European Journal of Pharmaceutics and Biopharmaceutics 165 (2021) 271–278

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Gold nanoparticle synthesis in contact lenses for drug-less ocular cystinosis treatment

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ARTICLE INFO

Keywords:
Gold nanoparticles
Nanoparticle synthesis
Soft contact lens
Cystinosis treatment

ABSTRACT

Purpose: To develop gold nanoparticles-loaded contact lens ("GoldinLens") to bind a significant mass of cystine on the surface of the gold nanoparticles (GNPs) for cystinosis treatment due to the reaction between cystine and gold.
Methods: The GoldinLens was manufactured by synthesizing GNPs inside the preformed contact lens matrix by first loading the lenses (Moist and TrueEye) with gold precursor followed by reduction (with sodium borohydride or trisodium citrate) to gold atoms, which nucleated to GNPs inside the polymeric matrix. The lenses were characterized by SEM, XRD, UV–Vis spectroscopy and mass of GNPs loaded in the lens was determined by direct measurement of mass. Manufactured lenses were soaked in cystine solution for cystine uptake in vitro.
Results: Results show that gold loading in the contact lens increases linearly with gold precursor concentration and number of repetitions of the manufacturing process. The stronger reducing agent sodium borohydride resulted in higher gold loading, with the loading being higher in the Moist lenses due to higher diffusivity of the reducing agent into the lens. However, GNPs were smaller in size and relatively monodispersed in TrueEye GoldinLens, resulting in higher cystine uptake of 47 μg/lens over 24 h (vs. 33 μg/lens for Moist GoldinLens). However, the rate of this uptake was higher for Moist GoldiLens (8.25 vs. 2.35 μg/h), with the maximum uptake occurring in one hour (vs. five hours).
Conclusion: A method for manufacturing GoldinLens, wherein small gold nanoparticles are trapped in contact lenses, has been developed for drugless cystinosis treatment. The lenses withdraw cystine molecules from the surrounding milieu, with the TrueEye GoldinLens being superior for the extent of, while Moist GoldinLens is superior for rate of cystine removal. GoldinLenses of this study can be used for drugless cystine removal cystinosis treatment with one- or five-hour wear at a time.

1. Introduction

Cystinosis is a metabolic disease, which affects around 1 per 100,000 to 200,000 newborns worldwide [1]. This disease is characterized by accumulation of cystine in the body because of the loss of the efflux of cystine from the lysosomes. Accumulated cystine molecules will crystalize in the lysosome, eventually causing cell death [2]. Cystinosis patients also have accumulation of cystine crystals in various ocular tissues, including cornea, conjunctiva, retinal pigment, etc. [1–5]. Different from the cystine crystals in most other organs, the majority of the needle-like cystine crystals are dispersed in the water-like stroma layer of the cornea extracellularly [6]. The ocular symptoms from cystinosis normally begins to show after sixteen months after birth and become more severe without appropriate treatment [7]. Over time, continuous accumulation of cystine crystals leads to photophobia and eventually more serious complications such as corneal scars which could cause irreversible damage and finally blindness [5,8,9].

The current treatment for ocular cystinosis involves instillation of eye drops of cysteamine (β-mercaptoethylamine), which can reduce cystine to cysteine and cysteine-cysteamine, which have higher solubilities in aqueous environment [7,10–14]. Also, cysteine-cysteamine complex can exit the lysosomes through transporters. While

Abbreviations: GNPs, gold nanoparticles; GoldinLens, gold nanoparticles-loaded contact lens; pHEMA/MAA, poly Hydroxyethyl Methacrylate/Methacrylic Acid; FESEM, field emission scanning electron microscopy; UV–Vis, ultraviolet-visible spectroscopy; XRD, X-Ray Diffractometer.

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https://doi.org/10.1016/j.ejpb.2021.05.019
Received 3 February 2021; Received in revised form 21 April 2021; Accepted 19 May 2021
Available online 24 May 2021

efficacious, the eye drops based must be instilled multiple times each day due to the low ocular bioavailability of 1–5%, which could result in poor compliance [12,15,16]. The corneal bioavailability can be significantly increased up to 50% when a contact lens is used as a delivering vehicle [17] affording the possibility of treating cystinosis with a vitamin E loaded contact lens [18]. The presence of vitamin E increases the release duration of cysteamine from a few minutes to a few hours while also showing some additional benefits of improving drug stability [18,19]. The drug released by the contact lens diffuses into the cornea to dissolve the crystals. Here we explore a novel idea of potentially dissolving cystine crystals in the eyes by designing a contact lens with a high affinity for cystine. It is hypothesized that the soluble cystine in the cornea (0.16 mg/mL) will diffuse out into the tears to bind to the contact lens reducing the concentration in cornea, thereby resulting in dissolution of crystals. While quantitative measurements of cystine permeability in cornea have not been reported, multiple publications have shown that cystine can diffuse into the cornea [6,19–23]. It is also feasible to release permeability enhancers from the contact lens to increase the permeability of cornea. Therefore, our goal is to design a novel contact lens with immobilized and stable nanomaterials, which can react with cystine. Amongst all the nanomaterials, we chose GNPs because of biocompatibility and high affinity for cystine [24–28].

