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공학석사학위논문

Iontophoretic transport of *poly*(lactic-co-glycolic acid) nanoparticles across in vitro rabbit cornea

이온영동방법을 통한
poly(lactic-co-glycolic acid)나노 입자의
토끼 각막으로의 전달양상

2017년 2월

서울대학교 대학원
협동과정 바이오엔지니어링전공

이재연

Abstract

Iontophoretic transport of *poly*(lactic-co-glycolic acid) nanoparticles across in vitro rabbit cornea

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College of Engineering,

The Graduate School

Seoul National University

Iontophoresis is a non-invasive technique used to transport substances of interest across tissues and this has drawn interest in ophthalmic fields to enhance delivery efficiency of topically administered drugs. Thus, there have been numerous trials to deliver small molecules across cornea into the eye using iontophoresis; however, few were reported to transport the nanoparticles. The purpose of this study was, therefore, to profile an iontophoretic transport of the nanoparticles made of a biodegradable polymer, *poly*(lactic-co-glycolic acid) (PLGA),

where the various conditions of iontophoresis were applied via the cornea of in vitro rabbit eyes. Also, iontophoresis was conducted on the simulated eyelid to minimize the potential problems caused by direct contact of an electrode to the sensitive eye surface. For this, the tablet formulation of fluorescence-tagged PLGA nanoparticles was applied onto the cornea, where the factors, such as the size of nanoparticles, amplitude of electric current and time for iontophoresis application, were varied. After the iontophoretic application of the nanoparticles, the fluorescence intensity of each of the cross-section layers of the cornea was observed with confocal fluorescence microscopy to assess the distribution of PLGA nanoparticles. The results show that there are significant differences in the delivered amount of PLGA nanoparticles into cornea according to the size of PLGA nanoparticles and time of formulation applications. Importantly, the particle size was observed to be one the most crucial factors in determining the transport of PLGA nanoparticles across cornea. In this study, however, although the intensity of electric current was varied at 0 mA to 0.5 mA, 1 mA and 2 mA, there were no statistically significant differences among the delivered amounts of PLGA nanoparticles. Under the iontophoresis conditions employed in this study, it appeared that the electric field was not properly focused on the cornea and the

surface charge of the nanoparticles was not very high (~ 2 mV). Therefore, transport of PLGA nanoparticles across in vitro cornea was mostly mediated by particle diffusion.

Keyword : cornea, diffusion, in vitro, iontophoresis, PLGA nanoparticles

Student Number : 2015-21218

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Chapter 1. Introduction

1.1. Study Background

Effective drug delivery to ocular tissues has been a challenging issue [1]. Due to a major physiological barrier in the eye, i.e., the cornea, only a small amount of drug can reach a target region with conventional drug delivery methods [1, 2]. Topical drug formulations, such as a solution, gel and ointment, are the forms mostly accepted for drug therapy for ocular diseases and they are preferred by patients because of their easy application [2, 3]. In spite of convenience, topical drug administration shows low drug bioavailability and thus, often requires multiple administrations to meet therapeutic efficacy [4, 5]. In case of gel and ointment, they increase residence time of drug hence enhancing drug bioavailability, yet it should be also applied multiple times and there could be a chance of applying wrong dosage and blurry vision due to nature of a dosage form [3, 6]. An oral administration and intravitreal injection are an alternative way of drug delivery to the eye, however it can cause systemic toxicity, pain and side effects [7, 8]. These limitations of conventional ocular drug delivery methods lead researchers to look for a better strategy for ocular drug delivery and one of them would be an iontophoresis-mediated drug delivery.

Iontophoresis is a non-invasive technique which involves low electrical current to control the delivery of substances across tissues [8, 9]. It has been extensively used in transdermal field because of its simple application, low possibility of side effects and high delivery efficiency to the targeted location [8, 10]. However, there have been attempts to apply an iontophoresis to ophthalmic field to overcome limits of conventional treatments [8, 11, 12]. Furthermore, iontophoresis technique combined with drug carrier technologies such as a dendrimer, liposome and nanoparticle [13, 14] are studied to seek synergetic effects and improved ocular drug delivery [7, 14, 15].

Even though an ocular iontophoresis has less chance of side effects than traditional treatment methods, it still can cause several side effects such as an epithelial edema, burn, pain and inflammation [8, 16, 17]. These side effects are caused due to non-optimized iontophoresis conditions. Moreover, in most previous trials, iontophoresis was applied directly to the preocular surface, where an electrode was placed on the open eye. This can lead cause patients inconvenience and sometimes damages to the sensitive eye surface [8].

1.2. Purpose of Research

In this study, a modified strategy of iontophoresis was proposed, where an electrode was applied on the eyelid to avoid the possible problems caused by a direct-contact ocular electrode. Also, I associate this indirect iontophoresis with a nanoparticle technology by applying poly (lactic-co-glycolic acid) nanoparticles on the cornea surface, envisioning various drug delivery patterns incorporated with drug-loaded nanoparticles. Polymeric nanoparticle has been widely investigated due to the advantages in controlled drug release, and enhanced drug stability and bioavailability [3, 7, 18, 19]. Especially, PLGA nanoparticles were extensively studied due to their biocompatibility and biodegradability [11, 20]. Moreover, as the characteristics PLGA can be easily modified by controlling the ratio of monomers, various patterns of drug release can be obtained and a relatively wide range of drugs can be encapsulated [21, 22].

While there are numerous reports about transport of drug carriers through cornea tissue after direct iontophoresis, a detailed analysis on movement of PLGA nanoparticles within cornea and study on iontophoresis with an electrode applied on the eyelid were scare [23-25]. Therefore, this study pursued to assess transport of PLGA nanoparticles through the eye with various iontophoretic

conditions, especially with an electrode attached on the eyelid. Three distinct factors were varied; size of PLGA nanoparticles, intensity of electric current and time of iontophoresis applications. Then, the distribution of delivered PLGA nanoparticles was evaluated semi-quantitatively by analyzing the confocal fluorescent images of the cornea and the effect of each varying factor was investigated.

Chapter 2. Materials and Methods

2.1. Materials

Polyvinyl alcohol (PVA) was purchased from Sigma (MO, USA). Whole rabbit eyes of albino rabbit were purchased from Pel-Freez Biologicals (AR, USA). PLGA nanoparticles tagged with Green fluorescence with sizes of 100 nm and 200 nm were obtained from Phosphorex (MA, USA).

2.2. Preparation of tablet formulation of PLGA nanoparticles

A dry tablet of PLGA nanoparticles were prepared following the protocol reported in the previous study [26]. Briefly, 2.5 mg of green fluorescence PLGA nanoparticles were dispersed in 0.25 % w/v PVA to prepare the PLGA nanoparticle, 250 ㎕ of which was dropped into a mold and frozen with liquid nitrogen for about 1 h. After being fully frozen, the resulting mold was freeze-dried for 24 h.

2.3. Characterization of **PLGA nanoparticles**

To examine a size and morphology of nanoparticles, scanning electron microscopy (SEM; JSM-7800F Prime, JEOL, Japan) was performed. Before SEM analysis, PLGA nanoparticle was dispersed in PBS (pH 7.4), a droplet of which was placed on a silicon wafer and dried in room temperature for 24 h. Then, the sample was sputter-coated with platinum for 1 min. Also, a zetasizer (Nano ZS system, Malvern, UK) was used to measure the size and zeta-potential of PLGA nanoparticles before and after a tablet formation process.

2.4. *In vitro* experimental setup

In this study, whole eyes of albino rabbit were employed, which were stored at - 80 ℃ until use. To thaw the eyes, they were left at 4 ℃ for over 3 h and at room temperature for an additional 1 h in a wet condition. To simulate the presence of the eyelid, a porcine skin tissue with the thickness of 1.8-2.3 mm, purchased from a local butcher shop, was immersed in HBSS for 1 h. The eye was then placed and fixed on a custom-made holder and a ground electrode was attached to the site of an optic nerve, as shown in Fig. 1. Then, a PLGA nanoparticle tablet was placed on top of the cornea,

on top of which a square-shaped piece of porcine tissue (2 cm x 2 cm) was placed to simulate the eyelid. Finally, a cathode electrode was attached on the tissue. A conductive gel (Super conductive, Sungheung, Korea) was applied between the electrode and tissue or eye surface.

Numerous conditions were applied, using an iontophoresis device (Activadose ll, ActivaTek, Canada) by varying three major factors; size of PLGA nanoparticles, intensity of electric current and time of iontophoresis application (Table 1). In this study, two distinct sizes of PLGA nanoparticles of 100 nm and 200 nm were used, respectively. The electrical current was varied at 0 mA (i.e., no electrical current), 0.5 mA, 1 mA and 2 mA. The iontophoresis was applied for three distinct periods of 5 min, 20 min and 60 min.

2.5. Semi-quantification of delivered PLGA nanoparticles

After iontophoresis, a simulated eyelid was removed and the surface of the cornea was gently washed with 10 ml of HBSS solution. Then, the cornea was enucleated from the eye and washed with HBSS solution again. The extracted cornea was imaged with a confocal laser scanning microscope (Leica TCS SP8, Leica, Germany), where the stacked fluorescence images were collected from five different areas from a single cornea. All images were

obtained under the same condition. To semi-quantitatively analyze the amount of PLGA nanoparticles permeated into the cornea, a total gray value was obtained with each of the fluorescence images.

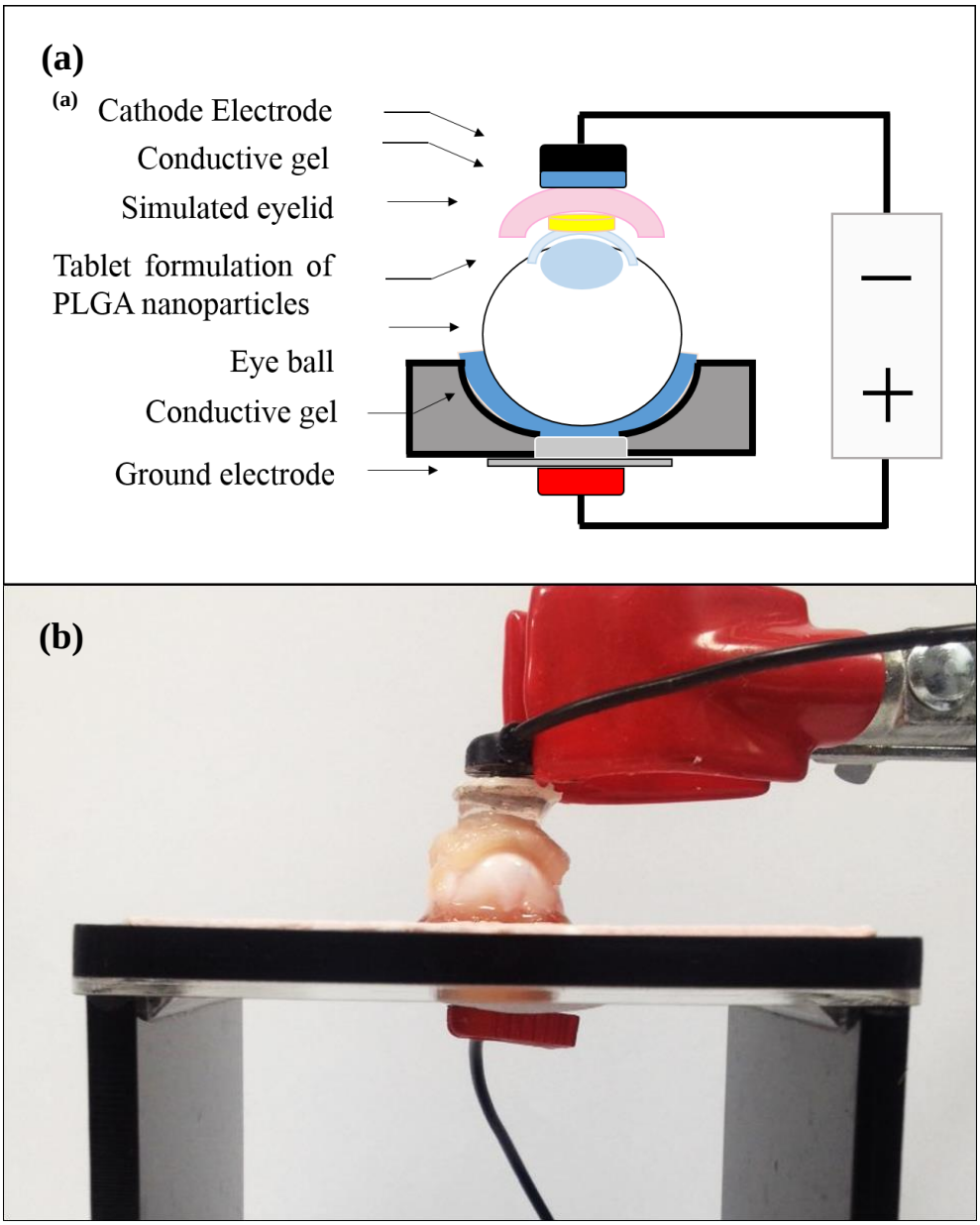


Figure 1. In vitro experimental setup for iontophoresis to a whole rabbit eye, using a tablet formulation of PLGA nanoparticles. (a) Schematic illustration and (b) optical image of the setup.

Size of PLGA nanoparticles (nm)	Current intensity (mA)	Time of application (min)
100	0	5
		20
		60
	0.5	5
		20
		60
	1	5
		20
		60
	2	5
		20
		60
200	0	5
		20
		60
	0.5	5
		20
		60
	1	5
		20
		60
	2	5
		20
		60

Table 1. Experimental conditions for iontophoresis.

