

Research Article

Effect of Gold Nanoparticles Size Capped with Surfactant on the Transformation of Plasmid into *Escherichia coli* Bacteria

Amr D*, Attia N, Seufi A and Galal A

Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt

***Corresponding author:** Dina Amr, Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt**Received:** August 16, 2021; **Accepted:** September 17, 2021; **Published:** September 24, 2021**Abstract**

Bacterial transformation has great importance in molecular biology, as it is used for introduction of foreign genes into bacterial cells either chemical or physical ways. Using calcium chloride to prepare competent cells and heat shock is the most widely used method for bacterial transformation. This method is an efficient and convenient technique but it has in some extent low transformation efficiency. Here we report the use of nanoparticles that significantly improve the transformation efficiency up to 10 times higher than the standard heat shock method by the assistance of ($\approx 15, 25$ nm) SDS capped gold nanoparticles in the transformation process that leads to the formation of temporary nano-channels across the bacterial cell wall to deliver plasmids into cells. Transformation of bacteria with plasmid was examined using β -galactosidase assay.

Keywords: Transformation; *Escherichia coli*; Competent cells; Plasmid; Gold nanoparticles

Introduction

Bacterial transformation steps have focal importance in the latest advancement in molecular biology by construction of recombinant strain. Recombinant plasmids are transformed into various bacterial host cells for replication and expression of exogenous gene, and to meet multiple varieties of research needs and commercial application [1,2]. It can be achieved using either chemical methods such as calcium chloride or physical methods such as electroporation. The drawback of these traditional transformation methods was that large numbers of bacteria was needed to compensate the high percentage of cell death in case of electroporation and in calcium chloride method has in some extent low transformation efficiency [3].

Hanahan's protocol is the most widely used chemical transformation method that use calcium chloride treatment to make bacterial cell competent [4]. There is limited attention in the use of synthetic inorganic gene nano-carriers in bacterial cell transformation [5]. Using Nanoparticles (NPs), either polymeric or inorganic, was resulted in increasing the transformation yield. Due to its condensation ability that cause DNA enrichment on to the cell surface [6]. It has been reported that non-toxic nanoparticles such as gold NPs, silica NPs, and carbon nanotubes facilitate the entrance of macromolecules into host cells [5,7,8]. Multi-walled carbon nanotubes (CNTs) that dispersed in water were used to target the bacterial surface to deliver plasmid DNA into the cells in the presence of microwave irradiation by developing of temporary nano-channels across the cell envelope [5].

In another study, the transformation efficiency of *E. coli* competent cells prepared with calcium chloride method was greatly improved by using amino modified silica-nanoparticles (a-SiNPs) as an aiding agent [9]. Also, Fe₃O₄ nanoparticles were used in plasmid delivery to *E. coli* competent cells prepared with calcium chloride

method and heat shock transformation standard method [10]. Magnetic nanoparticles were used in introduction of exogenous DNA into bacteria in the presence of pulsed magnetic field [11].

Chitosan nanoparticles and chitin nano-whiskers facilitates *E. coli* transformation and plasmid DNA delivery [12,13]. Specifically, gold nano-particles (Au NPs) are promising tool to use in gene delivery as these particles easily conjugated with biomolecules at a high packing density [14]. Delivering genes to bacteria can be done via electrospray of gold nanoparticles [15]. Gold nanoparticles functionalized with glutathione were used to deliver plasmid DNA into *E. coli* cells without the need for competent cells preparation [16]. So, the focus of research now is in the development of novel plasmid DNA delivery methods to improve the efficiency of the existing methods. Transformation efficiency improvement had a great importance in shotgun, as every single DNA fragment had a great importance in genomic DNA or complete cDNA library construction. So, if any DNA fragment lost during genome sequencing projects results in decreasing the technique accuracy [10]. Our aim in this study is to determine the effect of using SDS-capped gold nanoparticles of two different sizes ($\approx 15, 25$ nm) and the effect of changing the sequence of addition of gold nanoparticles through the transformation process on the efficiency of transformation of plasmid into *E. coli* competent cells prepared by calcium chloride and transformed by heat shock method.

Materials and Methods

Gold (III) chloride trihydrate ($\geq 99.9\%$) was obtained from Sigma. *Escherichia coli* bacterial strain (JM109) (endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ (lac-proAB) e14- [F' traD36 proAB+ lacIq lacZ Δ M15] hsdR17 (rK-mK+)), plasmid DNA pGEM'-5Zf (+) and PureYield™ Plasmid Miniprep Kit were obtained from Promega (USA). All reagents were prepared using ultrahigh pure water from

an ultra-pure water system Milli-Q Plus (Millipore Co.).

