Colloids and Surfaces B: Biointerfaces 129 (2015) 107–113

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

# Hybridization of different antisense oligonucleotides on the surface of gold nanoparticles to silence zinc metalloproteinase gene after uptake by *Leishmania major*

Ali Jebali [a], Mohammad Hosssein Anvari-Tafti [b,*]

[a] Department of Laboratory Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[b] Department of Pathobiology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

ARTICLE INFO

*Article history:*
Received 16 January 2015
Received in revised form 6 March 2015
Accepted 10 March 2015
Available online 18 March 2015

*Keywords:*
Antisense oligonucleotide
Gold nanoparticles
Zinc metalloproteinase
Uptake
*Leishmania major*

ABSTRACT

The use of antisense oligonucleotides is a novel strategy to treat infectious diseases. In this approach, vital mRNAs are targeted by antisense oligonucleotides. The aim of this study was to evaluate the effects of gold nanoparticles hybridized with different antisense oligonucleotides on *Leishmania (L) major*. In this project, gold nanoparticles were first synthesized, and then conjugated with primary oligonucleotides, 3′-AAA-5′. Next, conjugated gold nanoparticles (NP1) were separately hybridized with three types of antisense oligonucleotide from coding reign of GP63 gene (NP2), non-coding reign of GP63 gene (NP3), and both coding and non-coding reigns of GP63 (NP4). Then, 1 mL of *L. major* suspension was separately added to 1 mL of different hybridized gold nanoparticles at serial concentrations (1–200 μg/mL), and incubated for 24, 48, and 72 h at 37 °C. Next, the uptake of each nanoparticle was separately measured by atomic absorption spectroscopy. After incubation, the cell viability was separately evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay. Also, the expression of GP63 gene was read out by quantitative-real-time PCR. This study showed that NP2 and NP3 had higher (5-fold) uptake than NP1 and NP4. Moreover, NP2 and NP3 led to less cell viability and gene expression, compared with NP1 and NP4. It could be concluded that both sequence and size of antisense oligonucleotide were important for transfection of *L. major*. Importantly, these antisense oligonucleotides can be obtained from both coding and non-coding reign of GP63 gene. Moreover, hybridized gold nanoparticles not only could silence GP63 gene, but also could kill *L. major*.



## 1. Introduction

Cutaneous leishmaniasis (CL) is a complicated disease, caused by *Leishmania* (*L*) *major* and *Leishmania tropica*. Nowadays, efficient vaccine and drug are not available for CL [1]. The use of antisense technology is a new approach to treat infectious diseases, by means of microRNA, small hairpin RNA (shRNA), small interfering RNA (siRNA), antisense DNA oligonucleotides (oligos), and antisense plasmid [2]. Although these biomolecules can effectively knock-down vital genes, they need specific carriers, e.g. viral and non-viral, for transfection. Generally, non-viral carriers, e.g. cationic lipids, polymers, peptides, and dendrimers are more applied. Meanwhile, nano-sized carriers, such as carbon nanotube, iron oxide, silica, gold, and chitosan nanoparticles, are another choices [3]. Because of higher physical and chemical properties of gold nanoparticles, they are more interested compared with other nanoparticles. Additionally, gold nanoparticles can be precisely synthesized and functionalized. They can be used not only for gene delivery but also for imaging [4].

Based on our previous project, although gold nanoparticles had antileishmanial activity, they could damage both macrophage and skin cells [5]. Moreover, it was found that modification of gold nanoparticles led to decrease of their toxicity and antileishmanial activity [6]. Modification of gold nanoparticles with oligonucleotides has been previously done for different medical applications, e.g. detection, imaging, and gene delivery [7]. It has been found that these conjugated oligonucleotides have higher uptake than naked nanoparticles [8].

In this study, gold nanoparticles were synthesized and conjugated with primary oligonucleotides. Then, conjugate gold nanoparticles were separately hybridized with three types of

* Corresponding author. Tel.: +98 35337256458; fax: +98 35337256458; mobile: +98 9131545131.
*E-mail address:* mh.anvari.tafti@gmail.com (M.H. Anvari-Tafti).

http://dx.doi.org/10.1016/j.colsurfb.2015.03.029
0927-7765/

antisense oligonucleotide, to silence GP63 gene of *L. major*. Here, after incubation, uptake assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, and gene expression were separately carried out.