Chapter 3. Results

3.1. PLGA nanoparticles characterization

Figure 2 shows the SEM images of the PLGA particles used in this work, all of which exhibited a spherical shape with a relatively narrow size distribution. For 200 nm particles, some larger particles with the size of about 500 nm were seen; however, a majority of the particles were observed to be within the size range at about 200 nm. To further confirm the size distribution of the nanoparticles herein, a zetasizer analysis was performed (Figure 3 and Table 2). As a result, the particle sizes were measured to be 112.4 ± 0.25 nm and 196.8 ± 4.93 nm, respectively (Figure 3(a)). After being formulated in a tablet, the particle size did not vary much, showing 114.2 ± 2.98 nm and 198.1 ± 4.16 nm, respectively (Figure 3(b) and (c)) The zeta-potentials of the particles were measured to be −2.32 ± 0.30 mV and −2.00 ± 0.42 mV for the 100 nm and 200 nm PLGA particles, respectively. Again, after tablet formulation, the zeta-potentials did not vary much, giving - 2.54 ± 0.27 mV and - 2.03 ± 0.27 mV, respectively.

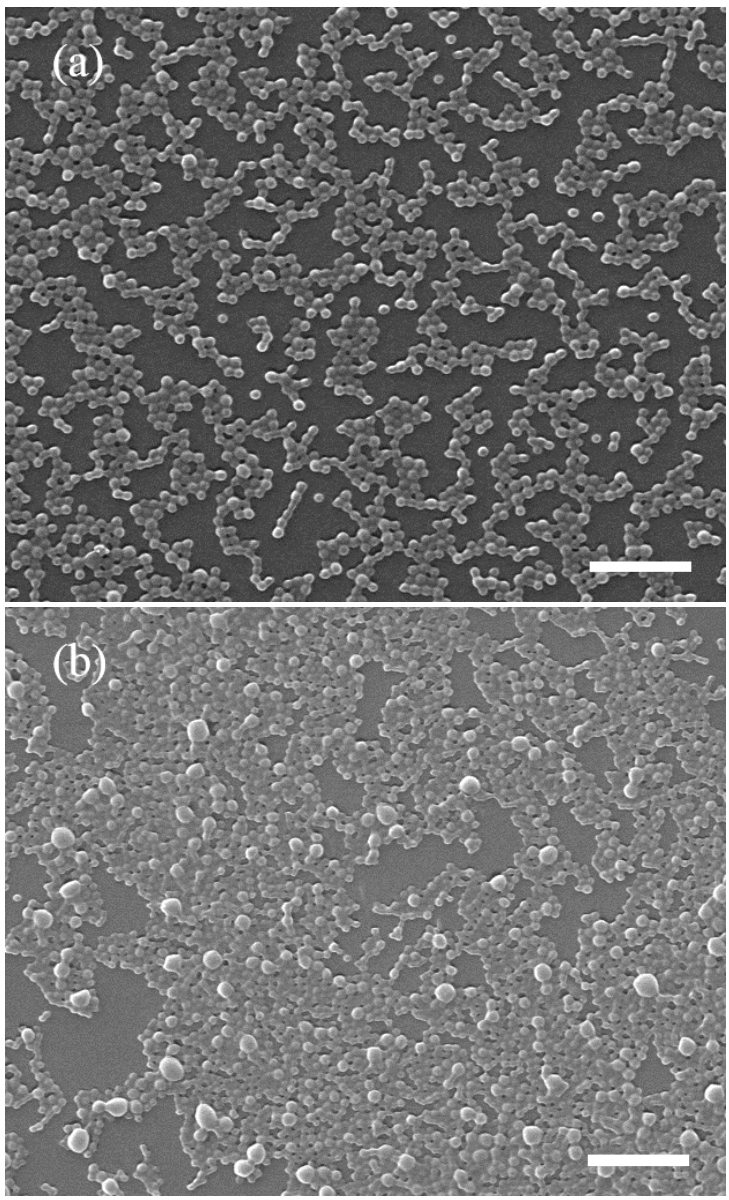
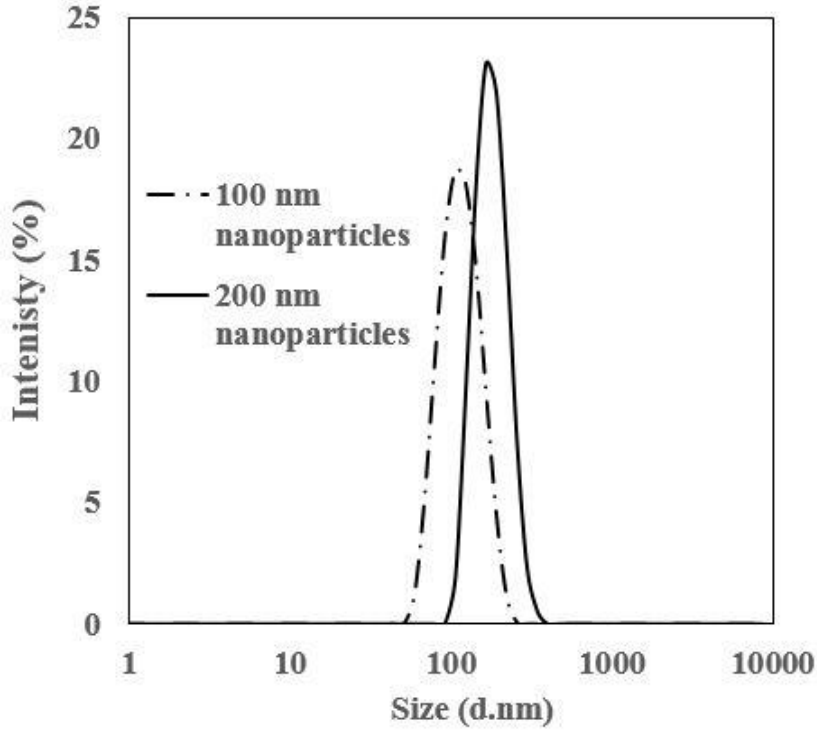
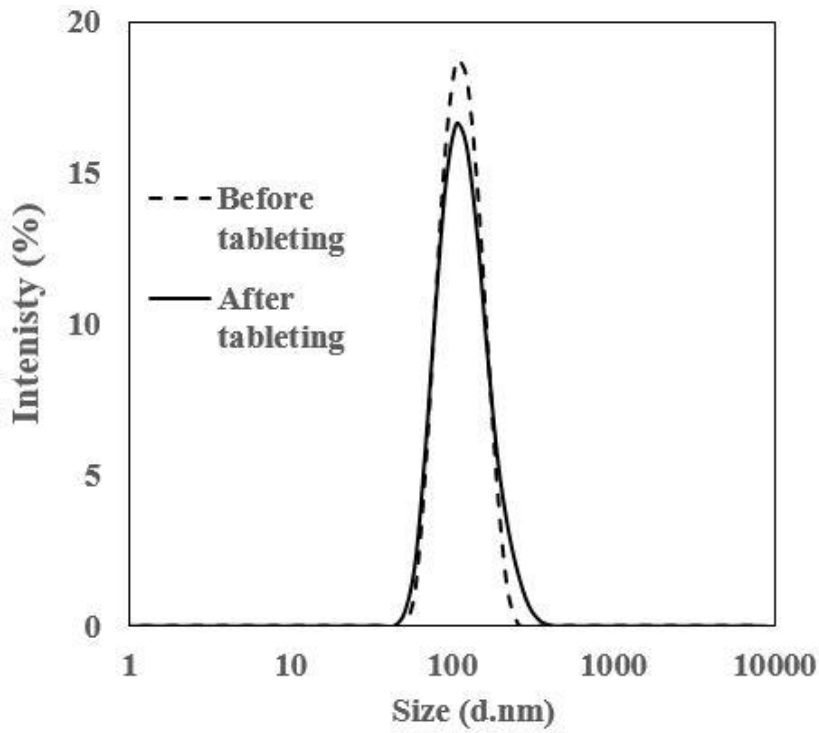


Figure 2. SEM images of the PLGA nanoparticles with the sizes of (a) 100 nm and (2) 200 nm. The scale bars are 1 ㎛.

(a)


25
20
15
10
5
0
Intenisty (%)
100 nm nanoparticles
200 nm nanoparticles
1
10
100
1000
10000
Size (d.nm)

(b)


20
15
10
5
0
Intenisty (%)
Before tableting
After tableting
1
10
100
1000
10000
Size (d.nm)

(c)

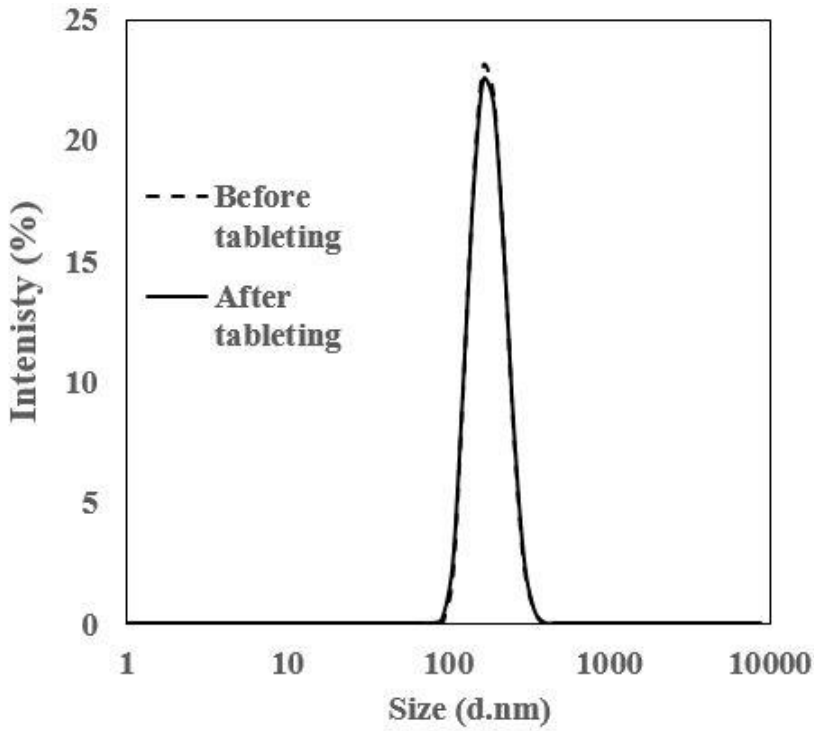


Figure 3. Size distributions of the PLGA nanoparticles used in this work. (a) Size distributions of the 100 nm and 200 nm PLGA nanoparticles before being formulated in a tablet. (b, c) Size distributions of the PLGA nanoparticles before and after being formulated in a tablet. The sizes of the PLGA nanoparticles were (b) 100 nm and (c) 200 nm.

Sample	Particle diameter (nm)	Zeta potential (mV)
100 nm intact nanoparticles	112.4 ± 0.25	- 2.32 ± 0.30
100 nm tableted nanoparticles	114.2 ± 2.98	- 2.54 ± 0.27
200 nm intact nanoparticles	196.8 ± 4.93	- 2.00 ± 0.42
200 nm tableted nanoparticles	198.1 ± 4.16	- 2.03 ± 0.27

Table 2. Properties of PLGA nanoparticles before and after being formulated in a tablet.

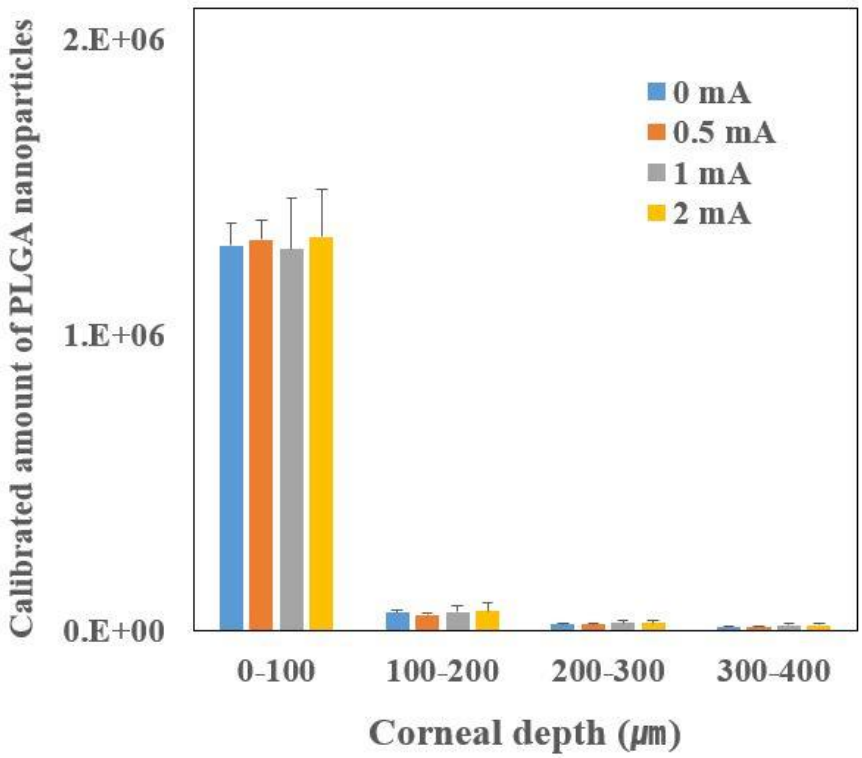
3.2. Effects of electric current intensity

Semi-quantification analysis of fluorescence is a widely used method to observe the distribution of a specific marker within tissues [27-29]. By slightly modifying this method, I assessed the amounts of PLGA nanoparticles delivered into the cornea and observe their trend under various iontophoretic conditions employed in this work. First, the effect of electric current intensity was investigated at 0, 0.5, 1 and 2 mA and assessed at each of iontophoresis application time.