Synthesis of SDS capped gold nanoparticles

SDS coated gold nanoparticles were prepared as previously reported procedure [17] (for more information see supplementary file section 1.1). The molar concentration of nanoparticle dispersion and nanoparticles number in it was calculated by using previous reported calculations [18,19] (for more information see supplementary file section 1.2, 1.3).

Competent cell preparation and transformation procedure

Competent cells *E. coli* cell (*JM109*) were prepared following "calcium chloride standard protocol" [1]. Transformation of plasmid DNA (pGEM⁺-5Zf (+) was applied in this study) into bacterial cells using nanoparticles was done by two different nanoparticles administration routes that differs on the sequence of addition of nanoparticles through the transformation process.

In the first nanoparticles administration route: before transformation, 1 μ L of the prepared nano-particles dispersed in deionized water (concentration $\approx 10^{-9}$ M, $\approx 10^8$ NPs) was mixed with 5 μ L plasmid DNA (2ng/ μ L) and allow the mixture to place on ice for 10min as to allow DNA/nanoparticles interaction and formation of nanoparticle/DNA complex. Then, this nanoparticle/DNA complex added on 100 μ L competent cells ($\approx 2.92 \times 10^8$ cells) then follow heat shock standard transformation protocol [1]. While, in the second nanoparticles administration route was done as the following, before transformation 1 μ L of the prepared nanoparticles dispersed in deionized water (concentration $\approx 10^{-9}$ M, $\approx 10^8$ NPs) was mixed with 100 μ L competent cells ($\approx 2.92 \times 10^8$ cells) and allow the interaction between NPs and competent cells by putting for 10min on ice. After that, follow the standard heat shock transformation protocol [1]. Finally, bacterial transformation efficiency calculated based on counting the numbers of colonies [20,21] (for more information see supplementary file section 2). The success of the transformation process was also confirmed indirectly by performing Plasmid DNA extraction (PureYieldTM Plasmid Miniprep Kit (Promega kit, USA)) following the manufacturer's instruction from blue colonies randomly chosen subculture. The extracted plasmid DNA band visualized on 0.8% agarose gel electrophoresis as the standard protocol [22] by gel

documentation system (Gel DocTM EZ System, Bio-Rad, USA) (for more information see section 2 in supplementary file).

Characterization by transmission electron microscope (TEM)

TEM sample preparation of either gold nanoparticles or bacterial ultra-thin sections were done following the protocols previously mentioned in literature [23,24] (for more information see section 3 in supplementary file).

Results and Discussion

Preparation and characterization of gold nanoparticles

Gold nanoparticles was prepared based on gold seeds synthesis, and growth of these seeds into larger particles by addition of SDS surfactant, more gold salt and ascorbic acid in small amount which act as weak reducing agent in the growth solution [17]. Transmission electron microscope (JEOL JEM-1400, operated at 80kV) used to determine the actual shape and size of the nanoparticle as shown in Figure 1a and 1b electron micrographs. From TEM images we found that SDS-stabilized gold nanoparticles have average particle diameter size 13 ± 2 nm (Figure 1a) and average particle diameter size 25 ± 2 nm (Figure 1b).

Bacterial transformation using SDS capped gold nanoparticles

Generally, the CaCl₂ solution that was used in competent cell preparations decrease membrane stability and increase its permeability by formation of multiple transient pores, which increase the transformation efficiency. The decrease in membrane stability facilitates the plasmid entrance into the bacterial cell [25]. In this study, the application of gold nanoparticles to *E. coli* cells to mediate transformation did not affect the bacterial cell growth and did not cause any damage to *E. coli* cells [26]. As the toxicity effect of gold nanoparticles capped with SDS were determined first on the growth of non-transformed *E. coli* cells overnight culture and it was found that the growth rate of *E. coli* cell culture supplemented with SDS capped gold nano particles were the same as the control experiment without nano particles addition while growth inhibition resulted when we test the use of chitosan capped gold nanoparticles (~ 15 nm). The non-toxic nature of SDS capped gold nanoparticles were more

