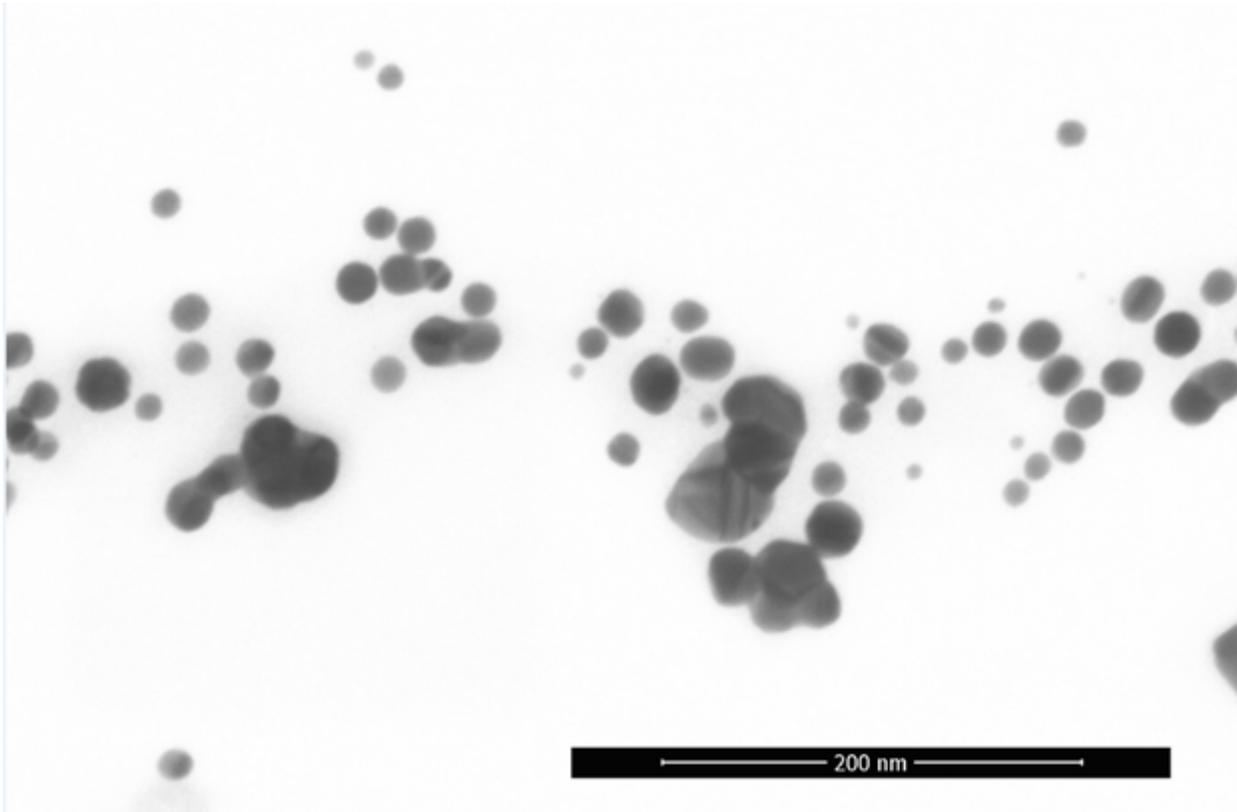


Figure 1

HR-STEM image of AgTANPs distribution and diameter

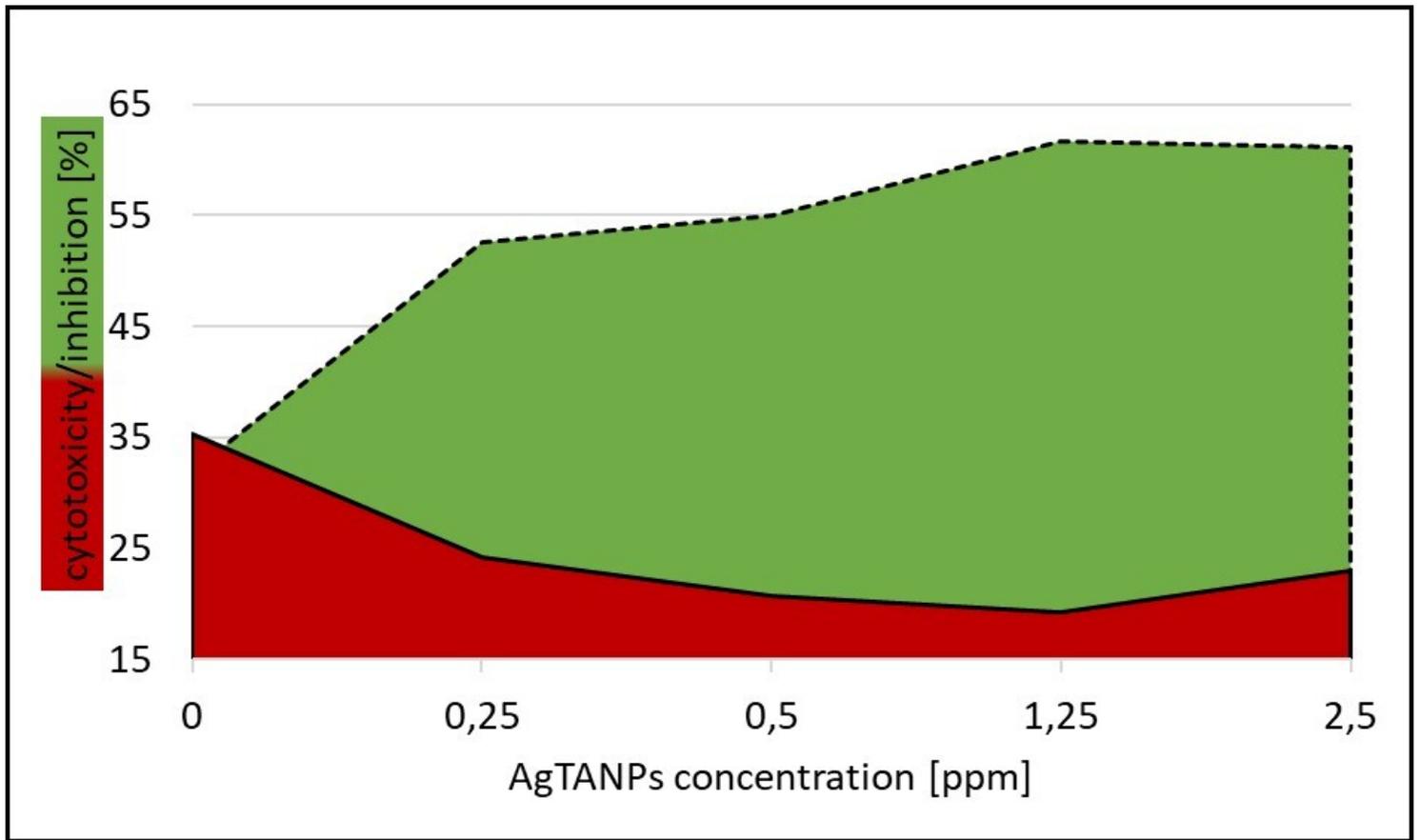


Figure 2

Anti-Acanthamoeba activity of AgTANPs conjugated with SCA contact lens solution after 6h of incubation in relation to cytotoxicity