## 2. Materials and methods

### 2.1. Material

All materials which used in this research were analytical grade. Ascorbic acid (AA), NNN medium, RPMI1640, MTT, isopropanol, SYBR Green, cetyl trimethyl ammonium bromide (CTAB), $HAuCl_4$, and Hank's balanced salt solution (HBSS) were purchased from Sigma–Aldrich Chemical Co, (St Louis, MO). Also, $HNO_3$ and isopropanol were provided from Merck, Germany. Both primary and antisense oligonucleotides were sourced from Takapo Zyst Company, Iran. Also, RNA extraction kit, DNase master mix, and cDNA Synthesis Kits were purchased from QIAGEN, Germany.

### 2.2. Synthesis of gold nanoparticles

First, 10 mL of 20 mM $HAuCl_4$ was added to 10 mL of 10 mM CTAB and 5 mL of 25 mM AA, and then incubated for 5 h at 37 °C. In the next step, the synthesized gold nanoparticles were centrifuged at 10,000 rpm for 15 min, and then washed three times by distilled water (DW). To characterize gold nanoparticles, UV-vis spectrophotometer (Novin gostar Company, Iran) and scanning electron microscopy (SEM) (Hitach, Japan) were used [9]. Here, 5 SEM images of gold nanoparticles were prepared, and then the size of 100 nanoparticles was recorded, and then the average size of nanoparticles was estimated.

### 2.3. Modification of gold nanoparticles

First, 0.1 g of synthesized gold nanoparticles was dissolved in 10 mL of DW. Then, 1 mL of primary oligonucleotide (1000 nM), 3′-AAA-5′ modified with thiol group, was added to the solution of gold nanoparticles, and incubated for 1 h at 37 °C. Next, conjugated gold nanoparticles were centrifuged at 10,000 rpm for 15 min, and washed three times with DW. In the next step, 100 mg of conjugated gold nanoparticles was separately mixed with antisense oligonucleotides 1, 2, and 3, as following:

Antisense oligonucleotide 1: 3′-TACTCTCGAC-5′, from coding reign of GP63 gene
Antisense oligonucleotide 2: 3′-TGCCGTACGT-5′, from non-coding reign of GP63 gene
Antisense oligonucleotide 3: 3′-TGCCGTACGTTACTCTCGAC-5′, from both coding and non-coding reigns of GP63gene (Fig. 1).

Then, each mixture was incubated at 55 °C for 10 min, in an annealing buffer containing 0.3 M NaCl, and slowly cooled to 25 °C for 1 h. At the final step, the hybridized gold nanoparticles were centrifuged at 10,000 rpm for 15 min, and washed three times by DW [10]. To confirm conjugation of primary oligonucleotides, FTIR was used. Also, to confirm hybridization, all conjugates were scanned under UV light, 330 nm.

### 2.4. Preparation of L. major promastigotes

The standard isolate of *L. major* (MRHO/IR/75/ER) was provided from Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The isolate was first incubated for 48 h at 37 °C in NNN medium enriched by RPMI1640. Then, all promastigotes were centrifuged