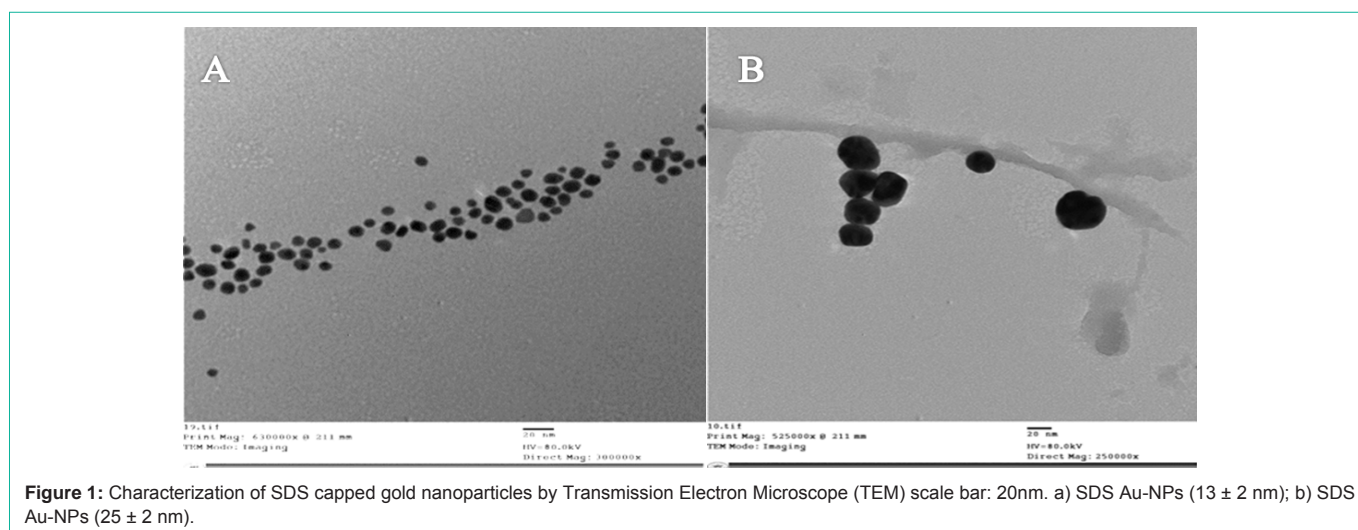
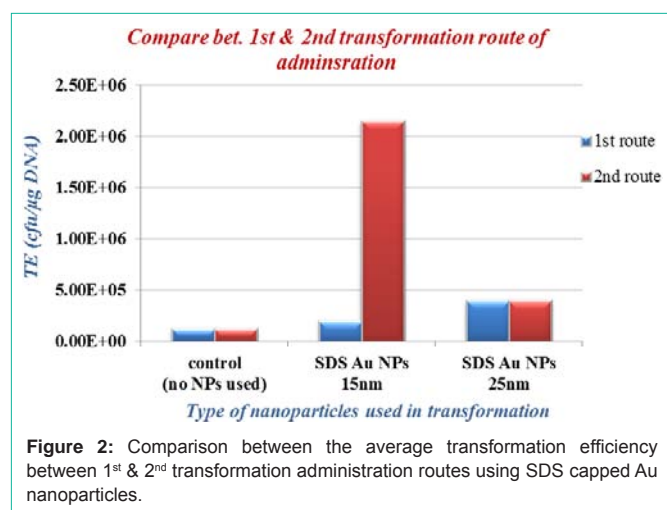


Figure 1: Characterization of SDS capped gold nanoparticles by Transmission Electron Microscope (TEM) scale bar: 20nm. a) SDS Au-NPs (13 ± 2 nm); b) SDS Au-NPs (25 ± 2 nm).

Table 1: Transformation efficiency experimental results.

Administration route of NPs	1 st NPs administration route	2 nd NPs administration route
Type of NPs used in transformation	Transformation efficiency	Transformation efficiency
Control experiment (no NPs)	1.23×10^5	1.23×10^5
SDS capped Au NPs (25nm)	3.95×10^5	3.94×10^5
SDS capped Au NPs (15nm)	1.95×10^5	2.15×10^6



confirmed from bacteria growth on LB plates as checked Colony-Forming Units (CFU) studies.

Therefore, it could be applied in biology as it has minimal cytotoxic effect [27]. Transformation of plasmids was examined using β-galactosidase assay and via antibiotic selection by bacterial blue colonies grown on LB agar/ampicillin plates [20].