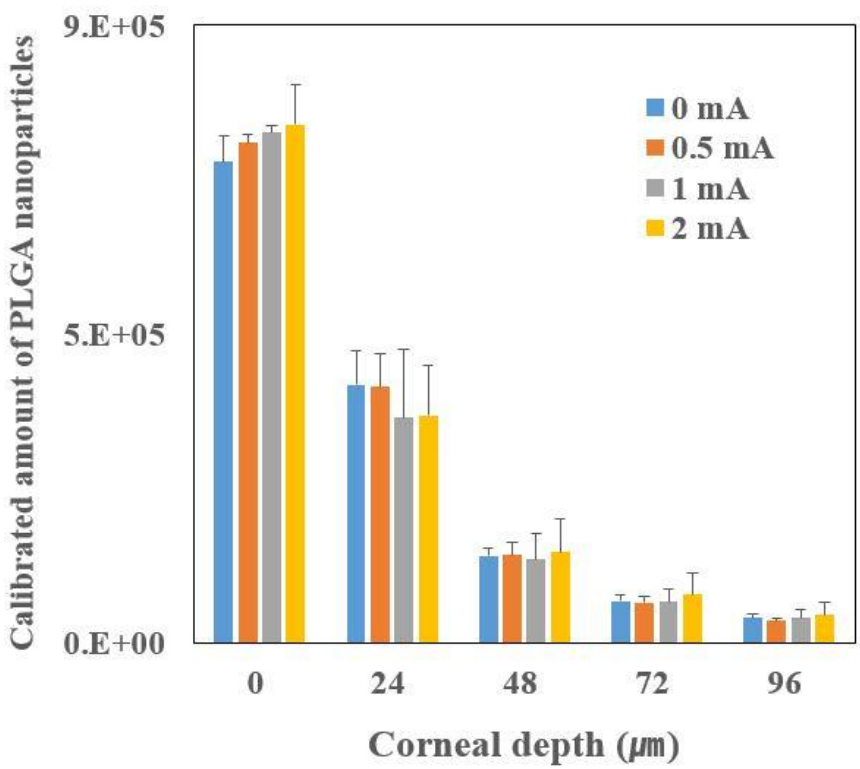
Fig 4. shows the delivered amount of 100 nm particles at varying electric current intensities. Interestingly, for each iontophoresis application time, there was no significant difference in amount of delivered PLGA nanoparticles with the change in electric current intensity. The result also revealed that most of PLGA nanoparticles were accumulated in the frontal layer of the cornea, up to 100 ㎛ in depth. Fig 5 shows the result with the 200 nm PLGA nanoparticles, where for each iontophoresis application time, a similar amount of particles were delivered into the cornea regardless of the change in electric current intensity. The fact that the particle delivery profiles at 0.5, 1 and 2 mA were similar to that at 0 mA implied that the nanoparticles be delivered into the cornea,

mostly mediated by diffusion, and the effect of electric current be minimal under the experimental condition herein.

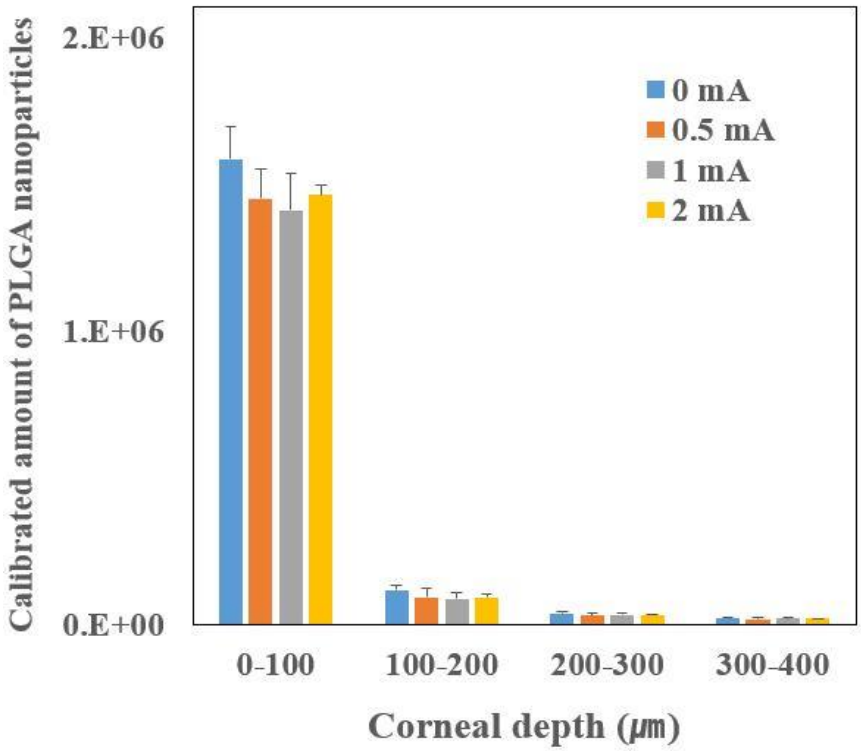
(a)


5 min
Calibrated amount of PLGA nanoparticles
2.E+06
1.E+06
0.E+00
0 mA
0.5 mA
1 mA
2 mA
0-100
100-200
200-300
300-400
Corneal depth (μm)

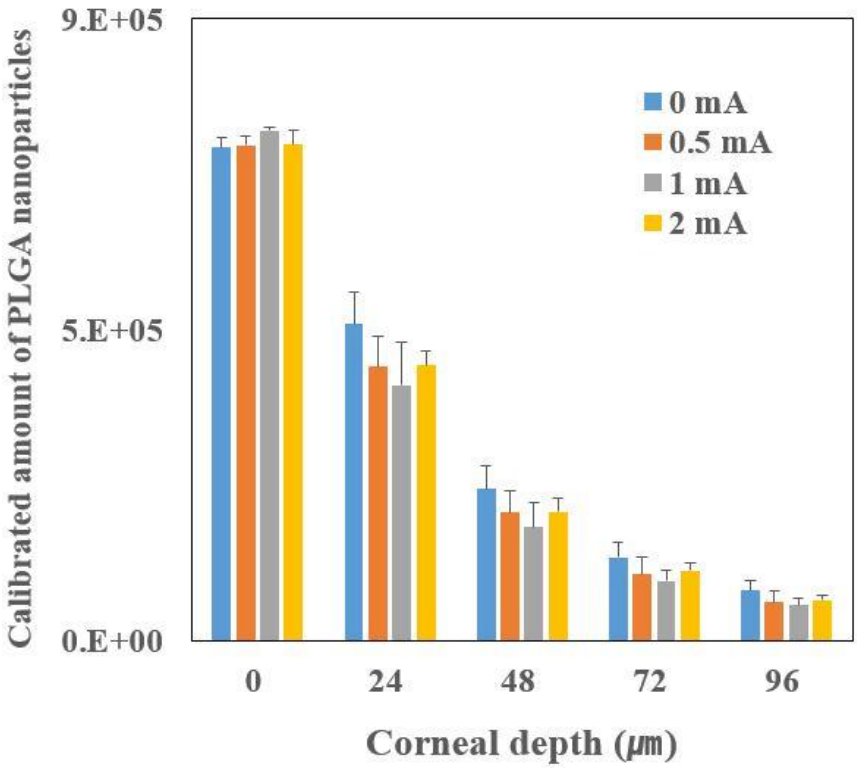
(b)


Calibrated amount of PLGA nanoparticles
9.E+05
5.E+05
0.E+00
0 mA
0.5 mA
1 mA
2 mA
0
24
48
72
96
Corneal depth (μm)

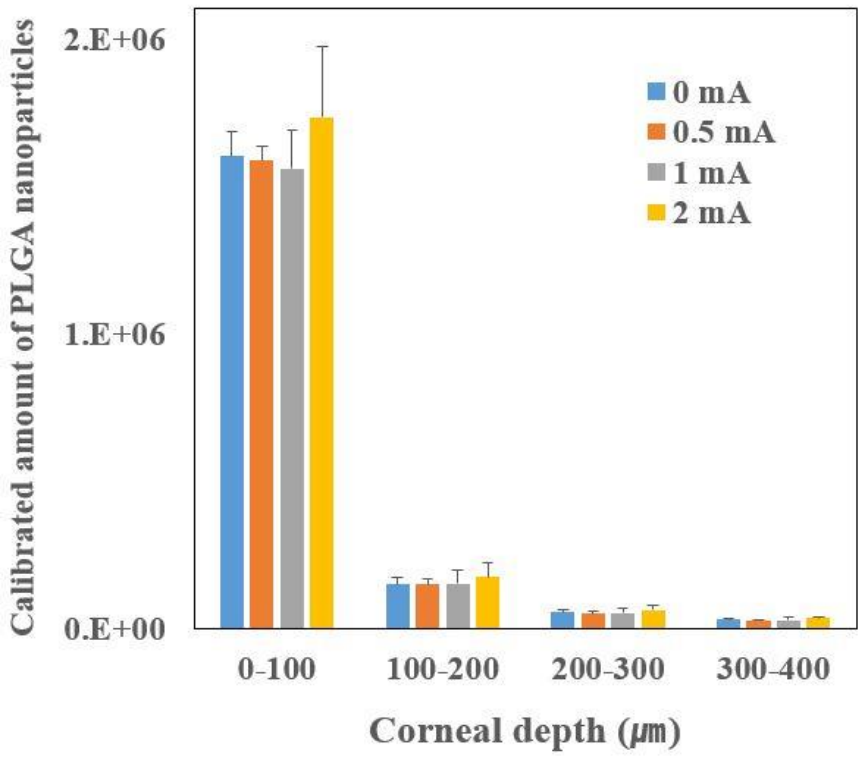
(c) 20 min


Calibrated amount of PLGA nanoparticles
2.E+06
1.E+06
0.E+00
0 mA
0.5 mA
1 mA
2 mA
0-100
100-200
200-300
300-400
Corneal depth (μm)

(d)


Calibrated amount of PLGA nanoparticles
9.E+05
5.E+05
0.E+00
0 mA
0.5 mA
1 mA
2 mA
0
24
48
72
96
Corneal depth (μm)

(e)


60 min
Calibrated amount of PLGA nanoparticles
2.E+06
1.E+06
0.E+00
0 mA
0.5 mA
1 mA
2 mA
0-100
100-200
200-300
300-400
Corneal depth (μm)

(f)