GNPs have been extensively explored for many applications including optical devices [29–32], surface-enhanced spectroscopy and catalyst [33,34], biomedical materials [35–37], etc. Recently, GNPs were incorporated into contact lenses for increasing loading of a glaucoma drug timolol [38]. Also, gold nanocapsules have been incorporated into contact lenses for laser protection [39]. Besides, gold loaded contact lenses could also be anti-bacterial which is a very useful feature in a biomedical device [40].

In previous studies, GNPs were incorporated into a contact lens by adding GNPs to the contact lens formulations followed by the free-radical polymerization [41,42]. However, this introduces limitations because GNPs may not be compatible with some monomers which could lead to segregation during polymerization. To address this limitation, the objective of this study was to manufacture a GNP-loaded lens ("GoldinLens") by loading the contact lenses with a gold precursor followed by reduction of the precursor using a suitable reducing agent to synthesize GNPs in preformed commercial contact lenses. This method will allow for incorporation of GNPs into contact lenses made of silicone hydrogel, a common contact lens material. During manufacturing, gold precursor in the contact lens can be reduced by different reducing agents (trisodium citrate and sodium borohydride), which have different capacities of reduction and diffusivities. Both reduction capacity and diffusivity of the reducing agent are expected determine the distribution and the morphology of GNPs in different polymeric matrices. The study characterized the lenses using transmittance measurements, XRD, SEM and measurement of weight increase to determine the mass of nanoparticles. Also, diffusion of gold precursor and reducing agent were measured because of their impact on the reaction dynamics. Finally, the mass of cystine removed by the lenses was determined for the optimized lenses, to establish the use of GoldinLenses as a drugless treatment approach for cystinosis.

2. Methods

2.1. Materials

Tetrachloroauric acid monohydrate ($HAuCl_4 \cdot H_2O$, 99.9985% assay with gold contents >49%) and trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, 99.9%) were purchased from Electron Microscopy Service (Fort Washington, PA, USA), and used without further purification. Sodium Borohydride ($NaBH_4$, ≥98.0%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercial silicone hydrogel contact lenses (Acuvue TruEye) and pHEMA/MAA contact lenses (Acuvue Moist) were purchased from Johnson & Johnson Vision Care, Inc. (Jacksonville, FL, USA).

2.2. GoldinLens manufacturing

The schemes of the GoldinLens manufacturing process are shown in Supplement Material (Fig S1). Commercial contact lenses of two different types (TruEye and Moist) were soaked in the gold precursor for two hours, which is sufficient to reach equilibrium because almost the same amount of gold precursor was released with a longer soaking duration of six hours (Table S1 in supplement material). The lenses were then taken out of the gold precursor solution and gently wiped by KimWipe to remove the gold precursor on the surface of the contact lenses. The gold precursor-loaded contact lenses were subsequently soaked in two different reducing agent solutions (50 mg/mL trisodium citrate and 5 mg/mL sodium borohydride). The GNPs synthesis took only 30 min with sodium borohydride compared to more than 5 h with trisodium citrate. Finally, GNP-loaded contact lenses were soaked in excess water to extract unreacted chemicals and then stored in deionized water for further characterization and use.

2.3. GoldinLens characterization

Optical transmittance of GoldinLens was measured by Avantes UV–Vis spectroscopy (Avantes, Louisville, CO, USA) over 200 to 1000 nm range. The crystallinity of the control and particle loaded lenses was tested with PANalytical PW3040 X-Ray Diffractometer (XRD) (Malvern, United Kingdom). The microstructure of the control and particle loaded lenses was explored using JEOL 7000 FESEM (Akishima, Tokyo, Japan). Contact lenses were fully dried out in an oven and then attached to the SEM stubs with vertical sidewalls (TED PELLA, Inc. Redding, CA) using a carbon tape. A thin carbon film was coated on the contact lens to enhance the conductivity of the contact lenses. SEM images were captured both for the surface and the cross-section.

2.4. Transport of solutes in contact lenses

Transport of the gold precursor and the reducing agent were measured in both contact lenses by loading the molecules from solution followed by release under sink conditions. Contact lenses were first soaked in a 5 mL solution with a known concentration of the chemical reagent until equilibrium. After wiping off excess solution on the surface, these contact lenses were subsequently placed in fresh DI water (3 mL) for measuring the release dynamics. The dynamic concentrations were determined by the measuring absorption spectra in the 200 to 350 nm range.

2.5. Cystine diffusion and binding in GoldinLens

Cystine was dissolved in water to a final concentration of 74.7 μg /mL under continuous stirring and heating. GoldinLens was soaked in a 3 mL cystine water solution and the cystine concentration as a function of time was measured using the UV–Vis absorption of cystine solution in the 250 to 300 nm range. The cystine uptake was assumed to be complete when the UV–Vis spectra did not change with time, which occurred after about five hours for TruEye GoldinLens and about one hour for Moist GoldinLens.

