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ORIGINAL ARTICLE

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

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Abstract

Introduction. Various *Acanthamoeba* species are free-living organisms widely distributed in the human environment. Amphizoic amoebae as facultative parasites may cause vision-threatening eye disease – *Acanthamoeba* keratitis, mostly among contact lens wearers. As the number of cases is increasing, and applied therapy often unsuccessful, proper hygienic measures and effective contact lenses disinfection are crucial for the prevention of this disease. Available contact lens solutions are not fully effective against amphizoic amoebae; there is a need to enhance their disinfecting activity to prevent amoebic infections. The use of developing nanotechnology methods already applied with success in the prevention, diagnostic and therapy of other infectious diseases might be helpful regarding amoebic keratitis. This study assesses the *in vitro* effect of selected contact lens solutions conjugated with nanoparticles against *Acanthamoeba* trophozoites.

Materials and method. Three selected contact lens solutions conjugated with silver and gold nanoparticles in concentration of 0.25–2.5 ppm were used *in vitro* against the axenically cultured ATCC 30010 type *Acanthamoeba castellanii* strain. The anti-amoebic efficacy was examined based on the oxido-reduction of AlamarBlue. The cytotoxicity tests based on the measurement of lactate dehydrogenase (LDH) activity were performed using a fibroblast HS-5 cell line.

Results. Enhancement of the anti-amoebic activity of contact lens solutions conjugated with selected nanoparticles expressed in the dose dependent amoebic growth inhibition with a low cytotoxicity profile was observed.

Conclusions. Results of the study showed that conjugation of selected contact lens solutions with silver nanoparticles might be a promising approach to prevent *Acanthamoeba* keratitis among contact lens users.

Key words

Acanthamoeba keratitis, contact lens solutions, silver/gold nanoparticles, *Acanthamoeba* trophozoites, anti-amoebic *in vitro* effect

INTRODUCTION

Protozoans of *Acanthamoeba* genus are free-living amoebae with various degrees of pathogenicity, ubiquitous in both natural and man-made environments. As amphizoic organisms – facultative human parasites, they may be transmitted from various environment sources to the ocular surface and cause progressive sight-threatening corneal infection – *Acanthamoeba* keratitis (AK) [1, 2, 3, 4, 5]. It is considered that wearing contact lenses, especially poor hygiene while cleaning and using the lenses and their cases, corneal damages, eye exposure to water or moist soil in which *Acanthamoeba* forms exist, are among the main reported risk factors for AK. Moreover, the lack of specific symptoms and the common occurrence of mixed infections result in diagnostic difficulties, and therefore a delay of adequate treatment. Current therapeutic approaches are mainly limited to prolonged application of diamides and biguanides which is often unsuccessful and highly toxic to the eye. Only prevention measures, including proper eye hygiene and effective contact lens disinfection are efficacious approaches to limit incidences of AK [1, 6, 7, 8, 9].

It is widely known that *Acanthamoeba* trophozoites are able to attach themselves to the surface of both contact lens cases and contact lenses. Previous studies have shown that the most popular multipurpose contact lens disinfection systems, commonly based on anti-microbial and anti-fungal agents, are not fully effective against *Acanthamoeba*. Therefore, there is an urgent need to broaden research in order to develop contact lens solutions with improved anti-amoebic activity [10, 11, 12].

At present, synthesized nanoparticles have been proposed as a new generation of anti-microbial, anti-viral and anti-fungal agents [13, 14], and nanoparticles activity against different protozoans, such as *Giardia intestinalis, Entamoeba histolytica*, *Cryptosporidium parvum* and *Leishmania* spp. has

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been already confirmed [15, 16, 17]. It has been demonstrated in previous studies in our laboratory that silver and gold nanoparticles were well absorbed and showed activity against *Acanthamoeba* strains belonging to the T4 genotype [18]. Other authors have also confirmed that nanoparticles may enhance the anti-amoebic effect of biguanides, for example, chlorhexidine digluconate and other compounds [19, 20, 21].

OBJECTIVE

This study aimed to investigate and evaluate the activity of selected multipurpose contact lens solutions conjugated with silver and gold nanoparticles against the trophozoite stage of *Acanthamoeba*, as well as to test the induced cytotoxicity of these conjugates.