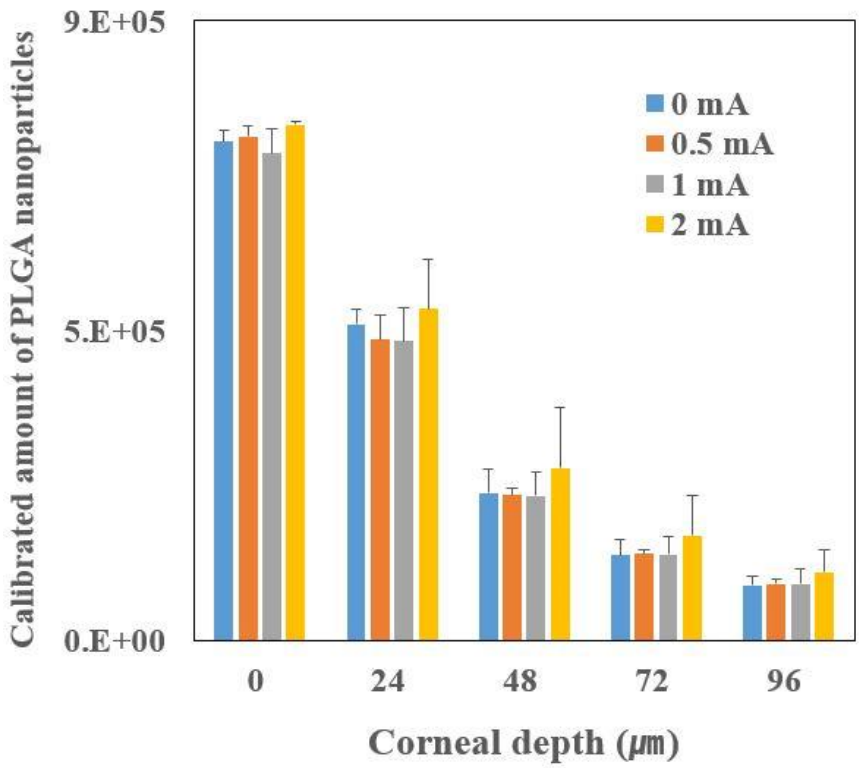
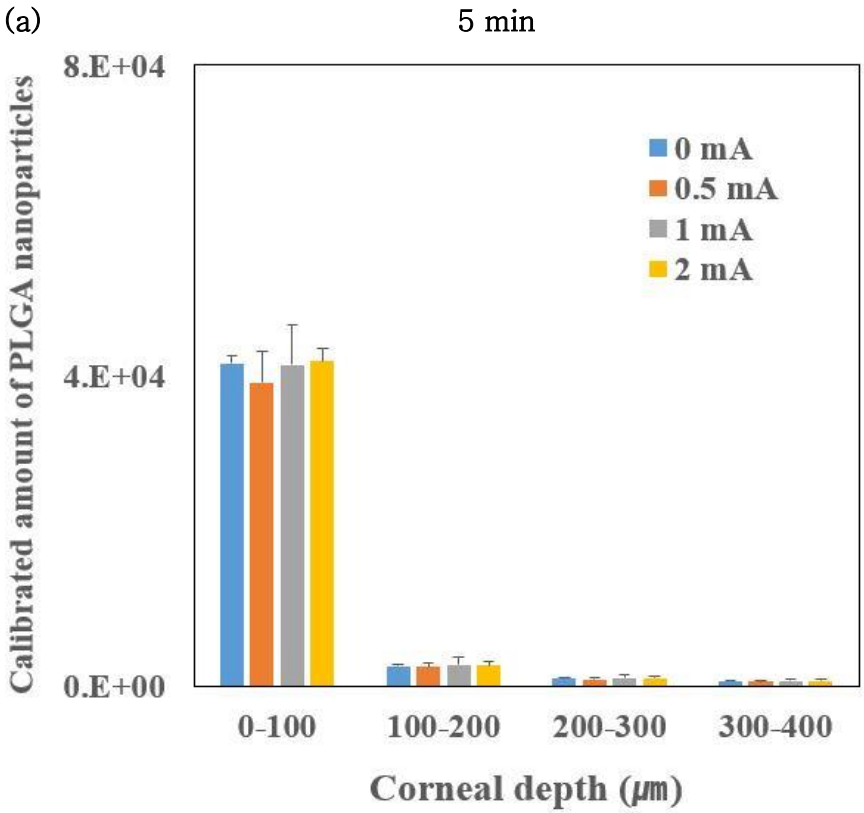
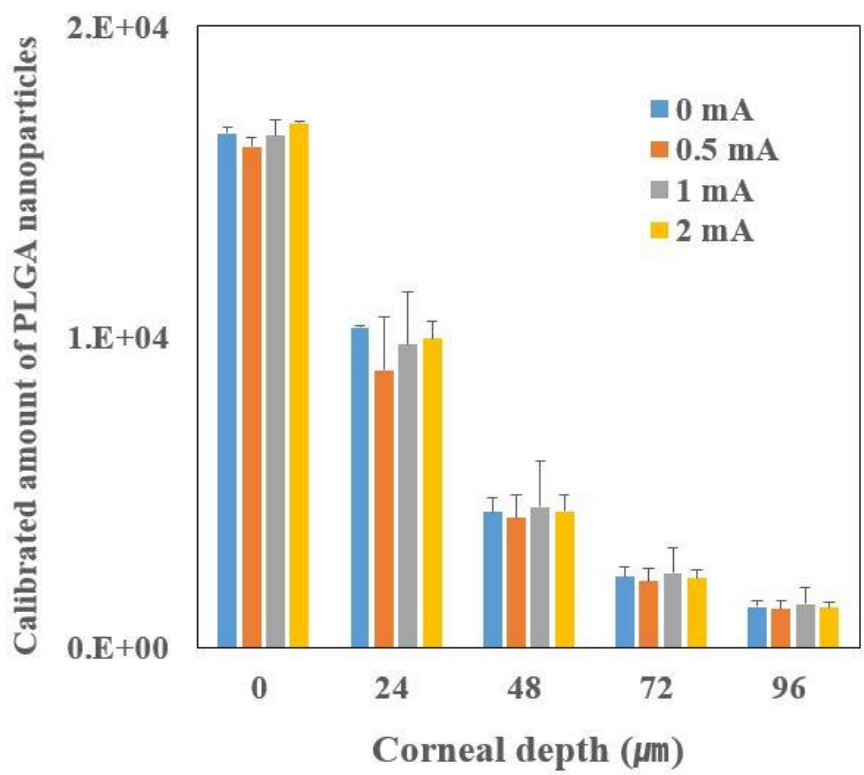

Calibrated amount of PLGA nanoparticles
9.E+05
5.E+05
0.E+00
0 mA
0.5 mA
1 mA
2 mA
0
24
48
72
96
Corneal depth (μm)

Figure 4. Profiles of calibrated delivered-amounts of 100 nm PLGA nanoparticles at different electric current intensities. The periods for iontophoresis application were (a, b) 5 min, (b, c) 20 min and (d, e) 60 min. Calibrated amounts of PLGA nanoparticles were plotted via (a, c, e) a whole depth (400 ㎛) and (b, d, f) frontal depth (100 ㎛) of the cornea.

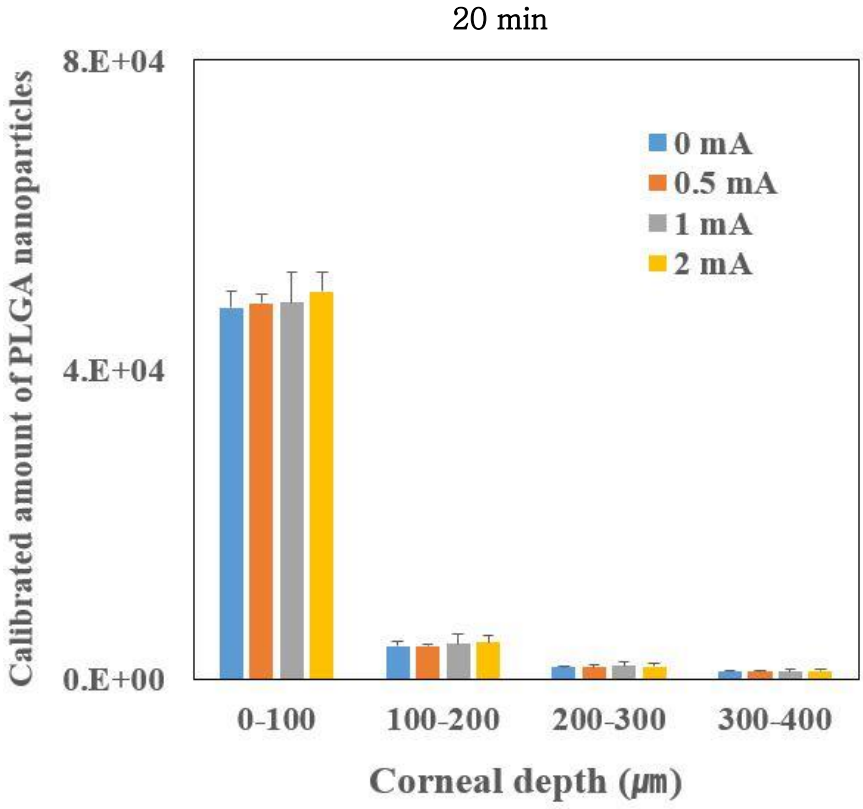
(a)


5 min
8.E+04
4.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
0 mA
0.5 mA
1 mA
2 mA
0-100
100-200
200-300
300-400
Corneal depth (μm)

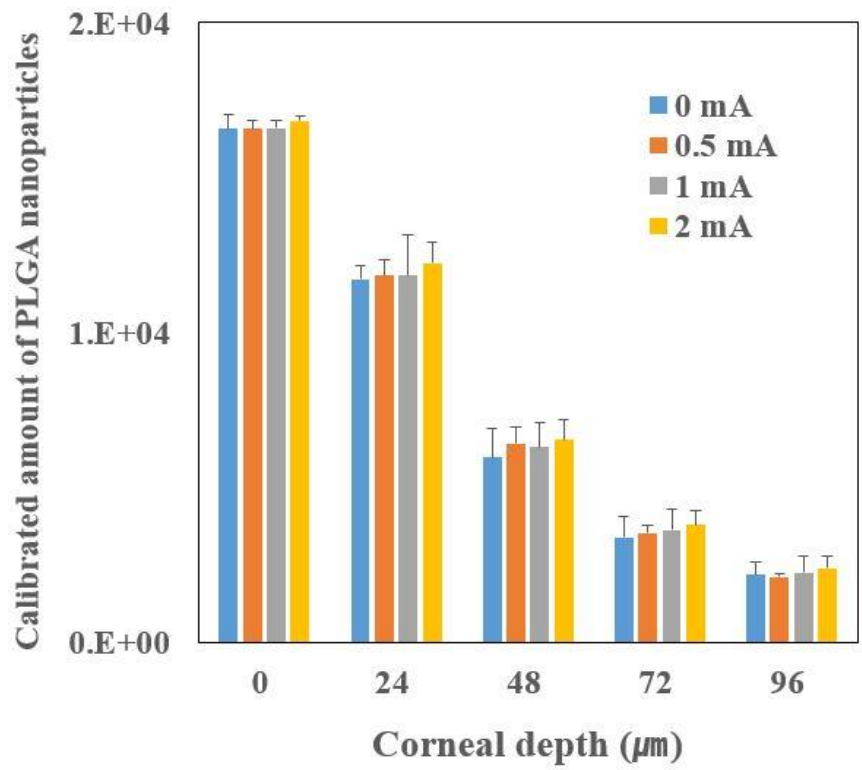
(b)


2.E+04
1.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
0 mA
0.5 mA
1 mA
2 mA
0
24
48
72
96
Corneal depth (μm)

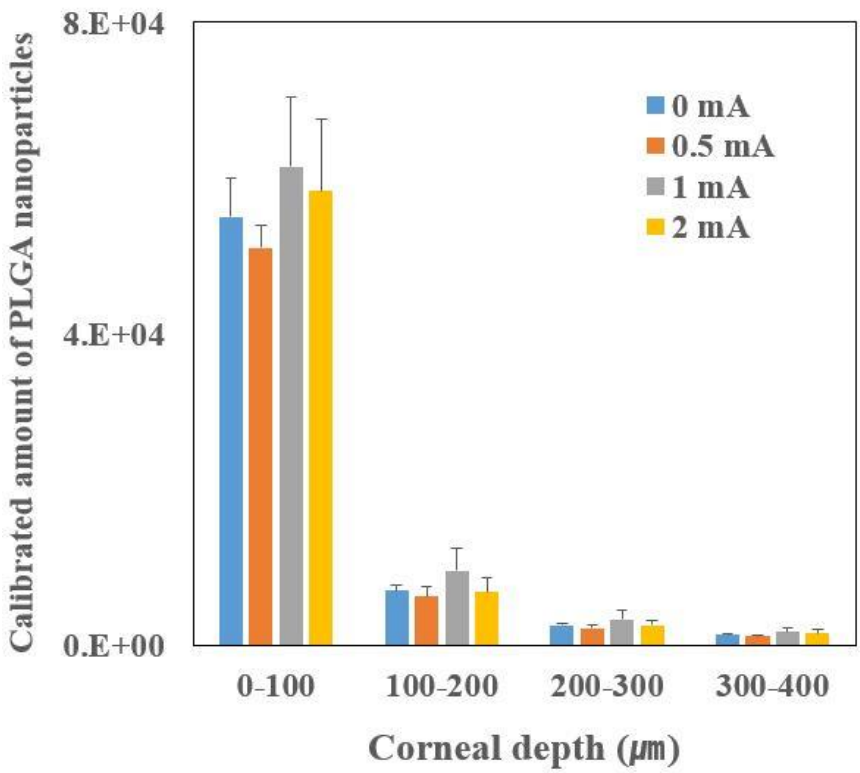
(c)


20 min
8.E+04
4.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
0 mA
0.5 mA
1 mA
2 mA
0-100
100-200
200-300
300-400
Corneal depth (μm)

(d)


2.E+04
1.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
0 mA
0.5 mA
1 mA
2 mA
0
24
48
72
96
Corneal depth (μm)

(e)


60 min
8.E+04
4.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
0 mA
0.5 mA
1 mA
2 mA
0-100
100-200
200-300
300-400
Corneal depth (μm)

(f)