3. Results and discussion

3.1. Effect of different reducing agents

Contact lenses of two different types (TruEye and Moist) were chosen for GoldinLens manufacturing. During the GoldinLens manufacturing process, blank contact lenses were first soaked in gold precursor solution till equilibrium was reached. Next, contact lenses were soaked in a reducing agent solution (trisodium citrate or sodium borohydride

solution) to allow for diffusion of the reducing agents into the lenses to cause reduction of the gold precursor to gold atoms and subsequent nucleation and growth to GNPs. During this step of reducing agent diffusion into the lens, gold precursor is expected to diffuse into the external loading solution where it will be reduced to form gold particles. Visual observation showed that the color of trisodium citrate solution turned red or purple due to the GNPs formation outside the contact lens, whereas the borohydride solution remained colorless. This qualitative observation suggested that the rate of formation of the GNPs in contact lenses is faster with borohydride, resulting in negligible loss of gold precursor to the external solution.

Visual observations of the lenses (Fig. 1) showed that the lenses prepared by sodium borohydride appear more uniform and darker, suggesting a more uniform and higher concentration of particles in agreement.

The above inferences were also supported by optical transmittance measures over a range of wavelengths (Fig. 1). The transmittance data for the Moist lens (Fig. 1(b)) shows that the absorbance decreases significantlyfor both citrate and borohydride reduced lenses and the decrease is significantly more for the borohydride reduced lens over the entire wavelength range. The transmittance data for the TruEye lens (Fig. 1(a)) shows that the absorbance decreases significantly for both citrate and borohydride reduced lenses and the decrease is significantly more for the borohydride reduced lens but only below about 800 nm. Based on the phenomena above, we hypothesize that particle concentration is higher in borohydride lenses and the particles are smaller in size, leading to less scattering at higher wavelengths compared to the citrate reduced lens.

This hypothesis was tested by characterizing the morphology of gold particles in the contact lenses using SEM. Fig. 2 shows that the gold particles in citrate reduced lenses are non-uniform both in size and spatial distribution, while particles in borohydride reducing lens are relatively uniform at about 100 nm in size. In citrate reduced Moist GoldinLens, GNPs were highly polydisperse with sizes ranging from 10 nm to 100 nm and flat gold nanodiscs can also be noticed (Fig. 2(e)). The particles are highly segregated in the citrate reduced TruEye lens, making estimation of size difficult (Fig. 2(b)). Therefore, we can conclude that borohydride reduced lenses have higher and more uniform loading of monodisperse GNPs.

3.2. XRD characterization

The GNP were characterized by XRD to determine the crystal structure. In Fig. 3, XRD patterns of both TruEye GoldinLens and Moist GoldinLens showed identical fcc crystal structure of the gold. The 2θ values of the diffraction peaks were around 38.5°, 44.3°, 65.5°, 78.6° and 82.2° degrees, which represented (111), (200), (220), (311) and (222) crystal planes, respectively. The XRD of Moist contact lens showed a broad peak at 27.2° signifying partial crystallinity in the Moist contact lens.

3.3. Mass loading

The density of GNPs in the contact lenses can potentially be increased by either increasing the concentration of gold precursor solution or repeating the manufacturing process multiple times. The mass of GNPs loaded in the lenses was determined by measuring the weight of the dehydrated GoldinLenses (Fig. 4) after loading both types of lenses using gold precursor solutions ranging from 0.25% to 1% and repeating the manufacturing process for up to 4 cycles. The mass increase in each lens was linearly related both to the gold precursor concentration and number of repeated cycles. The slope of the weight gain per cycle and the precursor concentration is higher for the Moist lens compared to TruEye lens. The results show that 0.12–0.50 mg GNPs form in TruEye lens with each cycle when the concentration of gold precursor solution was changed from 0.25% to 1%. The mass of GNPs was higher in Moist lens with 0.32–0.94 mg GNPs per manufacturing cycle when the concentration of gold precursor solution ranged from 0.25% to 1%.

3.4. Optical transmittance and GNP morphology

The GNP concentration in the lens impacts the transmittance (Fig. 5). The transmittance of the GoldinLens decreases with increasing concentration of gold precursor solution (Fig. 5(b)(d)) and the number of manufacturing cycles (Fig. 5(a)(c)). The spectra display absorbance maximum (minimum transmittance) around 530 nm due to the localized surface plasmon resonance (LSPR) from the GNPs. The LSPR peak is sharp at low loadings but becomes broader at high loadings of particles possibly due to broadening of the GNP size distribution. To assess our hypothesis, we increased the GNP density by these two approaches and the morphology of GNPs in the lenses was determined by SEM. An increase in concentration of gold precursor from 0.25% to 1% did not significantly impact the morphology of GNP particles in both GoldinLenses (TruEye and Moist) (Fig S2 in Supplement Material). Thus, with the concentration of the gold precursor increased from 0.25% to 1%, the GNP density increased without significantly affecting the morphology of GNPs. Thus, more GNPs were generated in the contact lens when the concentration of gold precursor solution was increased, which caused the attenuation of the intensity of LSPR. When the manufacturing process was repeated, the morphology of GNPs in the contact lens changed (Fig. 6). As for TruEye GoldinLens, after the first manufacturing cycle, relatively monodisperse GNPs were generated, which were approximately 100 nm (Fig. 6(a)). When the manufacturing was repeated once, new GNPs of a secondary population were formed, which were much smaller than the GNPs of the first population (Fig. 6(b)). After third manufacturing, the majority of GNPs were as small as 10 nm (Fig. 6(c)). As for Moist GoldinLens, around 100 nm GNPs were formed and maintained the same morphology after the first two manufacturing processes (Fig. 6(d)(e)). However, a more complicated gold structure was noticed after the third cycle. From Fig. 6(f), except for the original 100 nm GNPs, complicated gold nanowires and structure are also generated, which are