MATERIALS AND METHOD

***Acanthamoeba* strain included – molecular identification and cultivation.** *A. castellanii* strain belonging to *Acanthamoeba* group II, monitored in the Department of Medical Biology at the Medical University in Warsaw, Poland, was identified using molecular techniques based on genotype associations of the 18S rRNA gene sequence as ATCC 30010 type *A. castellanii* Neff T4 genotype, was included in this study.

The protists were *in vitro* cultured axenically in 25 cm^2 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. The *Acanthamoeba* populations investigated were sub-cultured twice a month and their samples regularly observed for their *in vitro* growth under light microscope. Amoebae were regularly sub-cultured twice a month and observed for their growth under light microscope using the Bürker haemocytometer.

Contact lens disinfecting solutions examined. The three multipurpose solutions included in the study represent the most common types of solutions used for contact lens care in Poland, namely: Solo Care Aqua, Menicon (**SCA**), ReNu MultiPlus, BAUSCH+LOMB (**ReNu**), Opti-Free, repleniSH (**O-F**); all tested solutions were purchased from authorized agents.

The characteristics of contact lens disinfecting solutions applied in this study, with the data about their components and minimum disinfection time recommended by manufacturers, are presented in Table 1.

Nanoparticles. Nanoparticles used in this study were kindly provided by the Department of Animal Nutrition and Food Science, Warsaw University of Life Sciences. The hydrocolloids of silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) were produced by an electric non-explosive method from high purity metals and de-mineralized water (Polish Patent 380649) obtained from Nano-Tech, Warsaw, Poland. The long-term stability of the colloidal dispersions of all tested NPs (zeta potential) was measured and confirmed by electrophoretic light-scattering method with a Zetasizer Nano ZS, model ZEN3500 (Malvern Instruments, Worcs., UK) [21, 22]. The well-dispersed nanofluids were used as a stock solution and appropriately diluted to various nanoparticles concentrations (ppm), ranging between 0.25–2.5 ppm, and used in subsequent assays of the cytotoxicity and anti-amoebic activity.

Table 1. Characteristics of contact lens disinfecting solutions included in this study

Contact lens care solutions	Components of the solution	Minimum disinfection time recommended
Solo Care Aqua	Polyhexanide 0.0001%, Hydrolock® (sorbitol, deksapenthenol), sodium phosphate, tromethamine, poloxamer 407, disodium edetate	4 h
ReNu MultiPlus	HYDRANATE® (hydroxyalkylphosphonate) 0.03%, boric acid, edetate disodium, poloxamine 1%, sodium borate, sodium chloride preserved with DYMED™ (polyaminopropyl biguanide 0.0001%)	4 h
Opti-Free	TEARGLYDE® (TETRONIC® 1304, nonannoyl ethylenediaminetriacetic acid), POLYQUAD (Polyquaternium-1) 0.001%, ALDOX (Myristamidopropyl Dimethylamine) 0.0005%	6 h

Cytotoxicity assays. The fibroblast HS-5 (ATCC CRL-11882) cell line was used in the cytotoxicity assays, as described in our previous studies [18]. 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.

Fibroblasts were incubated with each contact lens solution separately, and contact lens solution + nanoparticles added in the same concentration as in the activity assays. To calculate the % of cytotoxicity, absorbance was measured at 490 nm and 680 nm.

Anti-amoebic activity assays. The pure contact lens solutions and contact lens care solutions conjugated with nanoparticles at concentrations 0.25, 0.5, 1.25 and 2.5 ppm were examined *in vitro* and assessed for their anti-amoebic activity. The protists from log growth phase, six days after sub-culturing were examined. To determine the anti-amoebic efficacy on trophozoites, the previously described [23] colorimetric 96-well microtitre plate assay, based on the oxido-reduction of AlamarBlue, was used. Subsequently, the plates were analyzed over a period of 6x h, 24 h, 48 h, 72 h and 96 h in a 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; results of the investigations were analyzed statistically (ANOVA, Student-Newman-Keuls); the level of statistical significance was set at $p < 0.05$.