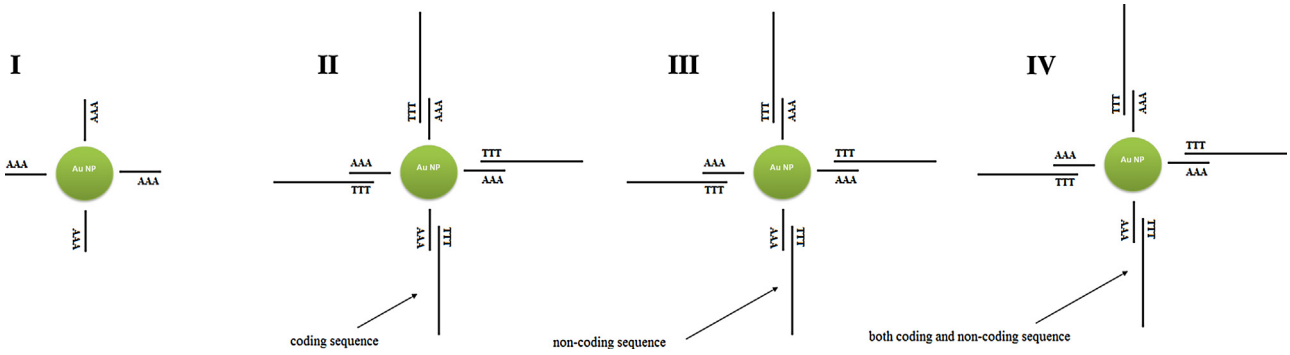


**Fig. 1.** The schematic of NP1 (I), NP2 (II), NP3 (III), and NP4 (IV).

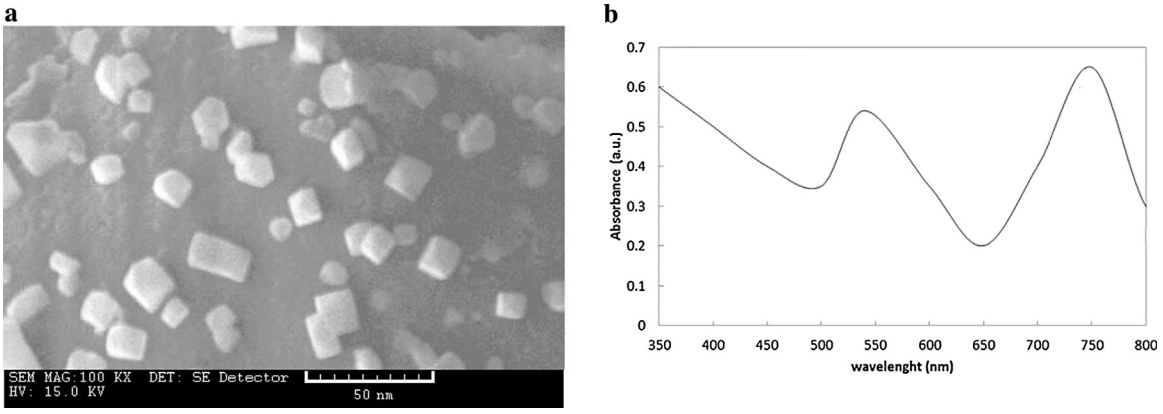


**Fig. 2.** The SEM image (a), and UV–vis spectrum (b) of gold nanoparticles.

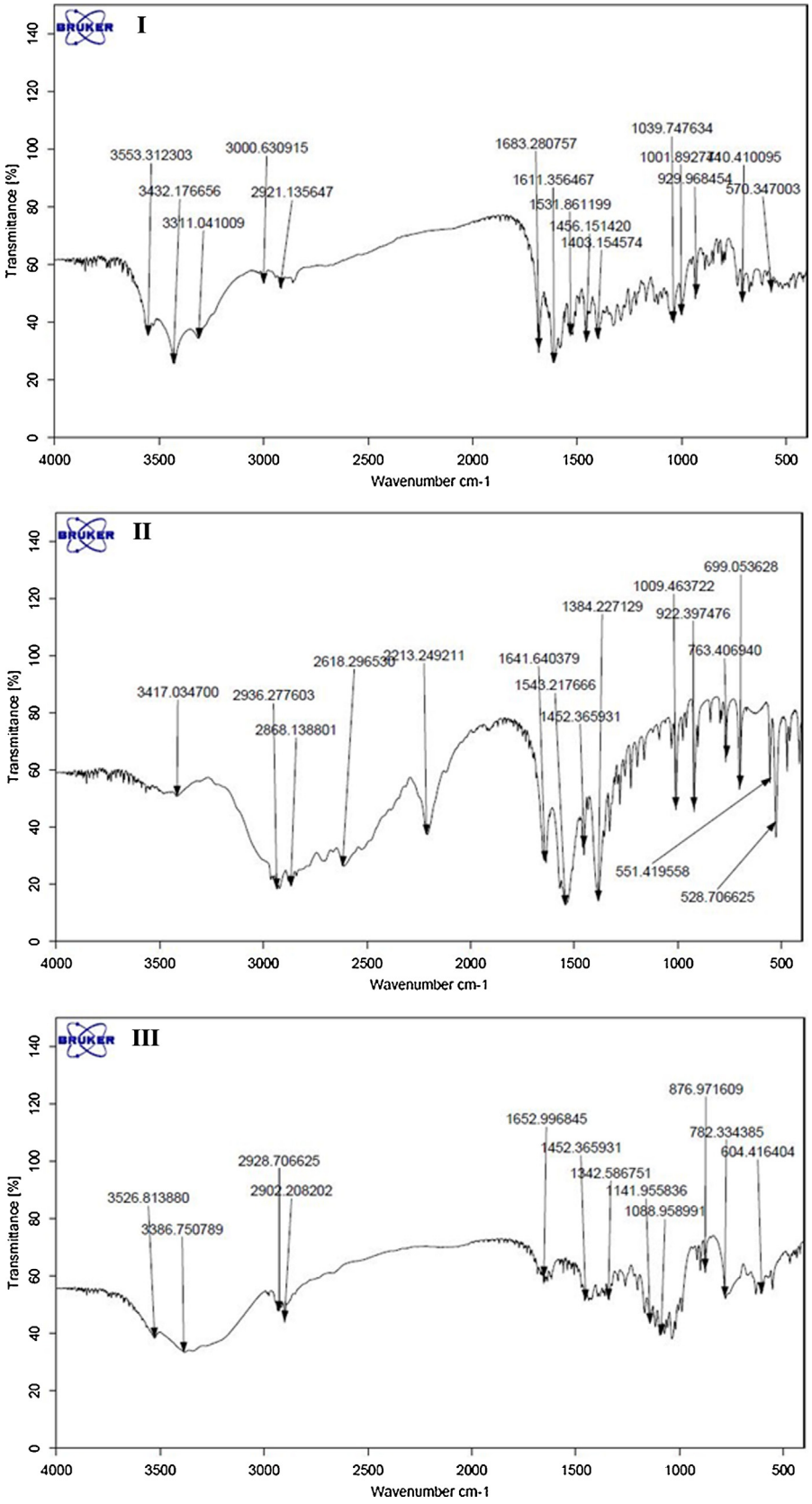


**Fig. 3.** The FTIR spectrum of conjugated gold nanoparticles (I), primary oligonucleotide (II), and gold nanoparticles (III).

at 3000 rpm for 5 min, and 10 mL of fresh RPMI1640 was added. Finally, the density of promastigotes was adjusted to $2 \times 10^5$ mL$^{-1}$ [5].

### 2.5. Exposure groups

One millilitre of *L. major* suspension was separately added to 1 mL of serial concentrations (1–200 μg/mL) of hybridized gold nanoparticles, and incubated for 24, 48, and 72 h at 37 °C. Four hybridized gold nanoparticles were enrolled in this study (Fig. 1), including:

(1) NP1: gold nanoparticles conjugated with 3′-AAA-5′.
(2) NP2: gold nanoparticles conjugated with 3′-AAA-5′ and hybridized with 5′-ATGAGAGCTG-3′.
(3) NP3: gold nanoparticles conjugated with 3′-AAA-5′ and hybridized with 5′-ACGGCATGCA-3′.
(4) NP4: gold nanoparticles conjugated with 3′-AAA-5′ and hybridized with 5′-ACGGCATGCAATGAGAGCTG-3′ [11].

### 2.6. Uptake assay

For uptake assay, 100 μL of treated promastigotes was centrifuged at 3000 rpm for 5 min, washed three times by normal saline. Then, 100 μL of 1 M $HNO_3$ was added to promastigote pellet, and incubated for 24 h at room temperature. Then, the concentration of gold ions was measured by atomic adsorption spectroscopy (AAS) [12].