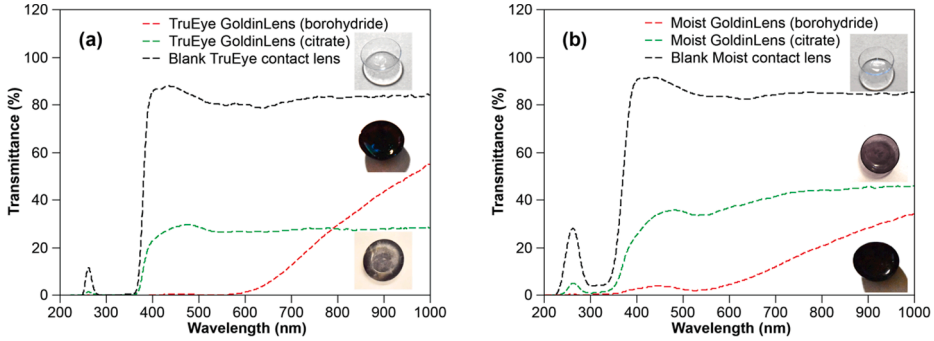

Fig. 1. Pictures of contact lenses (0.5% gold precursor, 1 cycle) and their optical transmittance spectra: (a) blank TruEye contact lens and TruEye GoldinLens; (b) blank Moist contact lens and Moist GoldinLens.

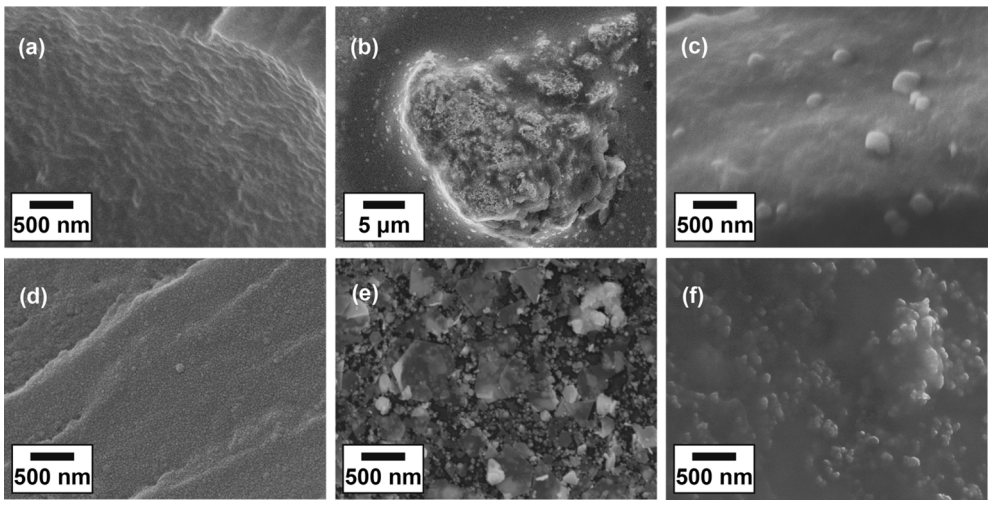

Fig. 2. SEM images of contact lenses and GoldinLenses (0.5% gold precursor, 1 cycle): (a) blank TruEye contact lens; (b) citrate reduced TruEye GoldinLens; (c) borohydride reduced TruEye GoldinLens; (d) blank Moist contact lens; (e) citrate reduced Moist GoldinLens; (f) borohydride reduced Moist GoldinLens.

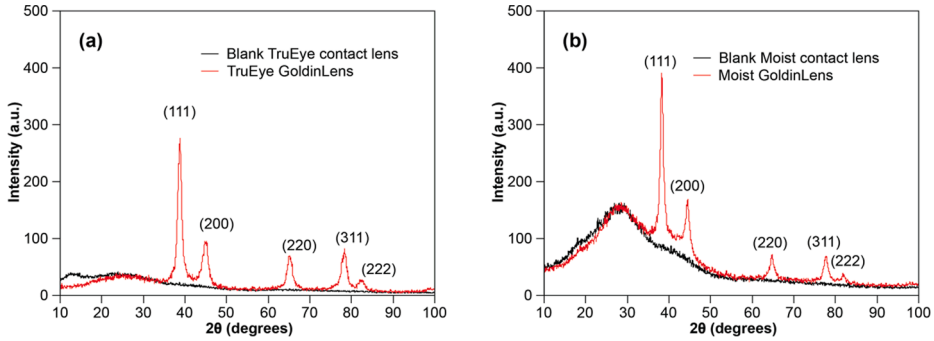

Fig. 3. Crystal pattern of GoldinLens and blank contact lens (0.5% gold precursor, 5 mg/mL sodium borohydride, 1 cycle): (a) Blank TruEye contact lens and TruEye GoldinLens; (b) Blank Moist contact lens and Moist GoldinLens.