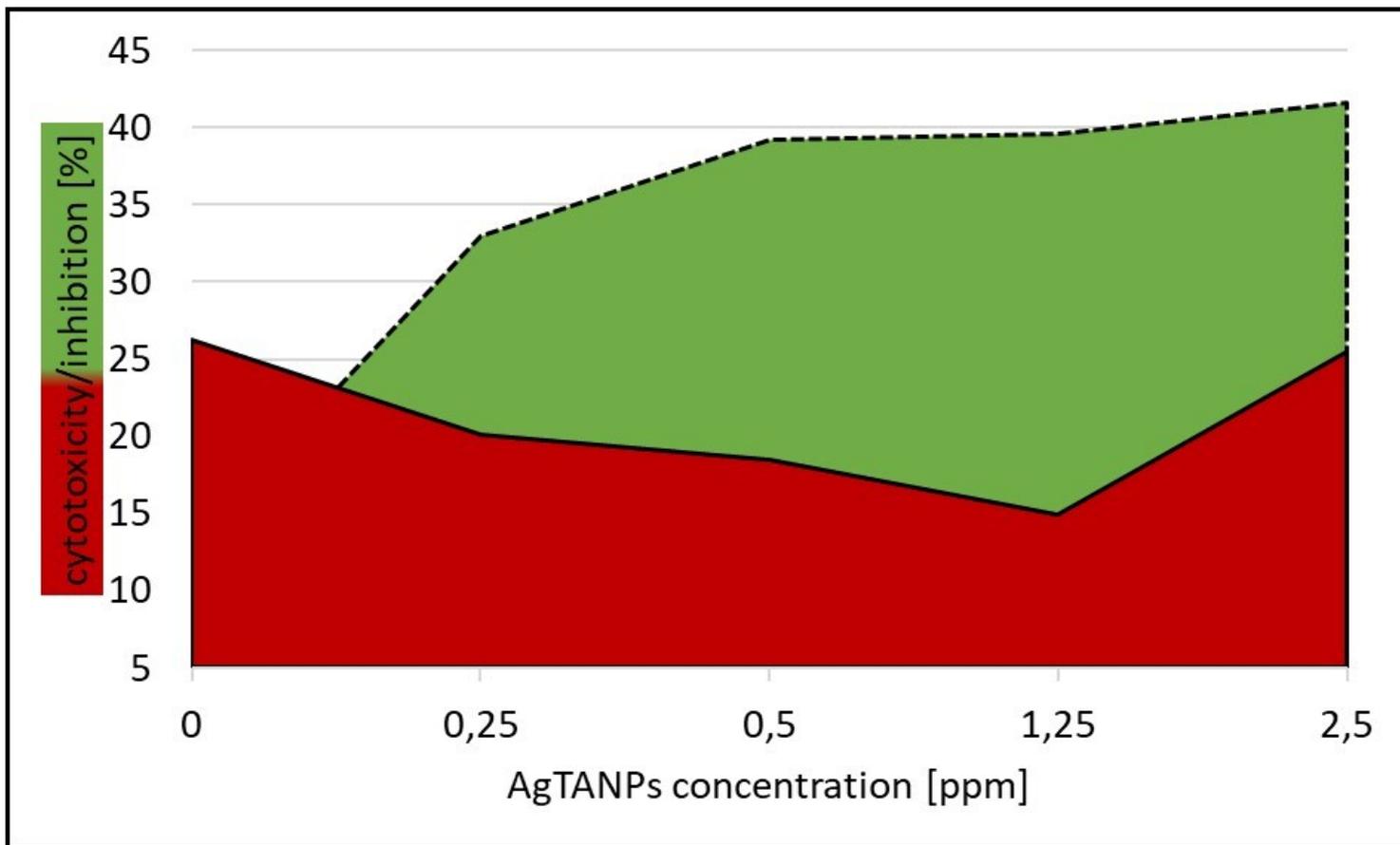


Figure 3

Anti-Acanthamoeba activity of AgTANPs conjugated with ReNu contact lens solution after 6h of incubation in relation to cytotoxicity