### 2.7. MTT assay

After incubation, 100 μL of treated promastigotes was centrifuged at 3000 rpm for 5 min, and supernatant was removed. Then, 100 μL of RPMI1640 and 25 μL of 5 mg/mL MTT were added to promastigote pellet, and incubated 3 h at 37 °C. Next, 100 μL of 70% isopropanol was added, and optical densities (OD) of wells were read by a micro plate reader (Novin Gostar, Iran) at 490 nm. Finally, the cell viability was measured for each treated promastigote. In negative control, the promastigotes were treated with normal saline [12].

### 2.8. RNA isolation, cDNA synthesis, and real-time PCR

Briefly, 1 mL of treated promastigotes ($2 \times 105$ cells) was centrifuged at 3000 rpm for 5 min, and washed by normal saline. Then, RNA extraction was done by RNA extraction kit, according to its protocol. The samples were first treated with DNase master mix to remove possible contaminated DNA. Then, the mixture was incubated for 15 min at 95 °C to inactive the DNase. Then, the OD of extracted RNA was read at 260 nm by a spectrophotometer (Clinic II, Novingostar, Iran).

Next, total RNA was reverse transcribed by cDNA Synthesis Kit. Briefly, 10 μL of extracted RNA was added to 10 of Super-Script II buffer, 5 μL of 10 mM dNTPs, 5 μL of 0.1 M DTT, 0.25 μL of 50 μM random primers, 1.25 μL of RNA guard, 1.25 μL of 200 U/μL Superscript II reverse transcriptase, and 1 μL of molecular biology water, and then incubated at 65 °C for 5 min. For reverse transcription, the mixture was incubated for 42 °C for 60 min, and then it was incubated for 10 at 75 °C, to inactivate the reverse transcriptase.