uniformly distributed inside the contact lens. Compared with the polymeric structure of blank Moist contact lens, the gold nanowire in the Moist GoldinLens showed similar structure, which matches the voids in the Moist contact lens. Thus, we conclude that greater GNP generation with higher concentration of gold precursor solution causes LSPR attenuation for both GoldinLenses. When the manufacturing process was repeated, more GNPs of different populations led to LSPR attenuation of the TruEye GoldinLenses. With the limitation of the special structure of the Moist contact lens, complicated gold nanowires were formed when the manufacturing cycles reached three, resulting in LSPR attenuation of Moist GoldinLenses.

3.5. *Transport of gold precursor and reducing agent in lenses*

To understand the significant differences in nanoparticle formation between the two lenses, we measured transport of both the gold precursor and the reducing agent (borohydride) in both types of contact lenses. Both lenses were loaded with the two components separately and then soaked in DI water to measure the release dynamics. Considering that both molecules are hydrophilic, the total mass released from the lens can be assumed to be equal to the total mass loaded in the lenses. From Fig. 7, the transport of the reducing agent was considerably faster in the Moist lens compared to the TruEye lens although both lenses were loaded with about 23 μg after soaking in 5 mg/mL sodium borohydride solution. There was considerable difference between gold release from TruEye and Moist contact lens, with about 550 μg released from a TruEye contact lens compared to 300 μg gold from the Moist contact lens. The gold precursor contains about 49% gold atoms by weight and thus based on the measured release of the gold precursor, we expect around 270 μg and 150 μg GNPs should be generated in TruEye and Moist contact lens, respectively. However, this phenomenon contradicted with the result from measurements of increase in mass. From weight measurement (Fig. 4), when contact lenses were soaked in 0.25% gold precursor solution, around 120 μg and 230 μg GNPs were formed in TruEye and Moist contact lens, respectively. The difference between gold precursor loaded and mass of gold particles formed in TruEye can be explained by the slower diffusion of the reducing agent. The diffusion time scale for borohydride is about 10 min in TruEye compared to about a few minutes in Moist lenses. The slower diffusion will result in a reduction in the net reaction rate resulting in higher diffusion of gold precursor from the lens to the bulk solution. The higher amount of gold particles generated in the Moist lenses compared to the measured mass of precursor released can only be explained by assuming that a fraction of the gold precursor remains trapped in the lens. We hypothesize that the gold precursor loaded in the Moist lenses is slowly reduced even in the absence of the reducing agent resulting in formation of gold particles during the uptake and release experiments. This hypothesis is supported by the color of the contact lenses loaded with the gold precursor (Fig S3 in Supplement Material). After 2-hour loading of gold precursor, TruEye lenses loaded with the gold precursor are slightly yellow in color, which is the identical color of the gold precursor, but Moist lenses loaded with the precursor appear purple, signifying reduction of gold to form particles which will not diffuse out from the lenses. After 5-hour release in 5 mL DI water, TruEye contact lens turned from slightly yellow to red, which indicated that some GNPs formed during the release experiment, whereas Moist contact lens remained purple in color. Therefore, we

