Penetration of Silicate Nanoparticles into the Corneal Stroma and Intraocular Fluids

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Purpose: The aim of this study was to investigate transmission of topical silicate nanoparticles (SiNPs) through the corneal stroma, anterior chamber, and vitreous fluids by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and inductively coupled plasma atomic emission spectrometry (ICP-AES), respectively.

Methods: SiNPs with a mean diameter of 40.6 ± 5.6 nm determined by dynamic light scattering were used in this study. The permeability of SiNPs was examined across isolated corneal buttons over a 30-minute period. To visualize the transport and diffusion of nanoparticles through the corneal tissue, SiNPs were applied over the corneal surface and evaluated at 5 and 30 minutes after SiNPs loading for SEM and 15 minutes for TEM. Sections of 10-μm thickness were cut and visualized using SEM. TEM study was performed on 70- to 90-nm-thick sections. ICP-AES was used to determine the concentration of SiNPs.

Results: The determined range of synthesized SiNPs by dynamic light scattering was 40 nm (41.9 ± 5.6 nm). Transmission of SiNPs through the corneal stroma was shown successfully with electron microscopic (SEM and TEM) images. The ICP-AES results revealed SiNPs in the anterior chamber and vitreous fluid.

Conclusions: Topical administration of SiNPs, as a noninvasive, and available modality with acceptable penetration through the corneal stroma and deep into the intraocular fluids including the anterior chamber and vitreous cavity, may be considered as a suitable alternative to invasive intravitreal injection of other expensive antineovascularization agents.

Key Words: transmission, topical silicate nanoparticles, antineovascularization agent, corneal stroma, intraocular fluids

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Nanotechnology has entered the field of medicine in recent decades and is used in different medical fields including diagnosis, biosensors, and drug delivery, and has thus provided novel nanomedicines and nanodevices.1–3 Nanomedicines use nanoscale technology for the treatment and prevention of diseases, which can pave the way for novel ophthalmologic therapeutic application with the ultimate goal of improving the quality of vision, and finally the quality of life. Although new drugs have been recently developed within the field of ophthalmology, drugs administered systematically have poor access to the inside of the eye because of the cornea, which acts as an effective barrier to drug penetration by completely surrounding and effectively sealing the superficial epithelial cells. Many nanostructured systems have been used for ocular drug delivery and have yielded some promising results.4

The corneal epithelium has a lipophilic nature with tight junctions between cells to restrict paracellular drug permeation.5,6 Usually, eye drops drain rapidly from the ocular surface and, therefore, the time for drug absorption is only a few minutes, which decreases bioavailability. Indeed, less than 5% of topically administered drugs reach intraocular tissues.7 Systemically administered drugs have poor access to the retina and corneal stroma because of the blood–ocular and blood–retinal barriers.8

Ocular neovascularization (NV) is a major sight-threatening condition and is caused by ischemia, infections, degenerative disorders, inflammation, and other traumatic insults and may lead to decreased vision.9,10 Multiple treatment modalities including laser photocoagulation, photodynamic therapy, fine-needle diathermy, conjunctival limbal grafts, and anti–vascular endothelial growth factor (VEGF) injections were used for the management of ocular NV.11–16

In this study, we investigated the ability of topical silicate nanoparticles (SiNPs) to pass through the corneal tissue and intraocular fluids by electron microscopy and inductively coupled plasma atomic emission spectrometry (ICP-AES), respectively, as a presumed anti-VEGF agent.

MATERIALS AND METHODS

Materials
t-arginine and tetraethoxysilane [(TEOS), reagent grade, ≥98%] were purchased from Sigma-Aldrich (St Louis, MO). Cyclohexane (99%) was obtained from Merck. Distilled...
deionized water was used for the preparation of all aqueous solutions. Human corneal buttons in Optisol solution were supplied by the Eye Bank of Islamic Republic of Iran to investigate the penetration of SiNPs into the corneal stromal tissue for the scanning electron microscopy (SEM) study. A fresh, intact, enucleated bovine globe was cleaned of extraneous tissue and was used for the evaluation of SiNPs penetration to the anterior chamber and vitreous fluid by ICP-AES method.

**Devices**

Fourier transform infrared spectroscopy was performed to indicate the hydrolysis of TEOS to SiO$_2$ in prepared nanoparticles. The mean particle size was determined by dynamic light scattering (DLS), and the morphology of the nanoparticles was investigated with the help of SEM. Cross sections were prepared through cryosections at 10-μm thickness. However, transmission electron microscopy (TEM) was performed to confirm the transmission of SiNPs through the corneal stroma. Additionally, ICP-AES was used to determine the concentration of the SiNPs suspension.