Then, 2 μL of synthesized cDNA and 1 μL of 50 μM forward and reverse primers were added to 18 μL of SYBR Green, and entered into real-time PCR machine (ABI 1 plus, USA). In this study, the expression of GP63 gene was analyzed. Here, 5′-CCGGTTTATA-TGAAGGAACAGT-3′ and 5′-GGGGAAGATAGAAGAACGAAA-3′ were forward and reverse primer of GP63, respectively. Also, 18s rRNA of *L. major* was considered as internal control. Its forward primer was 5′-CGTTACCATATGAAGGGGCAACAGT-3′, and its reverse primer was 5′-GATTAGATAGAAGGGCCAAAAGCTA-3′. All were obtained from National Center for Biotechnology Information (NCBI). PCR amplification had an initial denaturation at 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and one cycle at 60 °C for 1 min. The promastigotes which were treated with normal saline considered as negative control [13]. At end, the comparative expression was measured by expression ratio formula.

$$\text{Expression ratio} = 2_t^{-\Delta\Delta C} = 2^{-\{(A-B)-(C-D)\}}$$

where $A$ is cycle of threshold ($CT$) of target gene in treated group, $B$ is $CT$ of internal control gene in treated group, $C$ is $CT$ of target gene in control group, and $D$ is $CT$ of internal control gene in control group.

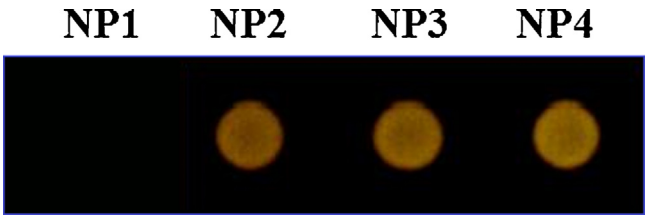


**Fig. 4.** The image of NP1, NP2, NP3, and NP4 after exposure to UV light. As seen, NP2, NP3, and NP4 have a sharp fluorescent intensity.

### 2.9. Statistical method

All tests were done five times and the results were reported as mean ± standard deviation (SD). To detect significant differences between groups, one-way ANOVA was carried out by SPSS software (V.16.0 for Windows; SPSS Inc., Chicago, IL), and $P$-values less than 0.05 were considered as significant significance.

## 3. Results

### 3.1. Characterization data

The shape of gold nanoparticles was cubic, and their size was approximately 15 ± 5 nm (Fig. 2a). According to Fig. 2b, synthesized gold nanoparticles had two sharp peaks at 540 nm and 750 nm. To confirm conjugation, FTIR was used (Fig. 3). As demonstrated, conjugated gold nanoparticle (I) and primary oligonucleotide (III) had approximately same peaks. To confirm hybridization, all conjugates were scanned under UV light. As seen, a sharp fluorescence is observed for NP2, NP3, and NP4 (Fig. 4).

### 3.2. Uptake assay

Fig. 5a shows the uptake of NP1, NP2, NP3, and NP4 after 24, 48, and 72 h. As the first finding, there was a direct relationship between concentration and uptake. As the second finding, at all concentrations, NP2 and NP3 had higher uptake than NP1 and NP4 ($P < 0.05$). As the third finding, at all concentrations, NP2 and NP3 had approximately similar uptake. Also, NP1 was the same as NP4.

### 3.3. MTT assay

Fig. 5b reveals the cell viability of *L. major* after incubation with NP1, NP2, NP3, and NP4. Like uptake assay, a dose-dependent pattern was seen for all types of nanoparticles, i.e. the increase of concentration led to decrease of cell viability. Also, at all concentrations, NP2 and NP3 had less cell viability than NP1 and NP4