RESULTS

Cytotoxicity assays measured for pure contact lens solutions showed similar results for SCA and O-F and reached 36%, while for ReNu the cytotoxicity was lower and reached 26%. (Tab. 2). Results of the assays performed for the assessment of a cytotoxic effect of contact lens solutions conjugated with silver or gold nanoparticles on the fibroblasts were variable and dependent on tested concentrations. Comparison of

Table 2. Cytotoxicity of pure contact lens solutions and contact lens solutions conjugated with silver nanoparticles [%]

	pure contact lens solution	+ 0.25 ppm AgNPs	+ 0.5 ppm AgNPs	+ 1.25 ppm AgNPs	+ 2.5 ppm AgNPs
Solo Care Aqua	35.3	40.49	38.41	32.26	32.21
ReNu MultiPlus	26.2	32.78	32.17	38.18	27.34
Opti-Free	36	40.33	37.86	38.2	39.5

Statistically significant differences in cytotoxicity of pure contact lens solutions in relation to data regarding solutions conjugated with silver nanoparticles are underlined

Table 3. Cytotoxicity of pure contact lens solutions and contact lens solutions conjugated with gold nanoparticles [%]

	pure contact lens solution	+0.25 ppm AuNPs	+0.5 ppm AuNPs	+1.25 ppm AuNPs	+2.5 ppm AuNPs
Solo Care Aqua	35.3	40.34	39.7	32.38	27.31
ReNu MultiPlus	26.2	32.04	31.61	37.96	26.14
Opti-Free	36	39.68	36.97	37.2	38.71

Statistically significant differences in cytotoxicity of pure contact lens solutions in relation to data regarding solutions conjugated with gold nanoparticles are underlined

cytotoxicity of pure contact lens disinfectant solutions and particular solutions conjugated with nanoparticles is presented in Tables 2 and 3.

In the activity assays, the clear anti-amoebic effect expressed in 32% amoebic growth inhibition was observed after 6 h of incubation with pure SCA. This favourable effect was confirmed after 24, 48, 72 and 96 h incubation. On the other hand, O-F and ReNu did not indicate an effect against *Acanthamoeba* after 6 and 24 h incubation; however, an anti-amoebic activity was observed at 48, 72 and 96 h incubation.

SCA conjugated with both AgNPs and AuNPs showed enhanced, dose dependent anti-amoebic effect. Furthermore, the highest effect with low cytotoxicity was achieved after 6 h incubation, which is nearly the minimum disinfection time recommended by manufacturers of the contact lens solutions. The enhanced anti-amoebic effect was prolonged up to 96 h incubation only for SCA + AgNPs. For SCA + AuNPs; the synergistic effect disappeared after 24 h incubation (Tab. 4).

ReNu showed the enhanced, dose dependent anti-amoebic effect only conjugated with AgNPs; ReNu conjugated with AuNPs did not show any synergistic anti-amoebic effect. O-F did not show any improved anti-amoebic activity conjugated with both types of nanoparticles

Table 4. Monitoring of anti-amoebic effect of pure contact lens solutions and contact lens solutions conjugated with silver or gold nanoparticles

solutions	dose dependent amoebic growth inhibition after incubation		duration of anti-amoebic effect after incubation
	6 h	48 h	
Solo Care Aqua	32%	37.5%	6–96 h
Solo Care Aqua + AgNPs	37%–55%	37.3%–47%	6–96 h
Solo Care Aqua + AuNPs	35%–46.5%	39%–42%	6–24 h
ReNu Multi Plus	14%	24%	48–96 h
ReNu Multi Plus + AgNPs	32%–42%*)	29%–44%	6–96 h
ReNu Multi Plus + AuNPs	26%–37%*)	27%–40%	6–24 h

* increased cytotoxic effect on the fibroblasts after 24 h; level of statistical significance set at $p < 0.05$; statistically significant differences in data regarding particular contact lens solutions and contact lens solutions conjugated with nanoparticles are underlined

Results of monitoring of anti-amoebic effect of pure contact lens solutions SCA and ReNu, and these contact lens solutions conjugated with silver or gold nanoparticles illustrating the dose dependent amoebic growth inhibition after 6 h and 48 h incubation and the duration of the effect after further incubation, are presented in Table 4.