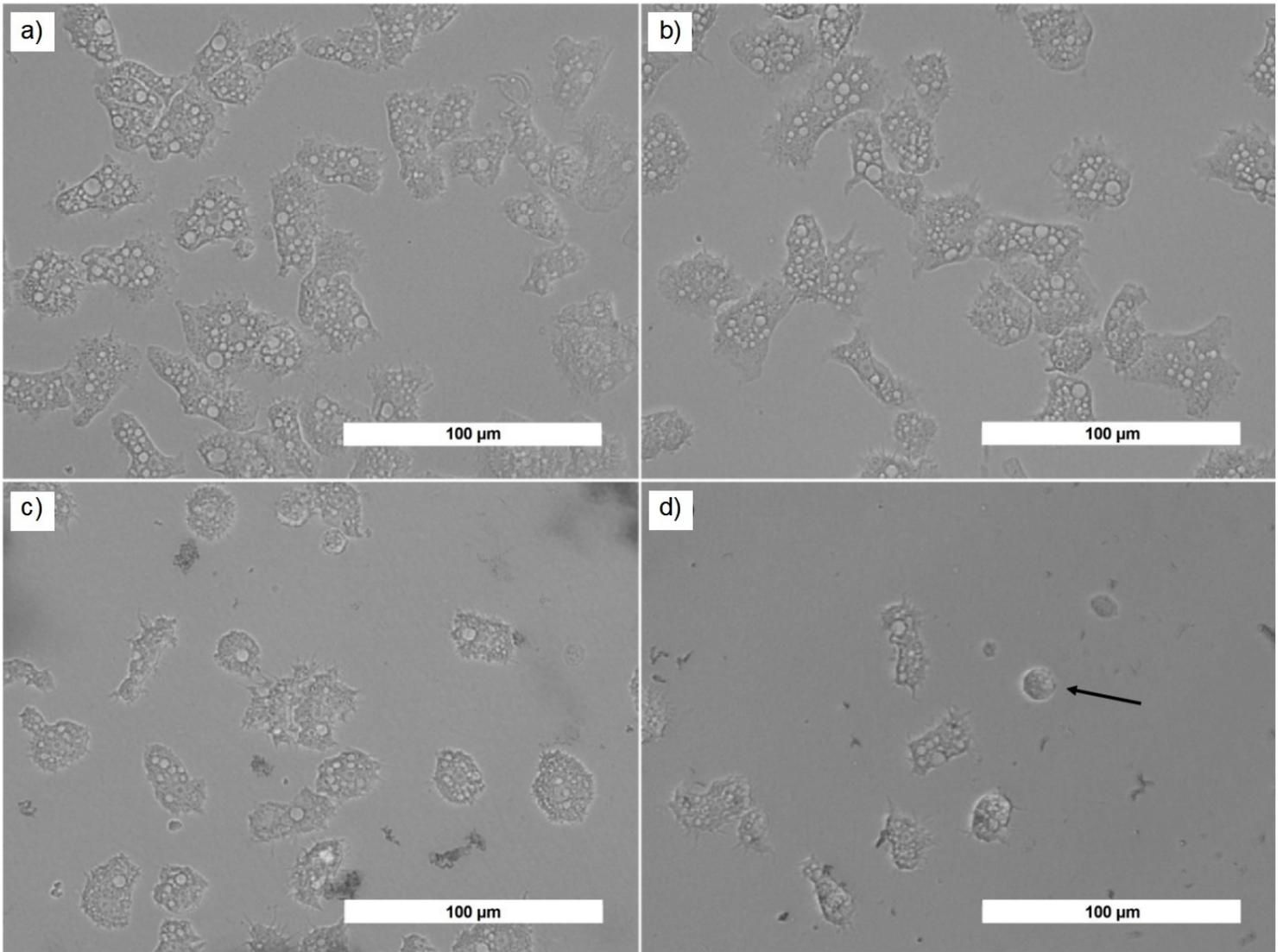


Figure 4

Acanthamoeba trophozoites after 6 h of incubation. a Control culture in PYG medium. b Incubation with AgTANPs. c Incubation with Solo Care Aqua. d incubation with AgTANPs conjugated with Solo Care Aqua. The arrow shows rounded form. All images ($\times 40$) represent of the population of treated amoebae and are based on the live cell imaging microscope EVOS FL cell imaging system