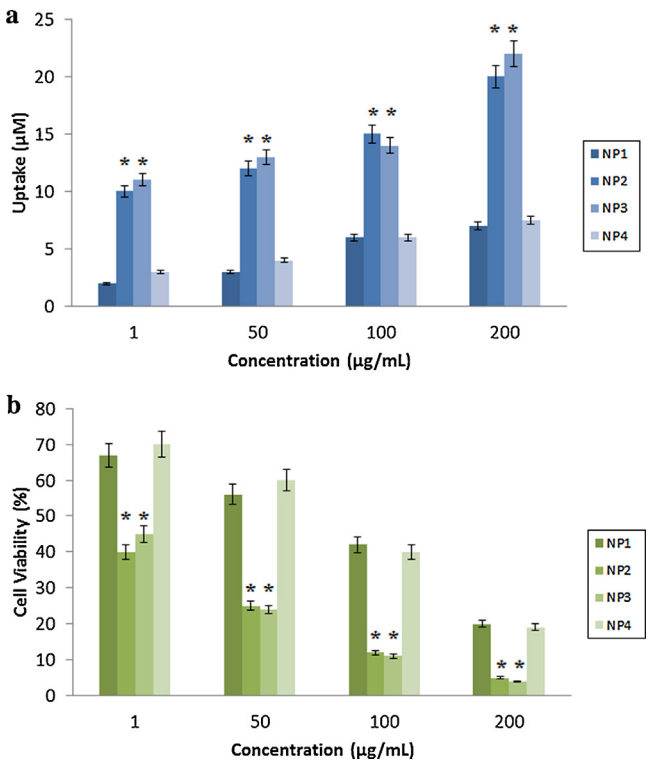


**Fig. 5.** The quantity of uptake (a) and cell viability (b) after incubation with NP1, NP2, NP3, and NP4. All data are shown as mean ± standard deviation, $n = 5$. Uptake and cell viability tests were done by AAS and MTT assay, respectively.

($P < 0.05$). Moreover, NP2 and NP3 had the same uptake. Also, NP1 and NP4 were the same.

### 3.4. Gene expression

Fig. 6 demonstrates the expression of GP63 gene in *L. major* when exposed to serial concentrations of NP1, NP2, NP3, and NP4. Such as uptake and MTT assay, a dose-dependent pattern was observed, i.e. the increase of concentration led to decrease of GP63 expression. Also, at all concentrations, NP1 and NP4 had higher gene expression than NP2 and NP3 ($P < 0.05$). It must be mentioned that although all nanoparticles could silence GP63 gene, NP2 and NP3 inhibited it more, than NP1 and NP4.

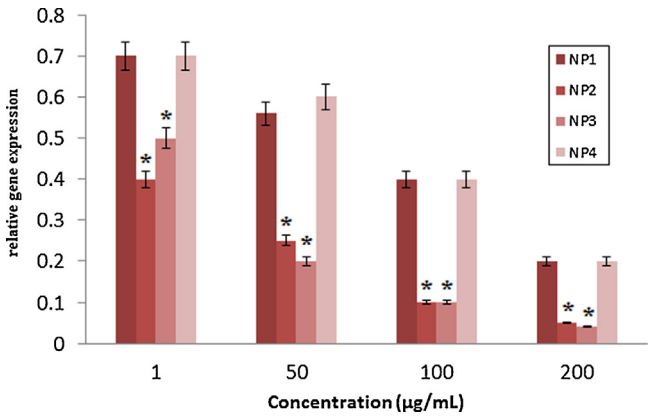


**Fig. 6.** GP63 expression after incubation with NP1, NP2, NP3, and NP4. All data are shown as mean ± standard deviation, $n = 5$. For gene expression, RNA extraction was first done, then cDNA was prepared, and real-time PCR was finally carried out. In negative control, the promastigotes were treated with normal saline. In this study, the comparative expression was measured by $2^{-\Delta\Delta C_t}$ method.

## 4. Discussion

GP63 is a vital gene of *L. major* and *L. tropica*, and codes a surface zinc metalloproteinase. Moreover, it is a key bio-molecule not only in promastigote–macrophage interaction, but also in complement cascade induction [14].

The aim of this study was to silence GP63 gene. As the first step, gold nanoparticles were synthesized by reaction between $HAuCl_4$ and AA. Considerably, gold nanoparticles had been previously used as an antisense carrier [15]. Here, gold nanocube was applied, because of better antimicrobial property [16]. Based on our previous experience, the shape of nanoparticles can change biological behaviors, as well as their size [16]. As the second step, gold nanoparticles were conjugated with primary oligonucleotides. Since antisense oligonucleotide should be released after uptake, 3′-AAA-5′ was used. Because 3′-GGG-5′ and 3′-CCC-5′ induce strong hydrogen binds, they were not suitable for our study.

Although there are different methods to conjugate various oligonucleotides on the surface of gold nanoparticles [17], the covalent binding between thiol and gold was applied [18]. As the third step, gold nanoparticles conjugated with primary oligonucleotides, were hybridized with three types of antisense oligonucleotide. Here, a sequence from coding reign, a sequence from non-coding reign, and a sequence from both coding and non-coding reigns were selected (Fig. 7). As mentioned in Section 2, these antisense oligonucleotides were hybridized at 55 °C in an annealing buffer. It must be mentioned that the secondary structure of antisense molecules may be affected by gold nanoparticles [19], and this affects both uptake and function of nanoparticles. After synthesis, conjugation, and hybridization, various characterization methods were used. SEM and UV-vis spectroscopy were applied to characterize naked-gold nanoparticles. To confirm conjugation of primary oligonucleotides, FTIR was used. Also, to confirm hybridization, all were scanned under UV light.