Further, confirmation for the success of plasmid transformation was done by plasmid extraction from *E. coli* cells (*JM109*) transformed cells “i.e., blue colonies”. From the experimental results of transformation efficiency as shown in Table 1, we found that using ($\approx 15, 25$ nm) gold SDS capped nanoparticles increase the plasmid transformation efficiency significantly by approximately 2 to 10 times more than control experiment (without using nanoparticles) in both administration routes. However, the transformation efficiency in the second route was significantly higher compared to the first nanoparticles administration route especially when using small size (≈ 15 nm) gold NPs as shown in Figure 2.

These results clearly shows an advantage of this protocol introduced in this study over the conventional methods as shown in Table 1, that also, compares between the results that we obtained from this study and previous work in comparison with the control experiment that done in the same conditions but without using nanoparticles. This increase in transformation efficiency by using NPs, were due to that NPs facilitate the binding and transportation of DNA into the bacterial membrane (*E. coli* cells) [28] by creating temporarily nano-channels into bacterial surface membrane, that permits the plasmid entrance into the cells. However, by incubating these cells in rich medium for a few hours result in repairing this temporary cell damage [5,15]. Also, NPs may cause increase in plasmids attachment contact time with bacterial surface membrane

thus plasmid transformation efficiency increased [29]. So, NPs are become promising vehicles to be used in gene delivery because these particles are easily conjugated with biomolecules at a high packaging density [14] facilitating DNA delivery into cellular membranes [30] but the precise mechanism is still unknown [31].

Transformation using SDS capped gold nanoparticles by the first route of administration

Transformation using SDS capped gold NPs by the first route of administration of two different sizes ($\approx 15, 25$ nm) significantly increase plasmid transformation efficiency in comparison with the control experiment as shown in Table 1. Using 25 ± 2 nm SDS capped Au NPs enhances the transformation efficiency compared to the use of 13 ± 2 nm SDS capped Au NPs in transformation. So, the increase in size of SDS capped Au NPs from 13 ± 2 nm to 25 ± 2 nm in the first administration transformation route improves the transformation efficiency two folds (P-value = 0.000048). This transformation enhancement is statistically significant (P = <0.001) and could not be done by chance. This means that transformation efficiency was affected by gold nanoparticles size up to some extent and this size dependency is also described in other studies [15]. Where an increase of gold nanoparticle size from 20nm to 50nm using electrospray process cause that transformation efficiency improved two-fold (P-value=0.007). In another study, it was demonstrated that gold nanoparticles mammalian cellular uptake was heavily dependent upon the size. The cellular uptake of gold nanoparticles of size 14nm is less than of size 30nm. The maximum uptake by a cell occurred at a nanoparticle size of 50nm [32]. The reason for size dependent endocytosis of Au NPs can be explained by the thermodynamic model of the many-NP-cell system [33]. Which reported that nanoparticles to up take by the cell requires thermodynamic driving force for wrapping which refers to the amount of free energy required to drive the NPs into the cell. So, NPs with a diameter smaller than 40nm, the docking of a single small NP will not produce enough free energy to completely wrap the NPs on the surface of the membrane. This could prevent the uptake of the single NP by endocytosis. For the smaller NPs to go in, they must be clustered together and thus take a long diffusion time. Therefore, the uptake amount is much smaller than 50nm NPs. Another studies reported that the size of nanoparticles was found to play a critical role in both the rate and extent of cellular uptake. It was found that 50nm transferrin coated gold NPs were taken up by mammalian cells at higher rates and extents compared to smaller and larger sizes in the range of 10-100 nm [34]. The explanation of this optimal size was based on the so called “wrapping effect”, which describes how a cellular membrane encloses NPs. Two factors dictate how fast and how many nanoparticles enter the cellular compartment via “wrapping”: the first is the free energy that results from ligand receptor interaction; the second is the receptor diffusion kinetics onto the wrapping sites on the cellular membrane.