**Methods**

**Nanoparticles Preparation**

A two-phase nucleation method$^{17, 18}$ was applied as a simple synthetic route for producing ultra-monodisperse silica nanoparticles. We used TEOS as an organic layer above the aqueous solution to limit the increased rate of silica monomer. In brief, 20 mg of l-arginine as catalyst was dissolved in 40 mL of water. To keep the aqueous phase undisturbed, 3 mL of cyclohexane and 3 mL of TEOS were added subsequently.$^{17}$ To form silicate spheres, solutions were slowly stirred for 24 hours at 70°C. The stirring rate was fixed such that the top organic layer was left almost undisturbed, and the water phase was well mixed. The size and size distribution of SiNPs were determined by DLS. The mean particle size of SiNPs was 40.6 ± 5.6 nm.

**Investigation of Intraocular Penetration of SiNPs**

The whole bovine globe was floated on SiNPs of concentration 1 mg/mL for 24 hours. After rinsing the globe with normal saline solution, 0.1 mL of the anterior chamber and vitreous fluid was tapped with a 23-gauge needle and was sent for detecting SiNPs and determining concentration. ICP-AES was used to determine the concentration of the SiNPs suspension.$^{19}$

**Investigation of Penetration of SiNPs to Human Corneal Stromal Tissue by SEM**

We used in vitro permeability studies to determine whether nanoparticles can penetrate through the cornea. To visualize the transport and diffusion of nanoparticles through the corneal stromal tissue, SEM was applied. After preparing corneal buttons, they were exposed to the nanoparticle suspension for 15 minutes and then embedded in optimal cutting temperature medium for frozen sections. Sections of approximately 10 μm in thickness were prepared and visualized by SEM. Further in this study, isolated corneal lenticules were exposed to 1 μL of nanoparticle suspension (1 mg/mL) for 5 and 30 minutes (on the epithelial side). Surfaces of the samples were then investigated by SEM.

**Investigation of Penetration of SiNPs to Human Corneal Stromal Tissue by TEM**

After 15 minutes of exposure to 1 μL of nanoparticle suspension (1 mg/mL), the tissue of interest was removed from the corneal lenticules and was placed in the primary buffered fixative solution of glutaraldehyde. Then, the samples were infiltrated with propylene oxide. For preparation of corneal tissue cross sections by ultramicrotomy, the corneal tissue was embedded in an epoxy resin. Then, 70- to 90-μm-thick sections were cut with a knife with a speed of 1 mm/s. Finally, the sections were stained with uranyl acetate and lead citrate, and then picked up on a lacy carbon–coated copper grid and were examined with TEM.$^{20}$

**RESULTS**

**Nanoparticles Size and Morphology**

SEM images showed spherical shapes of particles. TEM images confirmed spherical shape of SiNPs in more detail (Figs. 1A, B). The presence of SiO$_2$ peak (bands appearing at 1109/cm and 473/cm) in nanoparticle Fourier transform infrared spectroscopy plot demonstrated appropriate hydrolysis of TEOS in prepared nanoparticles (NPs) (Figs. 2A–C). According to DLS results, the mean size and polydispersity index of the prepared SiNPs were 40.6 ± 5.6 and 0.312 ± 0.11, respectively (Fig. 3).

**Investigation of Penetration of SiNPs Through the Corneal Stroma**

SEM and TEM images revealed that SiNPs in the above range were successfully transmitted to the deep corneal stromal tissue (Figs. 1, 4). SEM images of untreated (Figs. 5A) and treated corneal lenticules with SiNPs after 5 minutes (Figs. 5B) and 30 minutes (Figs. 5C) of treatments revealed penetration of SiNPs through the corneal epithelium. As seen in these figures, the density of SiNPs is significant after 5 minutes of exposure to the NPs (Fig. 5B) and markedly decreases after 30 minutes of exposure (Fig. 5C), which is similar to the virgin untreated corneal tissue (Fig. 5A).

**Investigation of Penetration of SiNPs to Anterior Chamber and Vitreous Fluids**

Anterior chamber and vitreous fluids both yielded positive results for SiNPs detected by ICP-AES. The concentrations of SiNPs in the anterior chamber and vitreous fluids were 0.44 mg/L and 0.03 mg/L, respectively.