In the next step, uptake, cell viability, and gene expression were evaluated. It was found that NP2 and NP3 had higher (5-fold) uptake than NP1 and NP4 ($P < 0.05$). Interestingly, there was no significant difference between uptake of NP2 vs. NP3, and between NP1 vs. NP4 ($P > 0.05$). In consistent with uptake assay, MTT assay showed that NP2 and NP3 had less cell viability compared with NP1 and NP4 ($P < 0.05$). Exactly, this pattern was seen for gene expression, i.e. *L. major* had less expression when exposed to NP2 and NP3, compared with NP1 and NP4 ($P < 0.05$). The authors hypothesize that three-dimensional structure of NP2 and NP3 leads to higher uptake, less cell viability, and less GP63 expression. Based on our study, the sequence of hybridized oligonucleotide can affect the uptake of nanoparticle. On the other hand, it seems that the size of antisense oligonucleotide is also an important factor.

To inhibit a specific gene in infectious agents, oligodeoxy nucleotides (ODNs), mRNA antisense, shRNA, and siRNA can be used [2]. Although *L. major* is an important parasite, limited works have been reported about its gene silencing. Kheirandish et al. showed the inhibition of *L. major* PTR1 gene using antisense plasmid. They declared that PTR1 gene could be inhibited by antisense mRNA [20]. Dumas et al. identified a new class of non-coding RNAs that are expressed specifically in the intracellular amastigote stage [21]. Ramazeilles et al. targeted the mini-exon sequence, presented at the 5′ end of every parasite mRNA, by phosphorothioate oligonucleotides [22]. The antileishmanial activity of naked gold nanoparticles was previously reported by us [5].

For the first time, the silencing of GP63 gene of *L. major* was done in this study. It was found that with antisense oligonucleotides could be up taken by *L. major*. This study showed that hybridized gold nanoparticles not only could silence GP63 gene but also could kill promastigotes.


**Fig. 7.** GP63 complete gene, from NCBI database [11]. The initial sequence is non-coding reign, and the later sequence is coding reign.

## 5. Conclusion

Taken together, this study showed that gold nanoparticle was a good substance to deliver antisense oligonucleotides into *L. major*. Importantly, these antisense oligonucleotides can be obtained from both coding and non-coding reign of GP63 gene. It was found that both sequence and size of antisense oligonucleotides were important for transfection of *L. major*. Moreover, hybridized gold nanoparticles not only could silence GP63 gene, but also could kill *L. major*.

## Conflict of interest

There is no conflict of interest to declare.

## Acknowledgments

The authors thank the laboratory staff of Pajoohesh Lab, and Department of Parasitology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. This study was financially supported by our team (Grant No. 2015-001).