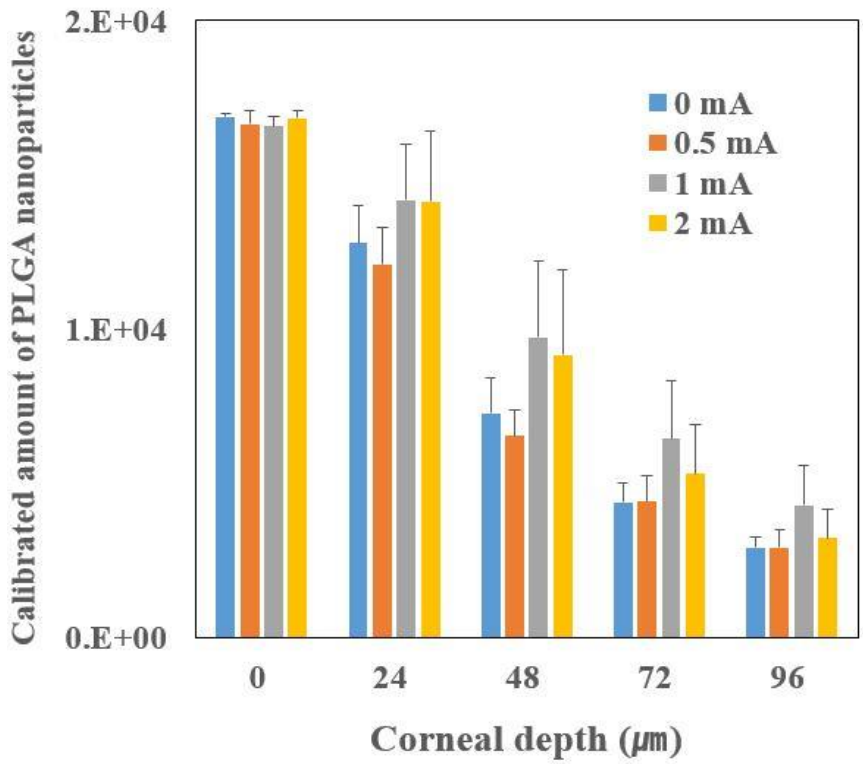
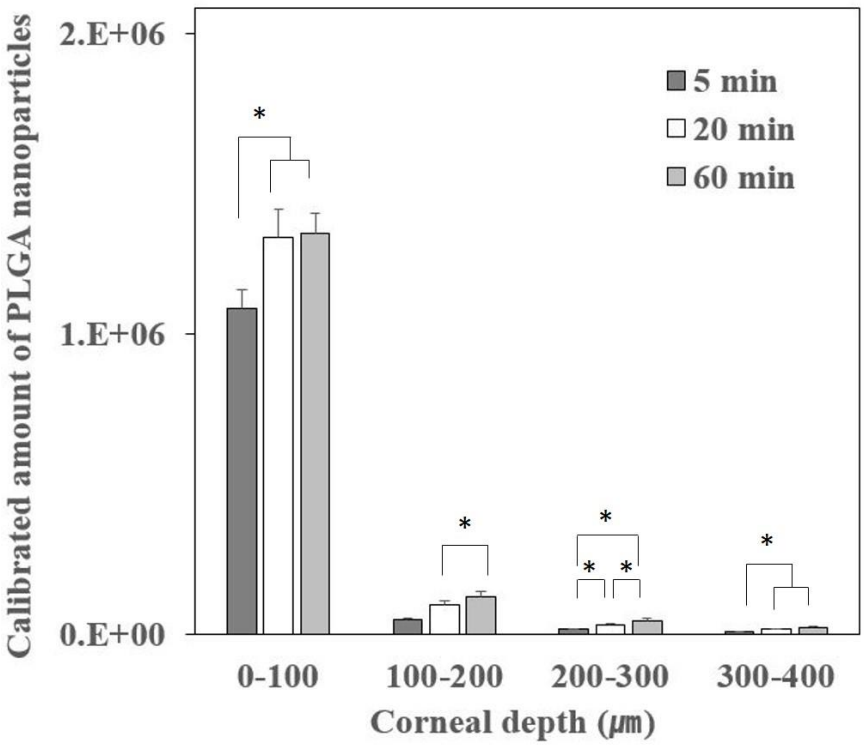

2.E+04
1.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
0 mA
0.5 mA
1 mA
2 mA
0
24
48
72
96
Corneal depth (μm)

Figure 5. Profiles of calibrated delivered-amounts of 200 nm PLGA nanoparticles at different electric current intensities. The periods for iontophoresis application were (a, b) 5 min, (b, c) 20 min and (d, e) 60 min. Calibrated amounts of PLGA nanoparticles were plotted via (a, c, e) a whole depth (400 ㎛) and (b, d, f) frontal depth (100 ㎛) of the cornea._

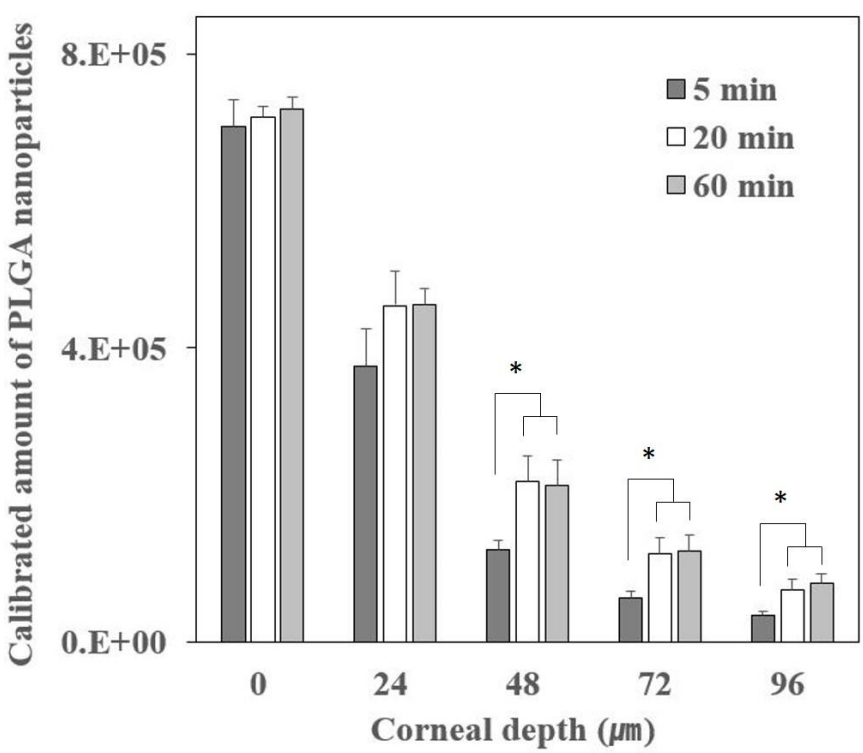
3.3. Effects of iontophoresis application time

To examine the effect of periods for iontophoresis application, the calibrated delivered-amounts of PLGA nanoparticles were replotted, as shown in Figures 6 and 7. For both sizes of PLGA nanoparticles, as the duration for iontophoresis application increased, the delivered amount of PLGA nanoparticles also increased. However, as stated above, the delivered amount of nanoparticles did not increase even with the presence of electric current and was rather similar to the condition at 0 mA. Therefore, the increase in delivered particle amount with the application period would be also mainly due to a longer time for particle diffusion into the cornea.

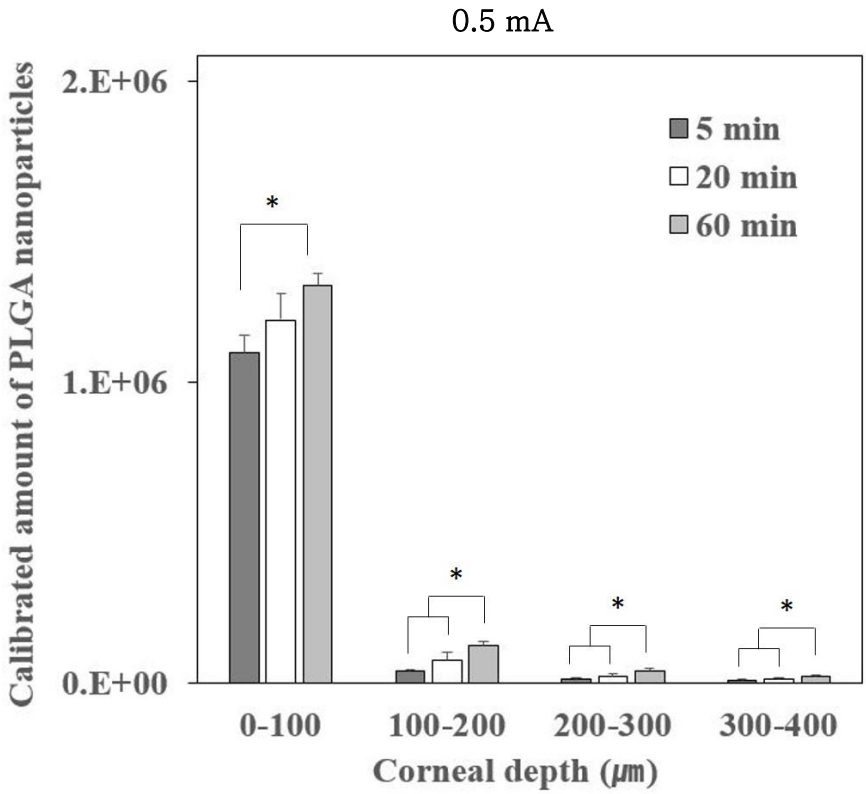
(a)


0 mA
Calibrated amount of PLGA nanoparticles
2.E+06
1.E+06
0.E+00
5 min
20 min
60 min
*
0-100
100-200
200-300
300-400
Corneal depth (μm)

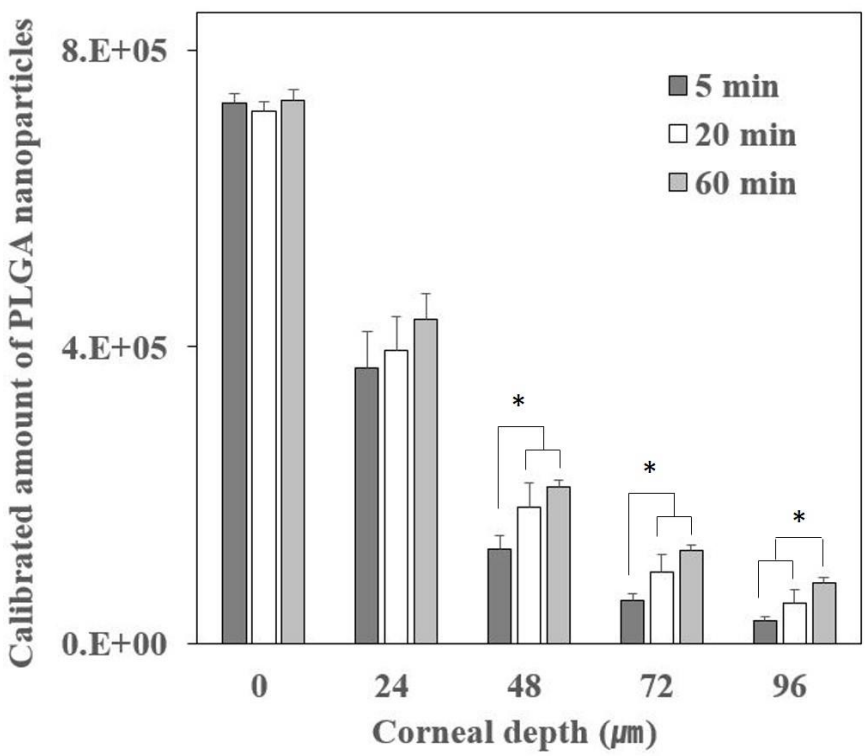
(b)


Calibrated amount of PLGA nanoparticles
8.E+05
4.E+05
0.E+00
5 min
20 min
60 min
*
0
24
48
72
96
Corneal depth (μm)

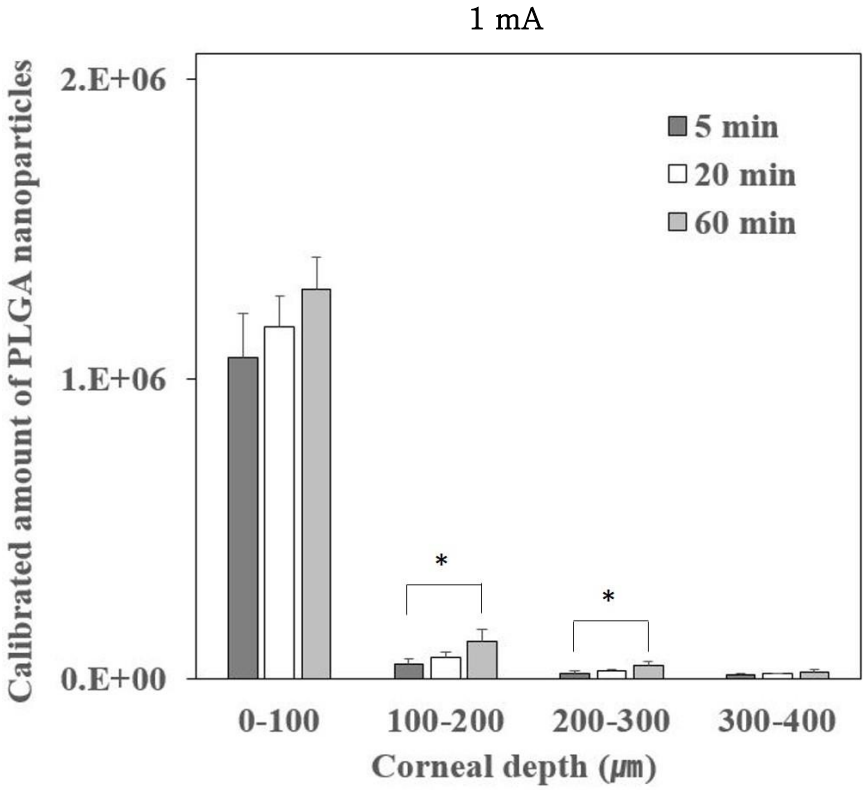
(c)


0.5 mA
Calibrated amount of PLGA nanoparticles
2.E+06
1.E+06
0.E+00
5 min
20 min
60 min
*
*
*
*
0-100
100-200
200-300
300-400
Corneal depth (μm)