DISCUSSION

Different strains of *Acanthamoeba* sp. play an important role in public health as causative agents of the vision-threatening corneal disease *Acanthamoebic* keratitis. Wearing contact lenses is an important risk factor in contracting the eye disease, and due to the popularity of contact lenses, and the to-date insufficient knowledge and awareness of their ubiquitous in human environments, AK incidents are increasingly recognized in various parts of the world, including Poland. Current therapies against AK are not fully effective and often require a lengthy treatment process which results in serious side effects due to high cytotoxicity. Among the risk factors for AK, apart from contact lens use, the corneal trauma as well as poor hygiene in the handling of contact lenses and their cases are listed [1]. In addition, a low activity against *Acanthamoeba* has been reported in most of the commonly used contact lens solutions [10, 11, 24, 25]. This last observation was confirmed by the results of the current study, since none of the tested contact lens solutions showed amoebicidal activity.

Progress in the development of nanotechnology resulted in an increased use of different nanoparticles as new antimicrobial, anti-fungal, anti-protozoal and anti-viral agents [13, 26, 27]. However, despite rapid advance in nanotechnology, only a few studies on the activity of nanoparticles against *Acanthamoeba* spp. have been previously performed and have shown that the tested nanoparticles are well absorbed by *Acanthamoeba* trophozoites and decreased their adherence ability and metabolic activity [18, 28, 29, 30]. However, the mechanism of action of AgNPs and AuNPs is still not fully understood. Nevertheless, according to previous studies conducted on bacteria, both types of nanoparticles cause cell membrane damages, inhibit DNA and RNA replication, deplete the levels of intercellular ATP and induce oxidative damages [31, 32, 33]. However, previous studies have shown promising effects of silver and gold nanoparticles against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and fungal corneal infections [14, 34]. The synergistic antimicrobial effect of nanoparticles conjugation with antibiotics and as well as disinfectants, e.g. chlorhexidine, has also been confirmed [19, 30]. With regard to amoebae, previous study has reported that AgNPs conjugated with plant extracts caused significant reduction of the *Acanthamoeba* trophozoites with low cytotoxic effect to human cells [18, 20]; additionally, nanoparticles coated on the surface of contact lenses caused a significant reduction in microbial colonization [29, 35, 36].

Contact lens solutions used in this study belong to multipurpose contact lens systems with different active ingredients. Nevertheless, all of them caused the formation of acid phospholipids that result in cell membrane perturbation [37, 38].

The current study shows that AgNPs conjugation with SCA and Renu, as well as AuNPs conjugation with SCA, enhanced anti-amoebic *in vitro* effect against *Acanthamoeba*

trophozoites within 6 h of incubation, the minimal disinfection time recommended by manufacturers. However, the enhanced anti-amoebic effect with favourable relation to the cytotoxicity was achieved only for AgNPs + SCA (Tab. 4). This conjugation acted in a dose-depended manner for the whole period of incubation. In other tested conjugations, the anti-amoebic effect of nanoparticles and contact lens solutions was lower than either overall cytotoxicity caused by the conjugation and/or anti-amoebic effect caused be the contact lens solution. The lowest activity against *Acanthamoeba* trophozoites in relation to the other solutions was showed for O-F, regardless if used alone or conjugated with nanoparticles.

The results obtained in this study suggest that the anti-amoebic synergistic effect of the conjugation might be related to the mechanism or level of disintegration of the cell membrane by contact lens solution, which influences the bioactivity of the nanoparticles. Based on the results of this study, and according to other studies conducted on bacteria, protozoans and fungi, it seems that silver nanoparticles have better antimicrobial potential than gold nanoparticles [14, 18, 29].

CONCLUSIONS

Results of the presented study show that conjugation of selected contact lens solutions with nanoparticles might effectively enhance their anti-amoebic activity. Moreover, AgNPs may be considered as a potential ingredient to use in contact lens solutions that increases their anti-amoebic activity, without increased toxicity to the human cells. Nevertheless, further studies should be conducted to elucidate the mechanism of action and activity of this conjugation against *Acanthamoeba* cysts. The study results show that conjugation of selected contact lens solutions with silver nanoparticles might be a promising approach to prevent *Acanthamoeba* keratitis among contact lens users.

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