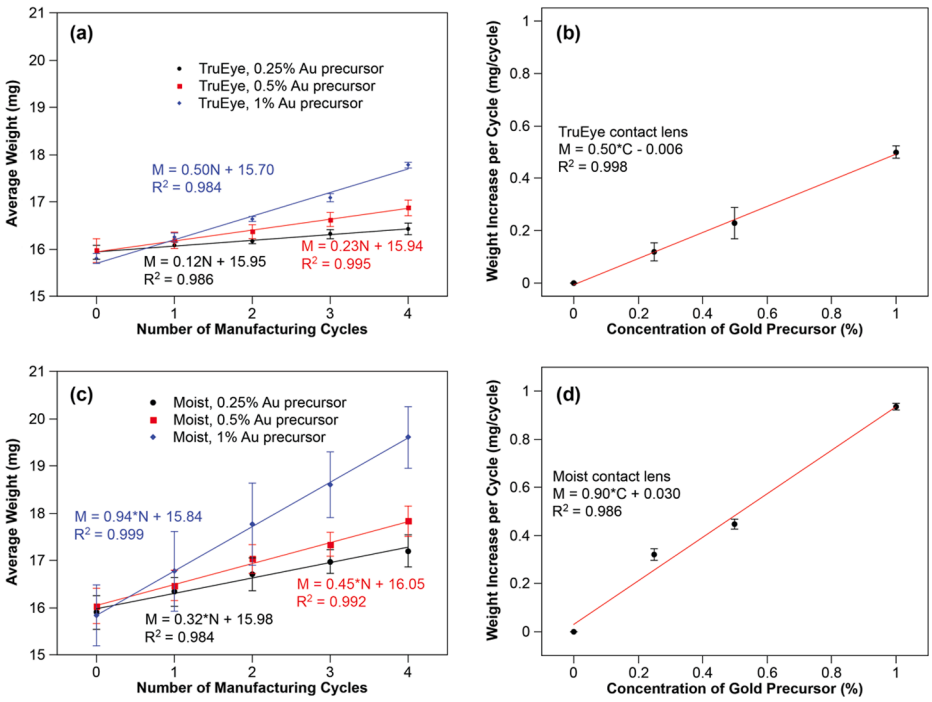

Fig. 4. Average weight of dehydrated contact lenses (reduced by 5 mg/mL sodium borohydride solution): (a) average weight of TruEye GoldinLens when repeating the manufacturing process; (b) weight increase of TruEye GoldinLens per manufacturing cycle; (c) average weight of Moist GoldinLens with repetitions of the manufacturing process; (d) weight increase of Moist GoldinLens per manufacturing cycle.

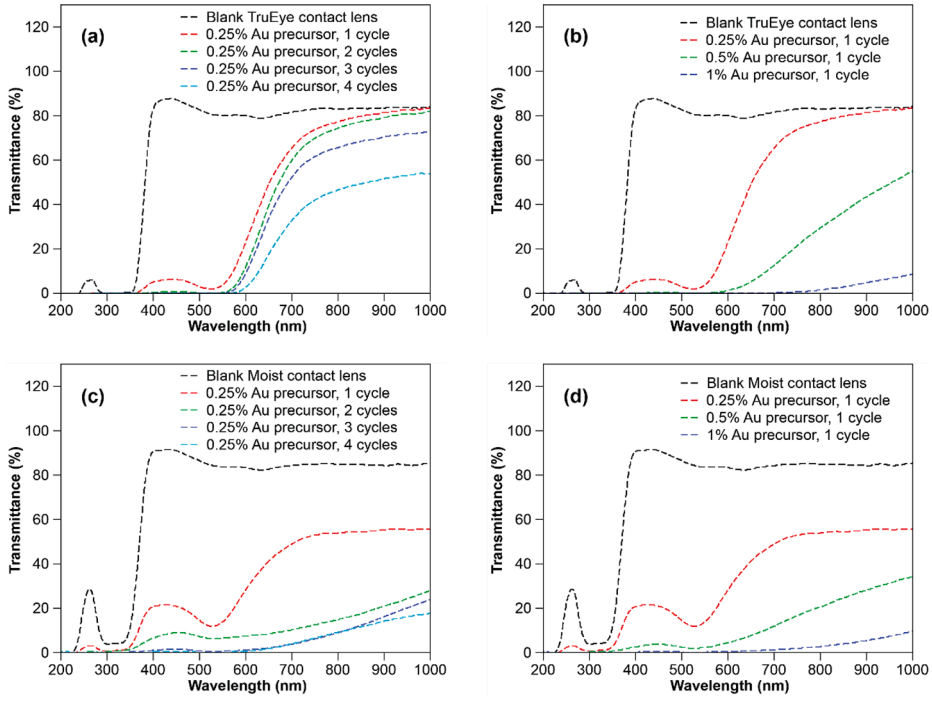

Fig. 5. Optical transmittance of GoldinLeses (reduced by 5 mg/mL sodium borohydride solution): (a) TruEye GoldinLenses with manufacturing repeated; (b) TruEye GoldinLens with concentration of gold precursor increased; (c) Moist GoldinLens with manufacturing repeated; (d) Moist GoldinLens with concentration of the gold precursor increased.

concluded that GNPs would be generated slowly from the reduction of the gold precursor by both contact lens matrices. Our hypothesis can be further supported by the distribution of these GNPs from SEM imaging (Fig S4 in Supplement Material). The SEM images show particle formation in both cases but mostly on the surface for the TruEye, compared to particles distributed throughout the lens thickness for the Moist lenses, showing that gold particles form in Moist lenses during the loading and release process.