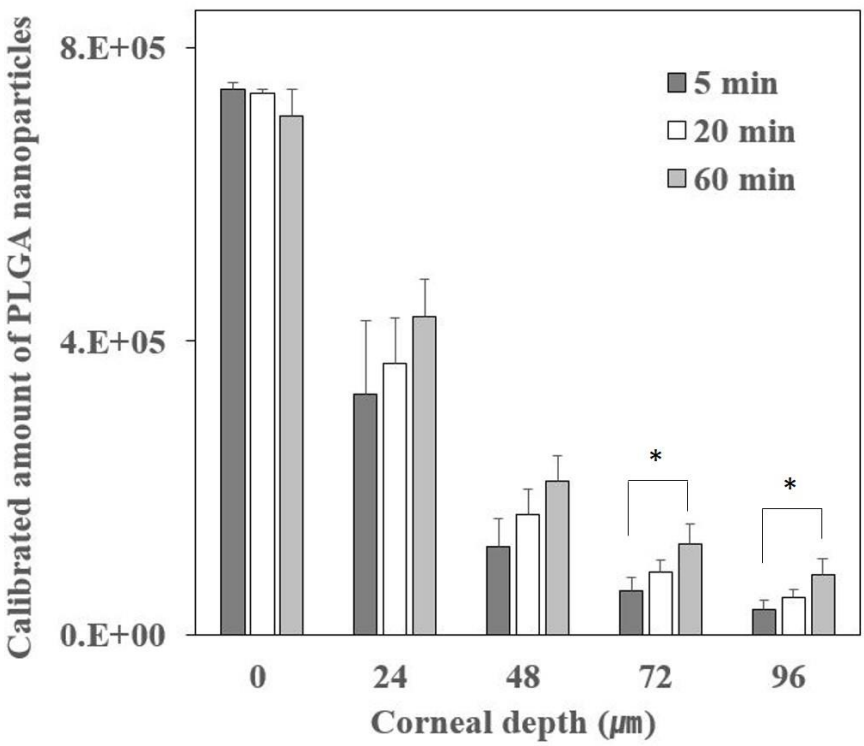
(d)


Calibrated amount of PLGA nanoparticles
8.E+05
4.E+05
0.E+00
5 min
20 min
60 min
*
*
*
0
24
48
72
96
Corneal depth (μm)

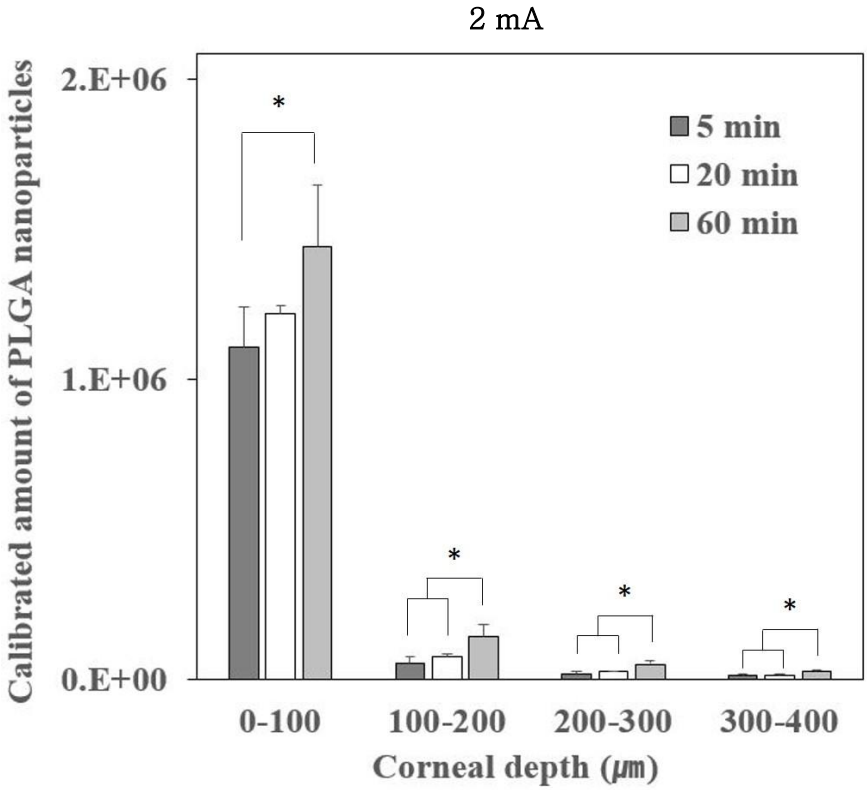
(e)


1 mA
Calibrated amount of PLGA nanoparticles
2.E+06
1.E+06
0.E+00
5 min
20 min
60 min
*
*
0-100
100-200
200-300
300-400
Corneal depth (㎛)

(f)


Calibrated amount of PLGA nanoparticles
8.E+05
4.E+05
0.E+00
5 min
20 min
60 min
*
*
0
24
48
72
96
Corneal depth (㎛)

(g)


2 mA
2.E+06
1.E+06
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
*
*
*
0-100
100-200
200-300
300-400
Corneal depth (㎛)

(h)

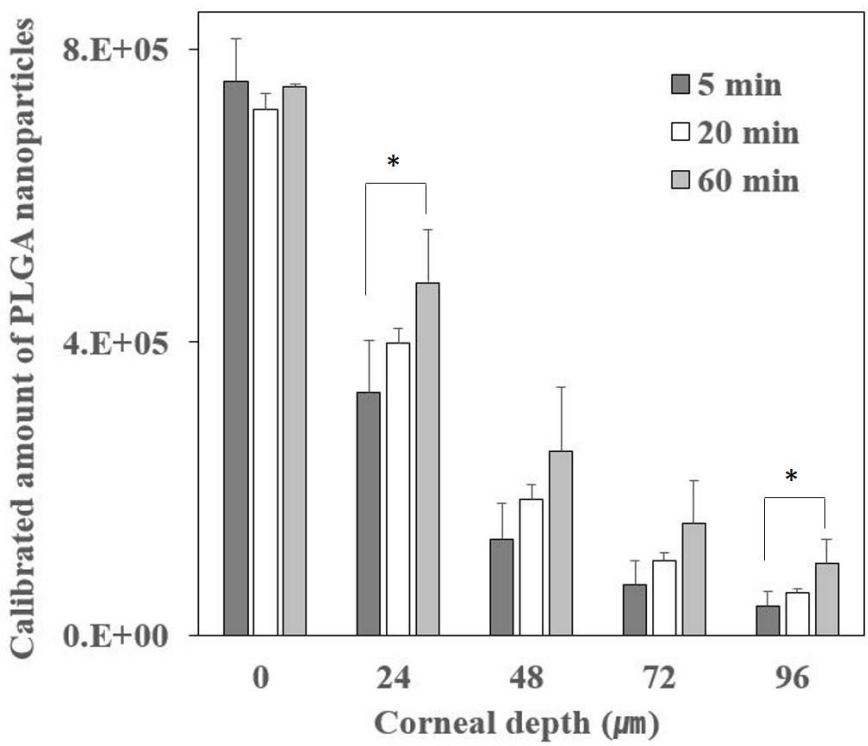
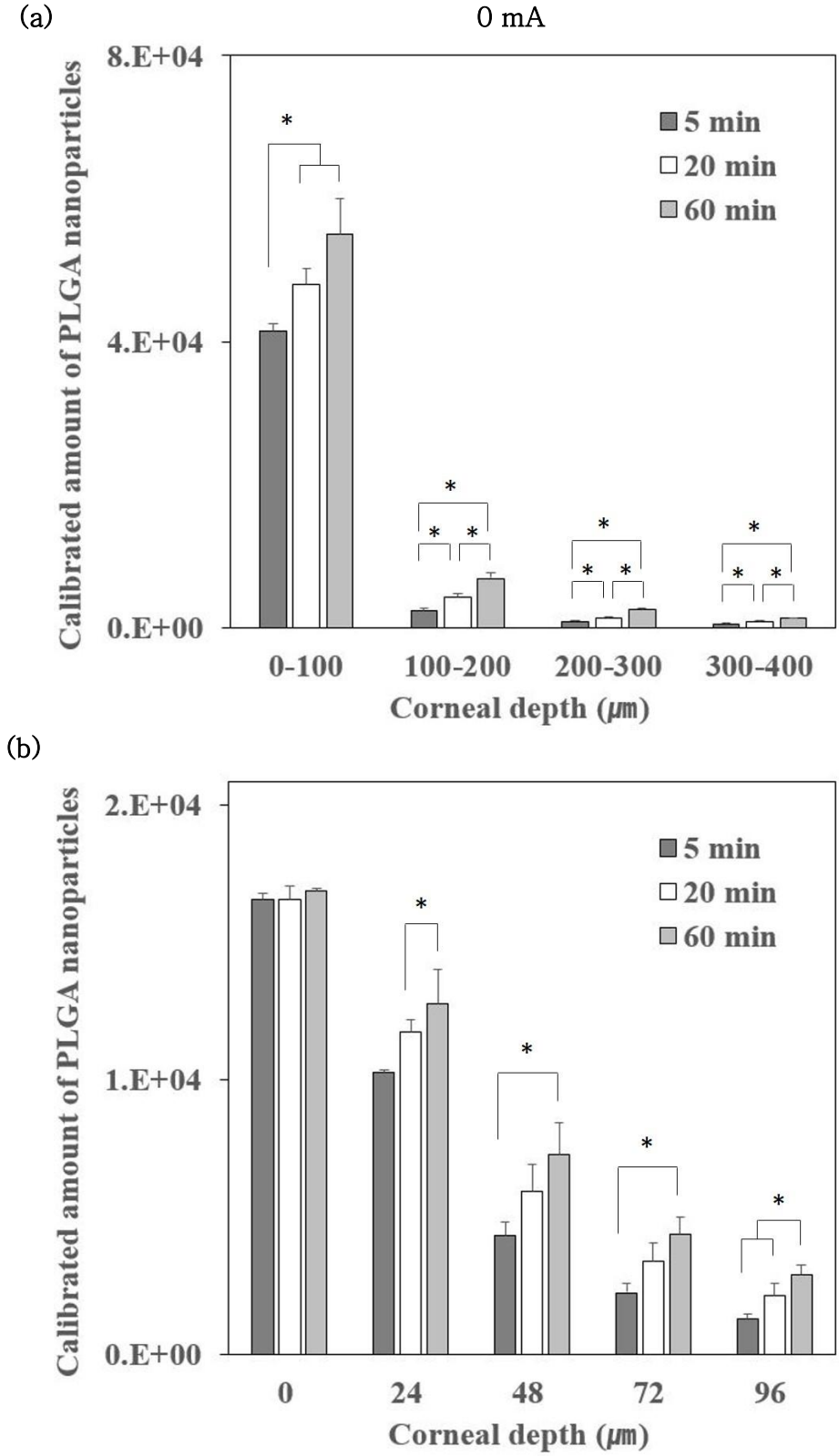
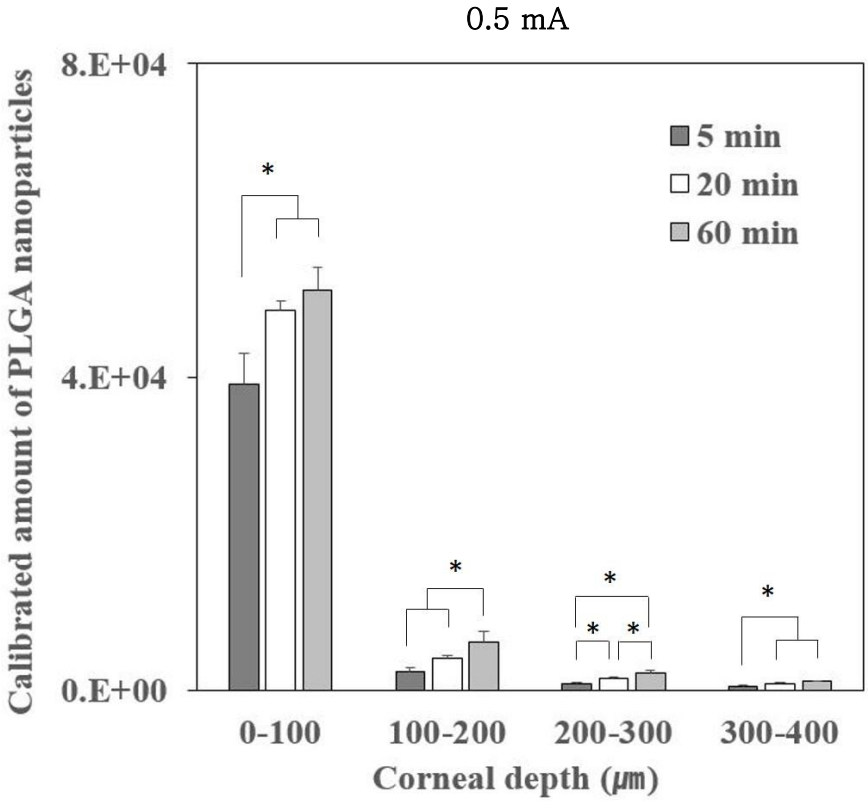

8.E+05
4.E+05
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
*
0
24
48
72
96
Corneal depth (㎛)

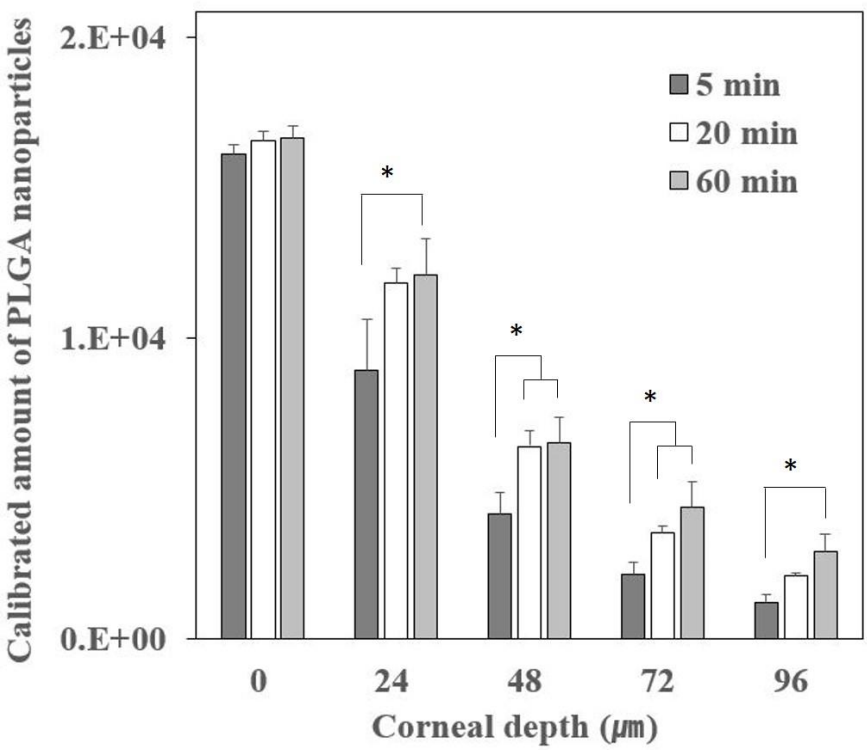
Figure 6. Profiles of calibrated delivered-amounts of 100 nm PLGA nanoparticles at different iontophoresis application periods. The electric current intensities were (a, b) 0 mA, (b, c) 0.5 mA, (c, d) 1 mA and (e, f) 2 mA. Calibrated amounts of PLGA nanoparticles were plotted via (a, c, e, g) a whole depth (400 ㎛) and (b, d, f, h) frontal depth (100 ㎛) of the cornea.