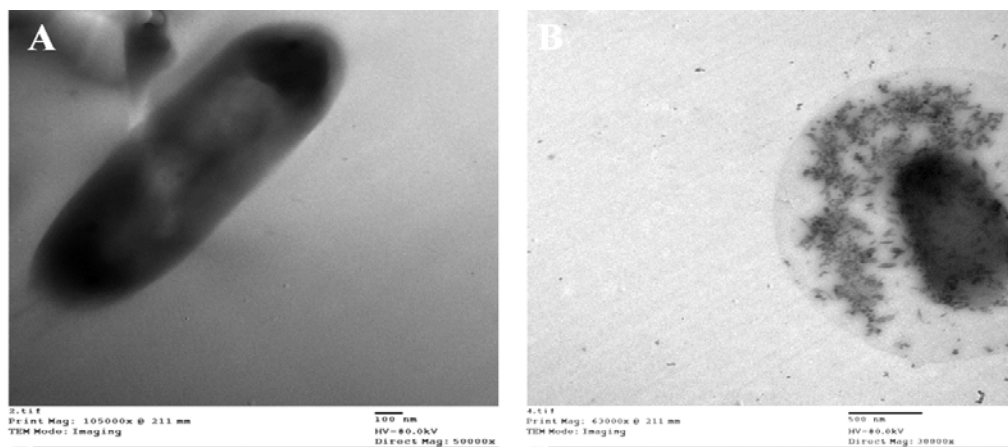


Figure 3: TEM image of bacterial cell of: a) Control transformation experiment, b) Transformation using SDS capped gold nanoparticles 13 ± 2 nm 1st administration route.

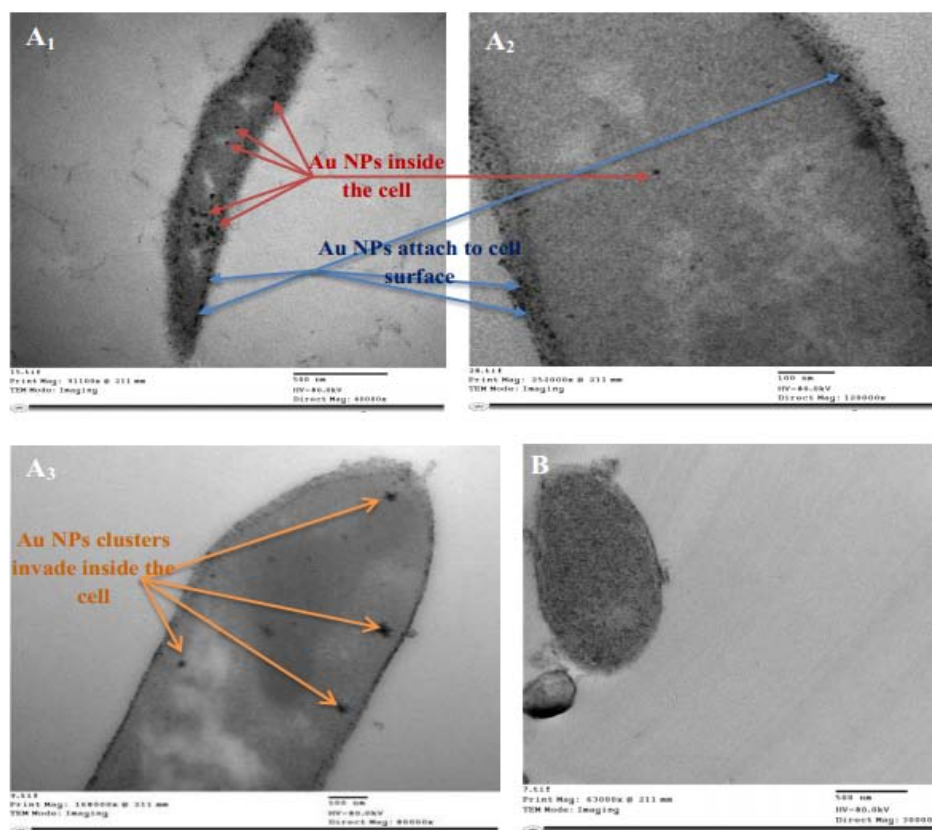


Figure 4: A1-A3): TEM images of bacterial cells transformed with the assistance of SDS capped gold nanoparticles 13 ± 2 nm by 2nd administration route; B) TEM of control bacteria.

Considering the contribution of these factors and using mathematical calculations, suggested that NPs with 27-30 nm diameter would have that fastest wrapping time and thus the fastest receptor mediated endocytosis [35]. But, we have to consider that the entry of NPs into mammalian cells was easier than bacterial cells due to the difference in cell size [36].

So, based on the above theory, the model of thermodynamic

for many NP-cell system with receptor mediated endocytosis the bacterial cell uptake to plasmid/NPs (≈ 25 nm) complex was more than the cellular uptake for plasmid/NPs (≈ 13 nm) SDS capped Au NPs complex in this study. Therefore, the plasmid transformation efficiency increases two times in case of (25 ± 2 nm) more than (13 ± 2 nm) SDS capped Au NPs in the case of first administration transformation route.

Table 2: Comparison between the previous studies for attaining higher transformation efficiency in bacteria using nanoparticles with the results obtained from this work.