3.6. Cystine uptake by GoldinLenses

Contact lenses loaded with GNPs could be useful for several

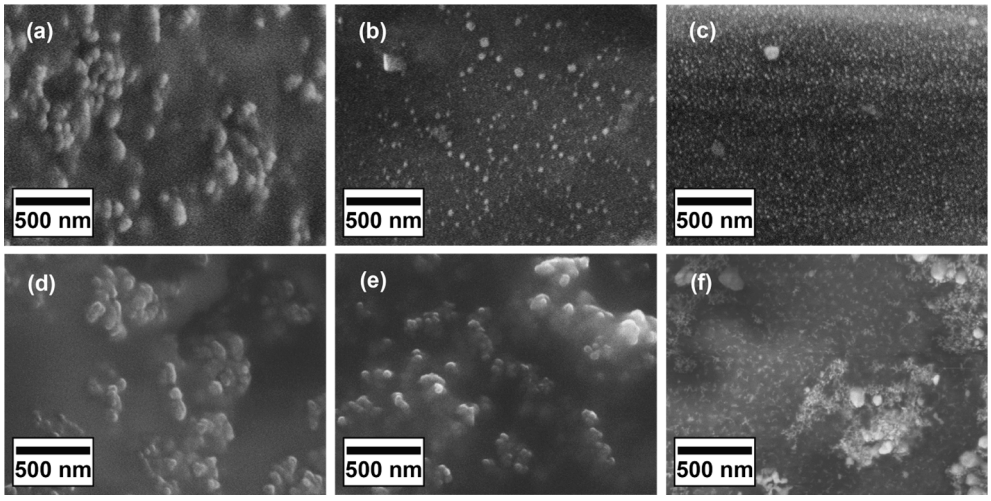

Fig. 6. SEM images of GNPs in the GoldinLenses (0.5% gold precursor, 5% sodium borohydride): (a) TruEye GoldinLens, 1-cycle manufacturing; (b) TruEye GoldinLens, 2-cycle manufacturing; (c) TruEye GoldinLens, 3-cycle manufacturing; (d) Moist GoldinLens, 1-cycle manufacturing; (e) Moist GoldinLens, 2-cycle manufacturing; (f) Moist GoldinLens, 3-cycle manufacturing.

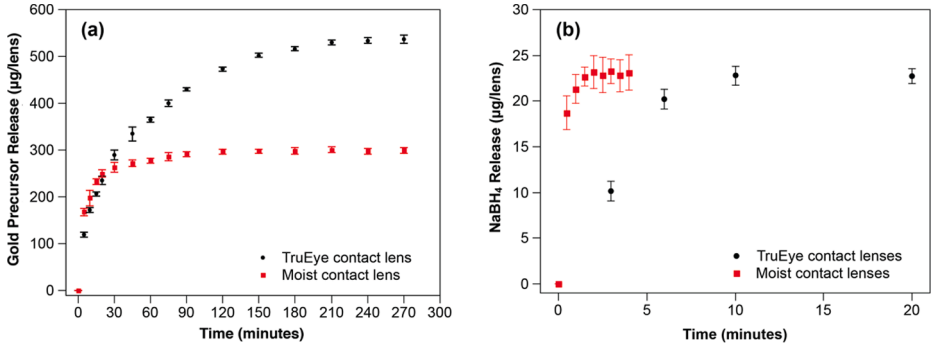

Fig. 7. Chemical reagents release from CLs: (a) gold precursor release profile, loading solution: 0.25% gold precursor solution; (b) sodium borohydride release profile, loading solution: 5 mg/mL sodium borohydride solution.

applications. Our specific goal here is to investigate the feasibility of designing lenses for binding cystine for treating cystinosis. Cystine uptake by lenses was measured by soaking the lenses in cystine solution followed by measuring the change in concentration with time. The amount of cystine taken up by the control commercial lenses was negligible (<1 µg). The mass of cystine taken up by both types of lenses with time is plotted in Fig. 8 for lenses subjected to 4-cycles of GNP manufacturing using gold precursor at 0.5 and 1%. TruEye lenses manufactured using 4-cycles of 0.5% and 1% gold precursor adsorbed 31 µg and 47 µg, respectively, compared with 23 µg and 33 µg, respectively, for the Moist GoldinLens. The higher cystine binding in TruEye despite less mass of GNPs is attributable to the smaller particle size, which results in larger surface area. The duration for reaching the maximum uptake is about an hour for the Moist GoldinLens which is significantly shorter than that for TruEye which is about 5 h. Thus, the rate of uptake is higher for the Moist lens. Under in vivo conditions, cystine will diffuse out from the cornea into the lens only from the posterior side. The equilibration time depends on the thickness and so if diffusion occurs from only one side the effective thickness over which molecules diffuse is twice that for the in vitro transport in which case the

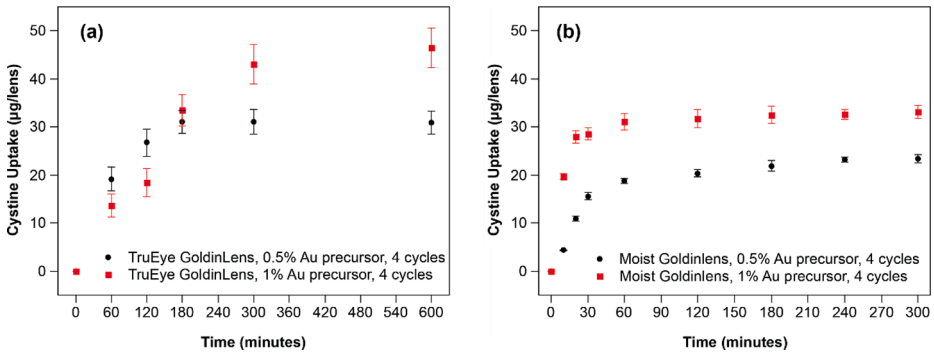

Fig. 8. Cystine uptake profiles of TruEye and Moist GoldinLenses (reduced by 5 mg/mL sodium borohydride solution): (a) cystine uptake of TruEye GoldinLenses; (b) cystine uptake of Moist GoldinLenses.

diffusion occurs from both sides. Since the diffusion controlled time for equilibration scales as square of the thickness, the total time for equilibration in vivo will increase 4-fold compared to in vitro to about 20 and 4 h for TruEye and Moist lenses, respectively. Thus, the average rate of uptake will be about 2.35 μg/hr and 8.25 μg/hr for the TruEye and Moist lenses, respectively.