## References

[1] Y. Daneshbod, A. Oryan, M. Davarmanesh, S. Shirian, S. Negahban, A. Aledavood, M.A. Davarpanah, H. Soleimanpoor, K. Daneshbod, Clinical, histopathologic, and cytologic diagnosis of mucosal leishmaniasis and literature review, Arch. Pathol. Lab. Med. 135 (2011) 478–482.
[2] D.M. Morens, A.S. Fauci, Emerging infectious diseases in 2012: 20 years after the institute of medicine report, MBio 3 (2012), e00494-00412.
[3] N. Saranya, A. Moorthi, S. Saravanan, M.P. Devi, N. Selvamurugan, Chitosan and its derivatives for gene delivery, Int. J. Biol. Macromol. 48 (2011) 234–238.
[4] Y. Shan, T. Luo, C. Peng, R. Sheng, A. Cao, X. Cao, M. Shen, R. Guo, H. Tomás, X. Shi, Gene delivery using dendrimer-entrapped gold nanoparticles as nonviral vectors, Biomaterials 33 (2012) 3025–3035.
[5] A. Jebali, B. Kazemi, Nano-based antileishmanial agents: a toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis, Toxicol. In Vitro 27 (2013) 1896–1904.
[6] A. Jebali, S. Hekmatimoghaddam, B. Kazemi, A. Allaveisie, A. Masoudi, K. Daliri, N. Sedighi, J. Ranjbari, Lectin coated MgO nanoparticle: its toxicity, antileishmanial activity, and macrophage activation, Drug Chem. Toxicol. (2013) 1–10.
[7] K. Saha, S.S. Agasti, C. Kim, X. Li, V.M. Rotello, Gold nanoparticles in chemical and biological sensing, Chem. Rev. 112 (2012) 2739–2779.
[8] M.M. Janát-Amsbury, A. Ray, C.M. Peterson, H. Ghandehari, Geometry and surface characteristics of gold nanoparticles influence their biodistribution and uptake by macrophages, Eur. J. Pharm. Biopharm. 77 (2011) 417–423.
[9] M. Luty-Błocho, K. Fitzner, V. Hessel, P. Löb, M. Maskos, D. Metzke, K. Pacławski, M. Wojnicki, Synthesis of gold nanoparticles in an interdigital micromixer using ascorbic acid and sodium borohydride as reducers, Chem. Eng. J. 171 (2011) 279–290.
[10] S.M. Ryou, S. Kim, H.H. Jang, J.H. Kim, J.H. Yeom, M.S. Eom, J. Bae, M.S. Han, K. Lee, Delivery of shRNA using gold nanoparticle-DNA oligonucleotide conjugates as a universal carrier, Biochem. Biophys. Res. Commun. 398 (2010) 542–546.
[11] M.B. Rogers, J.D. Hilley, N.J. Dickens, J. Wilkes, P.A. Bates, D.P. Depledge, D. Harris, Y. Her, P. Herzyk, H. Imamura, T.D. Otto, M. Sanders, K. Seeger, J.C. Dujardin, M. Berriman, D.F. Smith, C. Hertz-Fowler, J.C. Mottram, Chromosome and gene copy number variation allow major structural change between species and strains of *Leishmania*, Genome Res. 21 (2011) 2129–2142.
[12] A. Jebali, F.H.E. Hajjar, S. Hekmatimoghaddam, B. Kazemi, J.M.D.L. Fuente, M. Rashidi, Triangular gold nanoparticles conjugated with peptide ligands: a new class of inhibitor for *Candida albicans* secreted aspartyl proteinase, Biochem. Pharmacol. 90 (4) (2014) 349–355.
[13] V. Iniesta, S. Belinchón-Lorenzo, M. Soto, J. Fernández-Cotrina, R. Muñoz-Madrid, I. Monroy, V. Baz, A. Gómez-Luque, J.C. Parejo, C. Alonso, Detection and chronology of parasitic kinetoplast DNA presence in hair of experimental *Leishmania major* infected BALB/c mice by Real Time PCR, Acta Trop. 128 (2013) 468–472.
[14] U.A. Wenzel, E. Bank, C. Florian, S. Förster, N. Zimara, J. Steinacker, M. Klinger, N. Reiling, U. Ritter, G. van Zandbergen, *Leishmania major* parasite stage-dependent host cell invasion and immune evasion, FASEB J. 26 (2012) 29–39.
[15] K.C. Bahadur, B. Thapa, N. Bhattarai, Gold nanoparticle-based gene delivery: promises and challenges, Nanotechnol. Rev. 3 (2014) 269–280.
[16] A. Jebali, F.H.E. Hajjar, F. Pourdanesh, S. Hekmatimoghaddam, B. Kazemi, A. Masoudi, K. Daliri, N. Sedighi, Silver and gold nanostructures: antifungal property of different shapes of these nanostructures on *Candida* species, Med. Mycol. 52 (2014) 65–72.
[17] K.H. Lee, F.M. Ytreberg, Effect of gold nanoparticle conjugation on peptide dynamics and structure, Entropy 14 (2012) 630–641.
[18] A.K.R. Lytton-Jean, R. Langer, D.G. Anderson, Five years of siRNA delivery: spotlight on gold nanoparticles, Small 7 (2011) 1932–1937.

[19] J.E. Gagner, M.D. Lopez, J.S. Dordick, R.W. Siegel, Effect of gold nanoparticle morphology on adsorbed protein structure and function, Biomaterials 32 (2011) 7241–7252.
[20] F. Kheirandish, M. Bandehpour, A. Haghighi, F. Mahboudi, M. Mohebali, B. Kazemi, Inhibition of *Leishmania major* PTR1 gene expression by antisense in *Escherichia coli*, Iran. J. Public Health 41 (2012) 65.
[21] C. Dumas, C. Chow, M. Muller, B. Papadopoulou, A novel class of developmentally regulated noncoding RNAs in *Leishmania*, Eukaryot. Cell 5 (2006) 2033–2046.
[22] C. Ramazeilles, R.K. Mishra, S. Moreau, E. Pascolo, J.J. Toulme, Antisense phosphorothioate oligonucleotides: selective killing of the intracellular parasite *Leishmania amazonensis*, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 7859–7863.