Method	Plasmid	Host organism	TE of control (without use of NPs) (cfu/ μ g plasmid DNA)		TE of experimental one (cfu/ μ g plasmid DNA)		Ref.
Chitosan NPs bounded to plasmid DNA	pSV- β -galactosidase (6820 bp)	<i>E. coli</i> (ATCC 25922)	-----		TE increase more than control		[12]
			β -galactosidase activity \approx 1500 mU/mg		β -galactosidase activity \approx 85000mU/mg		
Chitin nanowhiskers (Yoshida effect)	pUC18	<i>E. coli</i> (JM109)	Transformation not occurs (no colonies formed)		2.1 \times 10 ⁶ cfu/ μ g plasmid DNA		[13]
CNTs & microwave irradiation	pUC19	<i>E. coli</i> (DH5 α)	Transformation not occurs (no colonies formed)		9 and 32 transformants/ μ g plasmid DNA		[5]
PEI-coated magnetic nanoparticles in pulsed magnetic field	pGEX-1 λ T	<i>E. coli</i> (DH1)	Transformation not occurs (no colonies formed)		64 to 99 cfu/ μ g plasmid DNA		[11]
(a-SiNPs) With heat shock & CaCl ₂ -treated bacteria	pBluescript SK+ plasmid DNA	<i>E. coli</i>	below 10 ⁶ cfu/ μ g plasmid DNA		10 ⁷ -10 ⁸ cfu/ μ g plasmid DNA		[28]
Electrospray of Au NPs (50nm)	pET30a-GFP	<i>E. coli</i>	0.3 \times 10 ⁶ cfu/ μ g		2 \times 10 ⁶ cfu/ μ g plasmid DNA (5-7 fold more than control)		[15]
Glutathione-functionalized Au NPs (5nm)	pUC 19 (3.2 ng/ μ l)	<i>E. coli</i> (DH5 α)	9.55 \times 10 ³ cfu/ μ g plasmid DNA		8.53 \times 10 ⁵ cfu/ μ g plasmid DNA		[27]
Glutathione-functionalized gold nanoparticles (Au NPs 2nm)	pUC 19 (1 ng/ μ l)	<i>E. coli</i>	2.3 \times 10 ⁵ cfu/ μ g plasmid DNA		4.2 \times 10 ⁷ cfu/ μ g plasmid DNA		[16]
Fe ₃ O ₄ NPs (19nm) with heat shock & CaCl ₂ treated bacteria	pCAMBIA (8,428 bp)	DH5 α JM107	2.9 \times 10 ⁴ cfu/ μ g	3.2 \times 10 ⁴ cfu/ μ g	3.0 \times 10 ⁶ cfu/ μ g	2.6 \times 10 ⁵ cfu/ μ g	[10]
	pGEM-T (3,000 bp)		3.8 \times 10 ⁵ cfu/ μ g	3.8 \times 10 ⁵ cfu/ μ g	2.4 \times 10 ⁶ cfu/ μ g	2.5 \times 10 ⁵ cfu/ μ g	
Electrospray of chitosan NPs (125nm)	PUC19 (2686 bp)	<i>E. coli</i> K12	Not written		1.7 \times 10 ⁸ cfu/ μ g		[48]
Ag NPs (100nm) with heat shock & CaCl ₂ treated bacteria	pUC18	<i>E. coli</i> DH5 α	2.3 \times 10 ³ cfu/ng		8.3 \times 10 ⁴ cfu/ng		[49]
	pBR322				8.0 \times 10 ⁴ cfu/ng		
	pCAMBIA				7.9 \times 10 ⁴ cfu/ng		
Mineral nanofibers with Sliding frictional forces treated bacteria	pUC19	TOP10 cells	Not written		1 \times 10 ⁶ to 2 \times 10 ⁶ cfu/mg		[50]
ZnO NPs with heat shock & CaCl ₂ treated bacteria	pGEM-T plasmid	<i>E. coli</i> K12	More than 9-fold higher compared to the control group without NPs				[51]
TiO ₂ NPs with heat shock & CaCl ₂ treated bacteria	pGEM-T plasmid	<i>E. coli</i> K12	Reduces the efficiency of transformation by more than 31 times compared to the control group without NPs				
AIOOH, CuO, and Fe ₃ O ₄ NPs with heat shock & CaCl ₂ treated bacteria	pGEM-T plasmid	<i>E. coli</i> K12	Do not have a significant effect on the transformation				
SDS capped Au NPs (25nm,15nm) (1 st route) with heat shock & CaCl ₂ treated bacteria	pGEM-5Zf (+) (3,000 bp)	<i>E. coli</i> (JM109)	1.23 \times 10 ⁵ cfu/ μ g	3.95 \times 10 ⁵ cfu/ μ g (25nm Au NPs)		This work	
				1.95 \times 10 ⁵ cfu/ μ g (15nm Au NPs)			
SDS capped Au NPs (25nm,15nm) (2 nd route) with heat shock & CaCl ₂ treated bacteria	pGEM-5Zf (+) (3,000 bp)	<i>E. coli</i> (JM109)	1.23 \times 10 ⁵ cfu/ μ g	3.94 \times 10 ⁵ cfu/ μ g (25nm Au NPs)		This work	
				2.15 \times 10 ⁶ cfu/ μ g (15nm Au NPs)			