**DISCUSSION**

The bioavailability of an instilled conventional drug onto the ocular surface is usually low, most of which are lost because of physiological mechanisms, such as tear drainage...
and blinking, a few seconds after instillation.\textsuperscript{21,22} Therefore, there is limited absorption of drug and limited access to intraocular tissues.\textsuperscript{23} Indeed, NPs offer the possibility of more feasible drug delivery and transport across tissues.

The intrinsic capacity of NPs to adhere to the ocular surface and interact with the epithelium has stimulated researchers to focus on therapeutic applications of NPs in ophthalmology. The possibility of controlled release of drugs

\begin{figure}
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\caption{A, SEM images of prepared SiNPs. B, TEM images of treated cornea with SiNPs (1 mg/mL) after 15 minutes of treatment.}
\end{figure}

\begin{figure}
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\includegraphics[width=\textwidth]{spectroscopy.png}
\caption{Fourier transform infrared spectroscopy plots of standard SiO\textsubscript{2} nanoparticles (A), arginine (B), and prepared SiNPs containing arginine as the catalyst (C).}
\end{figure}
that surpass the ocular barriers and effectively reach the target makes them applicable to treat eye diseases. Nanoparticle systems improve the delivery of poorly water-soluble drugs and significantly reduce toxicity compared with the free drug. There are several different modalities for ocular drug administration, including topical applications such as eye drops, subconjunctival or sub-Tenon, and intravitreal injections.

NPs of various molecular sizes, such as gold and silver, have been reported to have antiangiogenic effect on pathological NV. SinPs have been used in drug delivery, gene therapy, biolabeling, and in combination with other treatment modalities. Some characters of nanosized silica including size, size distribution, and morphology are of great importance for their applications. The large sizes are usually not effective for biomedical applications because the cell uptake will be limited. Another important requirement for biomedical application of SinPs is their aqueous suspension. One significant challenge for the successful development of therapeutic nanoparticles is rapid clearance during systemic delivery. Therefore, the factors that could affect the clearance and biodistribution of nanoparticles, such as particle physicochemical properties and targeting legends, should be carefully considered for the optimal design of therapeutic nanoparticles. Jo et al. demonstrated that SinPs exhibited negligible acute toxicity in retinal neuronal cells, retinal endothelial cells, and the retinal tissue at concentrations 100 times more than the effective therapeutic dosage. Also, they reported that SinPs had antiangiogenic effects.

Other reports suggested antiangiogenic effects of 50-nm gold and silver NPs. These results suggest that the concentration, shape, or size of NPs could be the key factors exerting their antiangiogenic effects. Because of tight junctions between the corneal epithelial cells, the small size of nanoparticles will be effective in increasing drug penetration into the deep corneal stromal layers, which suggests its efficacy. The physicochemical characteristics of SinPs are critical for cellular uptake, intracellular trafficking, and interaction with plasma proteins. Nonetheless, the biodistribution of NPs should be addressed for their biomedical application. Therefore, biodegradation and biodistribution of NPs should be investigated before clinical application.

It is presumed that SinPs are able to inhibit phosphorylation of ERK 1/2, a signaling molecule of the MARK pathway, not that of AKT. Furthermore, the mass production of SinPs may be more feasible and cost-effective than monoclonal antibodies such as Avastin or bevacizumab. Also, SinPs could have modifiable size and concentration. Using topical, subconjunctival, and corneal intrastromal injection of SinPs as an anti-VEGF therapy seems to be effective in suppressing new vessel-formation and vascular leakage, which can improve visual function.

According to the above-mentioned evidence and the proven antiangiogenic effects of intravitreal injection of
SiNPs as a nontoxic agent, it seems reasonable that topical administration of SiNPs can be a novel treatment for corneal NV. Because it could also be detected in the anterior chamber and vitreous fluid after topical administration, it may also be investigated for noninvasive management of choroidal NV with minimal side effects.

Since the concentration, shape, and size of SiNPs could be the key factors exerting their antiangiogenic effects, we recommend 40-nm-scale SiNPs to enhance their ability to pass through the corneal tight junctions and reach the new vessels in the corneal stroma and then deep into the interior eye. Further studies can be performed on the efficacy of SiNPs on other ocular segments to control neovascular glaucoma and retinal NV due to age-related macular degeneration, diabetic retinopathy, and vascular occlusive disorders.

In conclusion, this study shows that SiNPs have acceptable permeability through the corneal epithelial tight junctions and through the corneal stroma documented by SEM and TEM studies. Further studies should be conducted to show the safety and efficacy of SiNPs as a novel modality in the prevention and treatment of ocular NV.

REFERENCES