The solubility limit of cystine is 0.16 mg/mL [43], while the total concentration of cystine crystals in the cornea could be as high as 1–10%. Cysteamine is currently delivered through hourly instillation of 0.55% (50 mM) eye drops. The concentration of drugs in the eye drops is limited to 0.55% because higher concentrations cause toxicity to the eyes [3,44,45]. The known bioavailability of other drugs typically ranges from 1 to 5% for delivery by eye drops [46]. Thus each 0.55% 25 μL cysteamine drop would be expected to deliver about 1.1–6.5 μg to the cornea. With an hourly eye drop instillation over 8 h, about 9–50 μg of drug will reach the corneal tissue each day which will lead to dissolution of about 27–150 μg of cystine crystals. The lenses developed here absorb about 50 μg of cystine suggesting further improvements may be useful to increase cystine binding.

The rate of cystine transport through cornea into tears can be estimated as $kC_{sol}A_{cornea}$, where k is the permeability of the cystine through cornea, C_{sol} (=0.16 mg/mL) is the solubility limit of cystine in water at neutral pH [43] and A_{cornea} (=1.3 cm^2) is the area of the cornea available for diffusion. The permeability across epithelium k is unknown for cystine, but it typically ranges from 10^{-6} to 10^{-8} m/s for small hydrophilic drugs. The epithelial permeability for timolol, a small hydrophilic drug (316 Da MW) is reported to be 1.2×10^{-7} m/s. Considering that cystine will likely follow the same paracellular diffusion path as timolol, we expect the corneal permeability of cystine to be about 10^{-7} m/s [47,48]. Based on the permeability of $1.2x10^{-7}$ m/s, the rate of transport of cystine across the cornea will be about 9 μg/hr (=215 μg/day), which is comparable to the rate of cystine uptake into the Moist GoldenLens. Based on these estimates, it would appear that 24-h or 5-h wear each day may be needed to extract cystine at rates comparable to the rates of dissolution achievable with cysteamine based eye drop therapy. The exact time depends on the permeability of cystine in cornea and bioavailability of cysteamine. This rate could be increased considerably by slow release of permeability enhancers from the contact lenses. While use of permeability enhancers in not optimal due to toxicity concerns, it is noted that current commercial formulations contain benzalkonium chloride which is a well-known preservative and permeability enhancer. Continuous wear of GoldinLens is not feasible because of the very low transparency but overnight wear may be feasible as is the case for commercialized OrthoK therapy. The lenses prepared in this study require one or five hours of wear for maximum effect, which is feasible during nighttime. Also, the wear duration could potentially be increased if lenses could be made transparent which is possible by using alternative approaches for binding cystine in the lenses. Future studies should be aimed at developing lenses that have a greater capacity for cystine removal with single wear.

4. Conclusions

Here, we developed a method for manufacturing GNPs-loaded contact lens (GoldinLens) by reduction of gold precursor loaded in the lenses. The approach was developed for two different types of commercial contact lenses and using two different reducing agents. The approach was more effective with trisodium citrate compared to sodium borohydride because sodium borohydride is a stronger reducing agent, which allows a rapid reaction and nucleation during the manufacturing. Therefore, generated GNPs are uniformly distributed inside the contact lens with less loss of the gold precursor when sodium borohydride is chosen as the reducing agent. The density of GNPs in the contact lens increases linearly with gold precursor concentration and repeating the manufacturing process multiple times. The mass of GNPs is more in Moist contact lens compared with TruEye because of higher diffusivity of the reducing agent which results in a faster reaction minimizing loss of the gold precursor. We explored the application of these GoldinLenses for cystinosos treatment due to the high affinity of cystine to the gold. The TruEye GoldinLens manufactured using 4-cycles with 1% precursor absorbed about 47 μg cystine in 5 h compared with 33 μg cystine uptake in 2 h for Moist lenses manufactured with the same approach. Based on estimates of cystine permeation across the cornea, it may be possible to extract 50 μg of cystine during overnight wear of the TryEye GoldinLens which may be clinically significant. While the approach of using contact lenses to bind cystine seems promising for treating cystinosis, further optimization may be helpful in increasing the rate of binding and making the lens more appealing to patients by increasing transparency which may be feasible by dispersing smaller size gold nanoparticles. Also, safety of the lenses will need to be established before finally testing efficacy in suitable animal models and in humans.

Funding

This research was funded by the CMMI program of the National Science Foundation, grant number: 1762625.

CRediT authorship contribution statement

Zhen Liu: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft. **Uday B. Kompella:** Writing - review & editing, Visualization. **Anuj Chauhan:** Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We acknowledge financial support from the CMMI program of the National Science Foundation (Grant number: 1762625)

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