Transformation using SDS capped nanoparticles by the second route of administration

Transformation using SDS capped gold NPs by the second route of administration of two different sizes (\approx 15, 25 nm) significantly increase plasmid transformation efficiency compared to control experiment. The transformation efficiency in the second administration route was significantly higher compared to the first administration route especially for small size (\approx 15nm) gold NPs as indicated in Table 1. This may be due to the fact that incubating the NPs with competent cells allows NPs to interact with bacterial membrane proteins. So, enhancement in the bacterial membrane permeability takes place. As it was hypothesized that some cellular functions stimulated by NPs instead of disrupting them, as the cellular machinery working level have to imply an increase to uptake NPs [37]. This also enhances the cell membrane permeability by the formation of penetration intermediates through cell membrane and creating temporary membrane disruptions that facilitate plasmid delivery into the cells leading to transformation [38]. This study reported that, the second transformation administration route that use gold nanoparticles of 13 \pm 2 nm size enhances the transformation efficiency more compared to the use of 25 \pm 2 nm gold nanoparticles and both were greater than the control experiment. So, small size

SDS capped gold nanoparticles more enhances the transformation efficiency compared to the use of big size nanoparticles in the second transformation route of nanoparticles administration.

This difference in transformation efficiency could be explained as nanoparticles had a negative surface charge due to the anionic SDS molecules that coating the gold nanoparticles. So, according to that the primary interaction of the nanoparticle with the bacterium is probably by an electrostatic attraction between the nanoparticle surface and positively charged regions such as the extracellular domains of integral outer membrane proteins on the *E. coli* surface [39]. The surface lipopolysaccharides carry a negative charge from phosphate residues on the inner and outer core polysaccharide chains [40]. Thus, nano particle binding by electrostatic attraction could occur at sites where the LPS (Lipopolysaccharides) molecules are interspaced by protein "landing pads". The crystal structures of some surface proteins including OmpF (8nm across) and OmpC (7nm across) [41,42]. This indicate that the surface domains of these proteins would be more than sufficient to accommodate the binding of 15nm gold nanosphere, assuming that the contact surface is less than the radius of the sphere. This is supported by another study that report a size dependent interaction of silver nanoparticles with *E. coli* cells where smaller nanoparticles binding were more preferentially

than larger nanoparticles that may not fit onto the exposed protein domains [43,44]. So, small size NPs might accommodate more efficiently in membrane pores and transfer plasmid DNA more than large size nanoparticles.

Comparing between the first and the second transformation administration route using SDS capped gold nanoparticles

As indicated in Figure 2 and in Table 1, the transformation efficiency of SDS capped Au NPs of average size $\approx 15\text{nm}$ in the second administration route was ≈ 10 times higher compared to the first administration route and this difference was statistically significant ($P \leq 0.001$). But, the difference in transformation efficiency when using SDS capped Au NPs of average size 25nm in both the first and second administration route was statistically insignificant (P value = 0.867) which is ($P \geq 0.05$). So, this very small difference may occur without controlling the pertinent factors and will not be further considered.

This increase in transformation efficiency in the 2nd transformation administration route may be due to that incubating nanoparticles with competent cells allow nanoparticles especially small nanoparticles to interact directly with bacterial cell membrane proteins that previously explained in the previous section (section of second administration route). So, this may enhance the bacterial membrane permeability and destabilize it without disrupting them by the formation of temporally channels and penetrating intermediates that allow the plasmid to enter more efficiently through the competent cells. The binding of small size NPs were more preferentially than large size NPs that may not fit onto the exposed protein surface domains. Therefore, the surface domains of these proteins would be more than sufficient to accommodate binding of 15nm nanosphere than 25nm NPs. Also, as the particle size gets reduced to the nanorange, the surface/volume (S/V) ratio increases and more atoms get exposed to the surface (35-40% for $<10\text{nm}$ and 20-25% for $>30\text{nm}$ particle size) [44]. So, small size NPs might accommodate more efficiently in membrane pores and transfer plasmid DNA more than large size nanoparticles.