(a)
0 mA
8.E+04
4.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
0-100
100-200
200-300
300-400
Corneal depth (μm)
(b)
2.E+04
1.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
0
24
48
72
96
Corneal depth (μm)

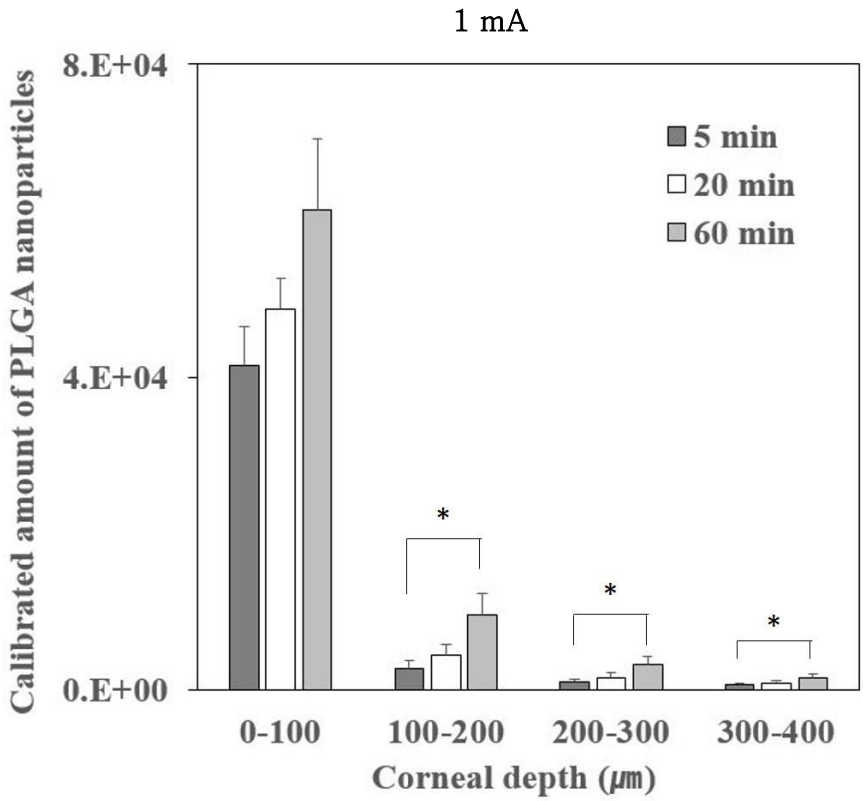
(c)


0.5 mA
8.E+04
4.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
*
*
*
*
*
0-100
100-200
200-300
300-400
Corneal depth (㎛)

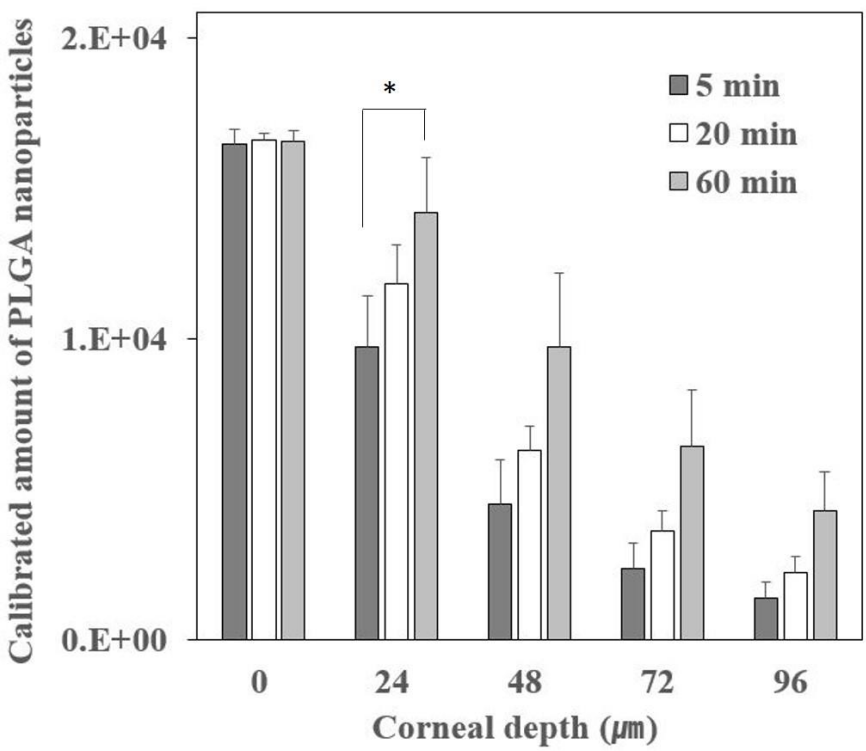
(d)


2.E+04
1.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
*
*
*
*
0
24
48
72
96
Corneal depth (㎛)

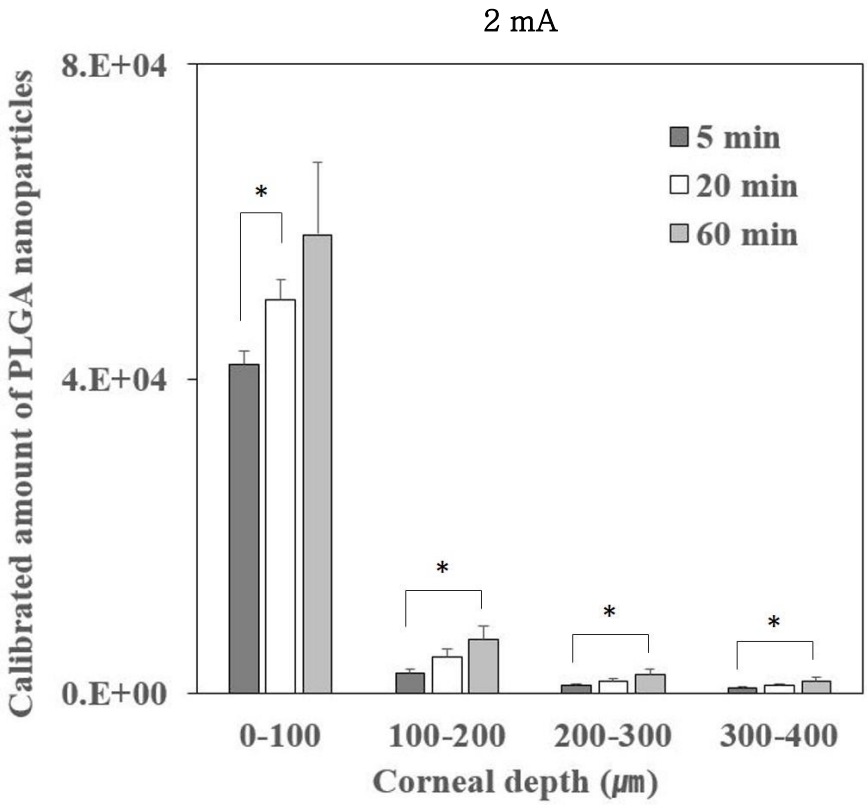
(e)


1 mA
8.E+04
4.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
*
*
0-100
100-200
200-300
300-400
Corneal depth (μm)

(f)


2.E+04
1.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
0
24
48
72
96
Corneal depth (μm)

(g)


2 mA
8.E+04
4.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
*
*
*
0-100
100-200
200-300
300-400
Corneal depth (㎛)

(h)

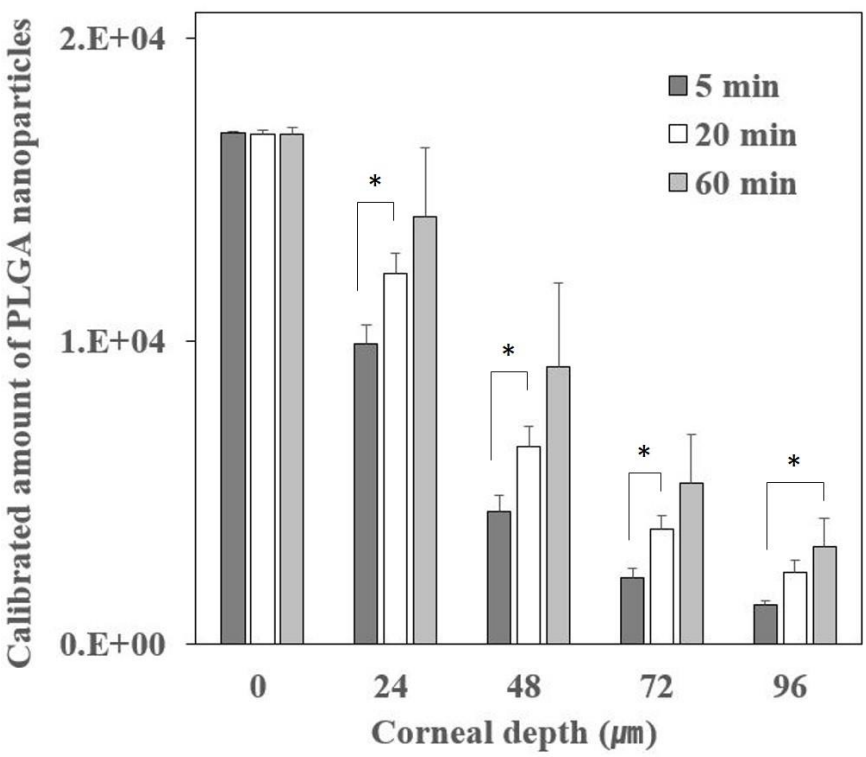
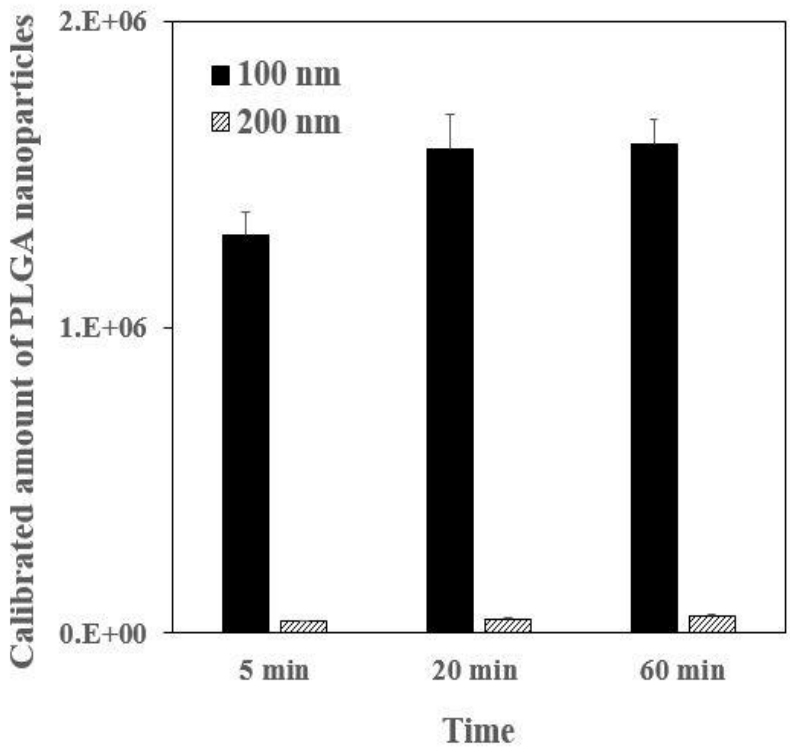

2.E+04
1.E+04
0.E+00
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
*
*
*
*
0
24
48
72
96
Corneal depth (㎛)

Figure 7. Profiles of calibrated delivered-amounts of 200 nm PLGA nanoparticles at different iontophoresis application periods. The electric current intensities were (a, b) 0 mA, (b, c) 0.5 mA, (c, d) 1 mA and (e, f) 2 mA. Calibrated amounts of PLGA nanoparticles were plotted via (a, c, e, g) a whole depth (400 ㎛) and (b, d, f, h) frontal depth (100 ㎛) of the cornea._