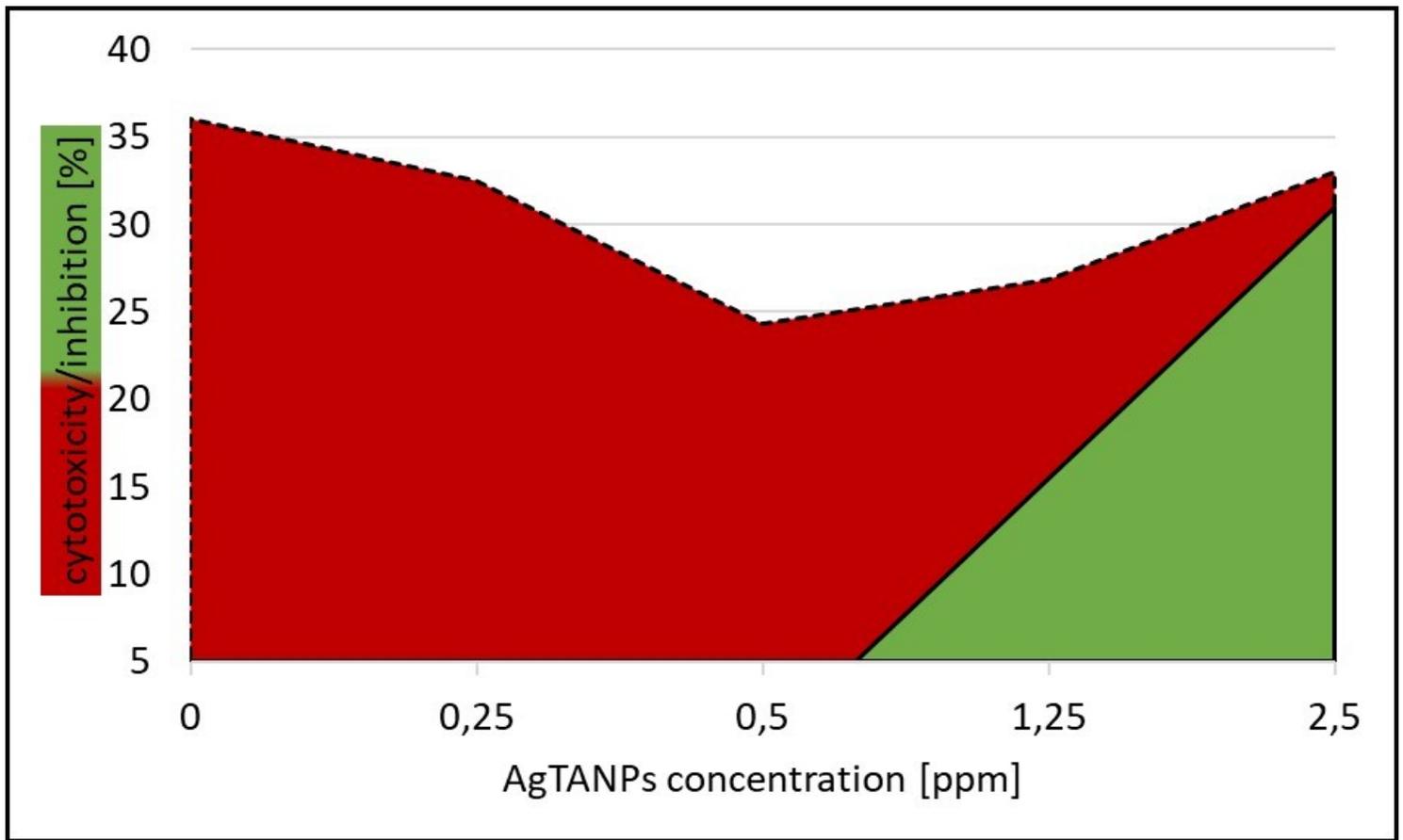


Figure 5

Anti-Acanthamoeba activity of AgTANPs conjugated with O-F contact lens solution after 6h of incubation in relation to cytotoxicity

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [graphicalabstract300dpi.tif](#)