Characterization of nanoparticle transformed bacteria

The cellular uptake for gold nanoparticles can be determined by making Transmission Electron Microscopy (TEM) in microtomed cell slices. Therefore, gold nanoparticles localization could be visualized inside the cells and around its membrane due to its high electron density. It will be also possible to allocate aggregations or agglomerations of particles within and outside the cells.

The TEM image of bacteria transformed with the assistance of gold nanoparticles ($\approx 15\text{nm}$) by 1st administration route (Figure 3b) shows that gold nanoparticles covers and invade the bacterial cell so, enhance the plasmid bacterial transformation if compared to control bacteria that shows overview of bacterial cell outer structure with their characteristic shape (Figure 3a).

The TEM images of bacteria transformed with the assistance of gold nanoparticles ($\approx 15\text{nm}$) by 2nd administration route (Figure 4A1-4A3) shows that several gold nanoparticles are evenly distributed and attached to the bacterial membranes and also distributed inside the cell and invade the bacterial cell as nanoparticles clusters if compared to TEM of control bacteria that shows the interior of the bacterial cellular components and its cellular membrane (Figure 4B). Also, a

temporary partial loss in bacterial membrane was observed which attributed to the action of CaCl_2 used in competent cells preparation. Previous study reported from the intracellular distribution of gold nanoparticles, the ability of nanoparticles to enter cells and to be trapped in vesicles, but are enable to enter the nucleus [34,36,45,46]. However, to date, there is still a lack of definite knowledge regarding the interaction of NPs with the bacterial cell wall and possible permeation of the NPs into the bacterial cells [47,52-55].

Statistical analysis

The statistical analysis was done on the experimental transformation efficiency results as indicated in Table 1. The data was normally distributed and this was confirmed by Skewness and Kurtosis analysis and represented as mean \pm standard error of mean (SEM) as shown in Table 1. Analysis of variance (ANOVA) and Post Hoc tests with multiple comparisons between different experiments were applied to ascertain significant effect in transformation efficiency when using different average size diameter of SDS-capped-Au NPs ($\approx 15\text{nm}$, 25nm) and by applying different administration routes (1st, 2nd) with various formulations using IBM SPSS software version 22 and Sigma Plot software, version 12 where, differences at P -value < 0.05 at ($\alpha = 0.05$) considered to be significant statistically.

Conclusion

This study focus on the importance and how to increase the efficiency of heat transformation method by using the assistance of SDS capped gold nanoparticles in plasmid delivery by using *E. coli* competent cells prepared with calcium chloride standard method. While most of other studies used nanoparticles assistance in other methods of transformation such as electroporation. Also, this work studies the effect of changing the size of nanoparticles ($\approx 15\text{nm}$ & 25nm) and the experimental results indicates that these factors had an effect on the resulted transformation efficiency while other studies only deals with only one nanoparticle and determine its effect on transformation efficiency. Moreover, in this work the used nanoparticles were administrated in the transformation experiment by two different sequences either mixed first with plasmid DNA (as in 1st administration route) or mixed with the competent cells instead of plasmid DNA (as in 2nd administration route) and compare the results which determines that the 2nd administration route gives higher transformation efficiency especially in 15nm nanoparticle size while other studies use only one route of nanoparticles administration and did not make comparative analysis with different factors as this work. (As indicated in "Table 2" which compare this work with other studies). It was found that the transformation efficiency not only depends on gold nano-particles size but also on the sequence of addition of nanoparticles through the transformation process (route of NPs administration) greatly affects the transformation efficiency. So, the use of SDS capped gold nanoparticles will increase the use of calcium chloride method for competent cells preparation with heat shock transformation method of *E. coli* cells. Therefore, it will result in facilitating gene delivery into cells, which is an essential step in different fields for example gene therapy and genetic engineering. Therefore, at this stage it is advisable to study the particle cell interaction with respect to the type and size of material used. Further research is encouraged to study the mechanism of action of this new transformation protocol as this study might help elucidate basic

concepts in DNA delivery through cellular membranes.

Acknowledgment

The authors would like to thank Cairo University Vice President Office for Research Funds for their financial support.

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