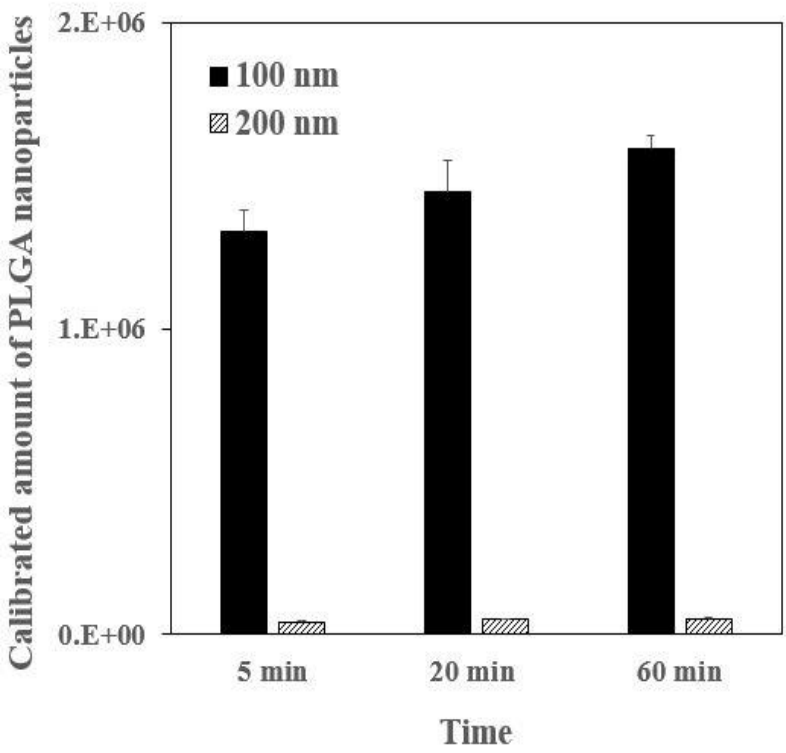
3.4. Effects of particles size

To compare the effect of particle size on their permeation into the cornea, I assessed the total of calculated delivered-amount of PLGA nanoparticles accumulated within a frontal layer of cornea up to 100 ㎛ in depth. As shown in Figure 8, there was a significant difference between 100 nm and 200 nm for all iontophoresis conditions employed in this work. The 100 nm particles exhibited about 30 times better permeation than the 200 nm particles. As the duration for iontophoresis application became longer, the accumulated amount of PLGA nanoparticles increased for both sizes; however, there was a noticeable difference in delivered amounts of PLGA nanoparticles between two different sizes.

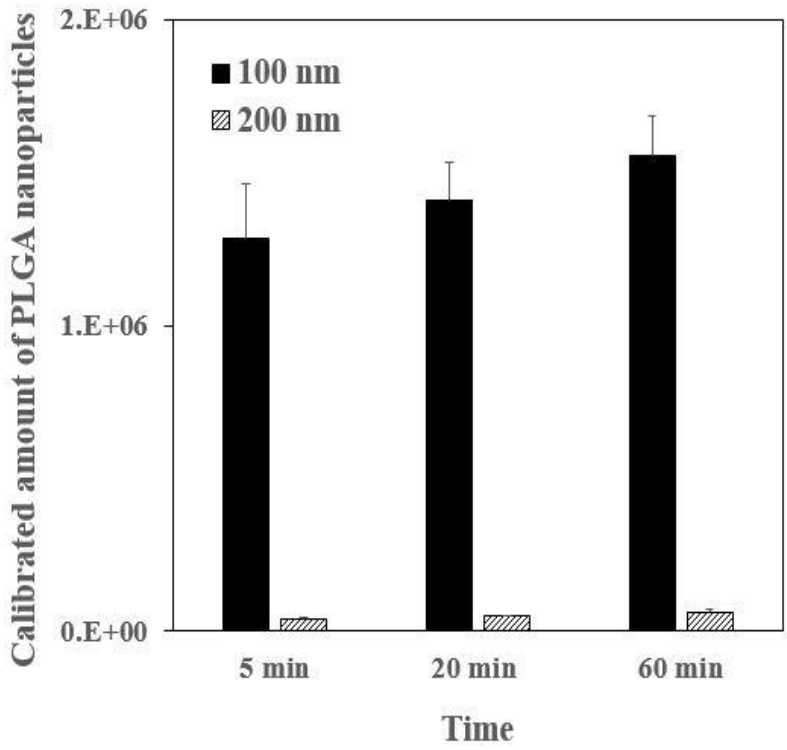
(a) 0 mA


2.E+06
1.E+06
0.E+00
Calibrated amount of PLGA nanoparticles
100 nm
200 nm
5 min
20 min
60 min
Time

(b) 0.5 mA


2.E+06
1.E+06
0.E+00
Calibrated amount of PLGA nanoparticles
100 nm
200 nm
5 min
20 min
60 min
Time

(b) 1 mA


2.E+06
1.E+06
0.E+00
100 nm
200 nm
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
Time

(c) 2 mA

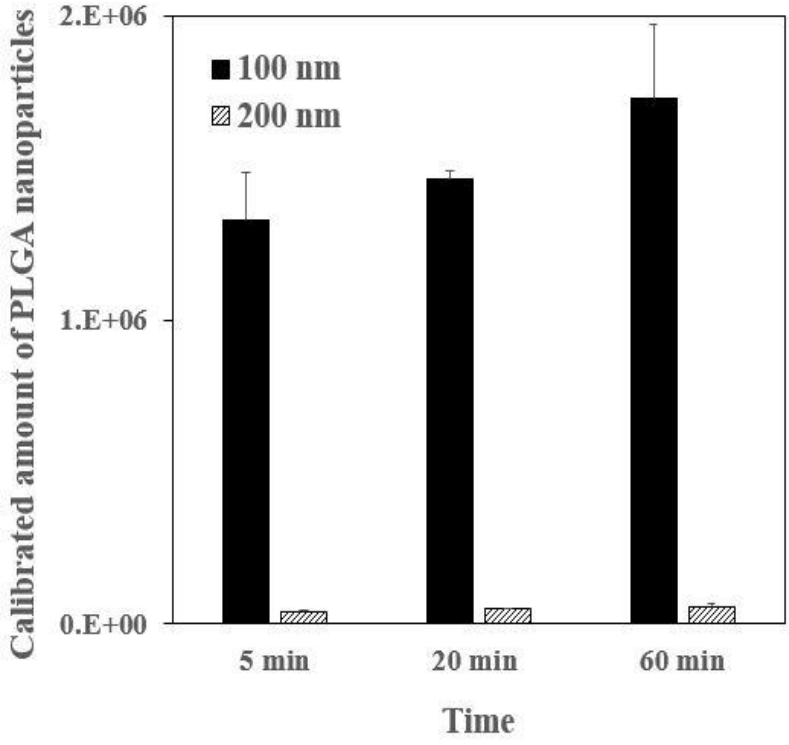

2.E+06
1.E+06
0.E+00
100 nm
200 nm
Calibrated amount of PLGA nanoparticles
5 min
20 min
60 min
Time

Figure 8. Calibrated delivered-amounts of the PLGA nanoparticles of two different sizes, 100 nm and 200 nm, accumulated within a 100 ㎛ frontal layer of the cornea. The electric current intensities were (a) 0 mA, (b) 0.5 mA, (c) 1 mA and (d) 2 mA._

Chapter 4. Discussion

Iontophoresis is expected to be a promising solution for tackling the issues raised by conventional ocular drug delivery methods, such as poor drug bioavailability. Therefore, to improve the iontophoresis methodology for ocular drug delivery, drug–delivery formulations such as using hydrogel or nanoparticles were often combined with iontophoresis. Among them, the PLGA nanoparticles has been drawing a great deal of attention: the nanoparticles could be efficiently delivered into the cornea via iontophoresis and after that, drug could be released in a controlled manner to eventually enhance drug bioavailability in ocular tissues [13, 27, 30].

Therefore, in this study, I pursued to investigate the profiles of iontophoretic transport of PLGA nanoparticles under various conditions and to be mimic ocular environment more realistically, a whole eye with a simulated eyelid was used to prepare the in vitro experimental setup.

Interestingly, the results herein revealed that with varying electric current intensities used in this work (0 - 2 mA), the particle transport did not vary much and this was not significantly different when compared with the current at 0 mA. This implied that under the experimental conditions employed in this work, the PLGA

nanoparticles be permeated into the cornea mainly by simple diffusion. Therefore, the amount of delivered particles increased with the duration for application and thus, the effect of particle size was much apparent.

Some previous studies reported that there are noticeable differences in a delivered amount of nanoparticle into the cornea when different experimental conditions were applied under a similar condition used in this work [14, 27]. However, those studies used the extracted cornea or performed the iontophoresis under a strictly controlled environment, e.g., using the Franz cells or direct contact of electrode to the eye surface, where an electric field was controlled to be focused specifically on the cornea. However, this study used a whole eye and considered the presence of an eyelid, where the density of electric field might be lower than expected in the cornea. In this aspect, therefore, my findings suggested the need for a more sophisticated design of the iontophoresis device and electrodes for drug delivery to the eye. In addition, the PLGA nanoparticles utilized in this study possessed a relatively low surface charge, compared to other nanoparticles developed as drug carriers [15, 27, 31]. For this reason, even if an electric field were well focused on the cornea, its effect could have been minimal.

Additionally, my findings indicated that the size of the PLGA

nanoparticles is most crucial for their transport into the cornea. The 100 nm particles exhibited about 30 times better permeation than the 200 nm particles. Considering their diffusion, this result could be explained by a diffusivity coefficient, which is inversely proportional to a diameter of particles [32]. However, under the experimental applications employed in this work, most of delivered PLGA nanoparticles were observed to be accumulated within the frontal 100 ㎛ layer of the cornea. However, all of those results again were obtained with the nanoparticle delivered into the cornea via diffusion. Therefore, to observe synergistic effects of combined iontophoresis drug delivery system, further studies would be needed, using the PLGA nanoparticles with a higher surface charge.

Chapter 5. Conclusion

In this study, I examined the iontophoretic transport of the PLGA nanoparticles applied to the in vitro whole eye with a simulated eyelid to better mimic the physiological condition. With varying iontophoresis conditions, the results indicated that the effect of electric current intensity was not apparent possibly due to a more complex geometry of a whole eye and a very low surface charge of the PLGA nanoparticles used in this work. Therefore, for the application time up to 60 min, the PLGA nanoparticles were permeated into the cornea mainly by diffusion, where the effect of particle size was seen to be prominent. Therefore, to be effectively transported via iontophoresis, the PLGA nanoparticles should possess a higher surface charge, and the arrangement of the electrodes needed to be carefully designed, considering their actual application. Overall, I expect that the results provided herein can contribute to finding an optimized condition for iontophoresis specifically for topical delivery of drug-loaded nanoparticles to the eye.

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국문초록

이온영동은 관심 있는 물질들을 조직으로 전달시키는 비침습적인 방법으로써, 국소적으로 전달시키는 약물들의 전달 효율성을 높이고자 안과분야에서 주목을 받아왔다. 이러한 이유로 이온영동을 이용하여 작은 크기의 입자들을 각막으로 전달하는 몇몇의 시도들이 있었다. 하지만 나노파티클의 전달을 다룬 논문들은 거의 없었다. 따라서 이번 연구는 생체적합성 폴리머인 poly(lactic-co-glycolic acid) (PLGA)로 만들어진 나노파티클이 다양한 이온영동 조건이 인비트로 상에서 주어졌을 때 각막으로 전달되는 양상에 대해 관찰하고 설명하고자 한다. 또한 이번 실험에서, 전극이 직접적으로 안구 표면에 닿으므로 인해 발생하는 문제들을 피하기 위해 모사 눈꺼풀 위로 이온영동을 인가하였다. 이를 위해, 형광이 탑재된 PLGA 나노파티클을 이용하여 만든 타블렛을 각막 위로 도포하고 입자의 크기, 인가하는 전류의 세기 그리고 이온영동 인가시간과 같은 요인들을 변화시켰다.

이온영동 후, 형광 입자의 분포를 평가하기 위해 컨포칼 레이저 현미경을 이용하여 각막의 단면들을 관찰하였다. 실험 결과들을 통해 본 실험에서 관찰한 요인들 중, 나노파티클의 사이즈와 이온영동 인가 시간의 변화가 전달되는 PLGA 나노파티클의 양에 의미 있는 영향을 끼친다는 것을 발견할 수 있었으며, 특히 입자의 사이즈가 PLGA 나노파티클 전달 양상을 결정하는데 있어서 중대한 요인 중 하나라는 것을 알 수 있었

다. 그러나 인가하는 전류의 세기가 0 mA 부터 0.5 mA, 1 mA, 2 mA 로 증가하여도 각 실험 군간의 전달된 PLGA 나노파티클 양에는 통계적으로 유의미한 차이를 발견할 수 없었다. 현재 실험에서 설정된 이온영동 조건에서는 전기장이 각막에 제대로 집중되지 않은 것으로 보여지며, 실험에 사용된 PLGA 나노 입자의 표면 전하가 매우 낮았다는 점(~2 mV)들로 미루어 보아, 확산이 나노파티클 전달을 하는 주 메커니즘으로 작용했다는 것을 확인할 수 있었다.

주요어 : 각막, 인비트로, 이온영동, PLGA 나노 입자, 확산

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