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The Inhibitory Effect Of Green Tea (*Camellia Sinensis*) On *Pseudomonas Aeruginosa* Treated Contact Lenses.

Mariah A. Bigaud
Department of Biology
Manhattanville College
Purchase, NY 10577 USA

Faculty Advisor: Anna K. Yeung-Cheung, Ph.D.

Abstract

Pseudomonas aeruginosa is the leading cause of ocular infections in those who wear contact lenses. Studies have been done using selenium-coated contact lenses to inhibit the growth of the bacteria to provide an opportunity for individuals to wear contact lenses for a prolonged period of time. Selenium has antioxidant properties that kill the bacteria, however, it is toxic even when used in small quantities. In this study, green tea which is a strong antioxidant was used to treat contact lenses. A disc diffusion assay was first done using different concentrations of green tea to study the inhibitory effect on *P. aeruginosa* and the results were compared to the inhibitory effects of black tea. The results demonstrated that green tea was significantly more effective than black tea in inhibiting the bacteria. The 100 mg/mL of green tea was the most effective concentration that maintained a uniform solution, producing an average of a 2.05 cm diameter of clear zone with 10^7 CFU of *P. aeruginosa*. Contact lenses were used and treated with 100 mg/mL of green tea for possible coating before being exposed to 10^6 CFU of *P. aeruginosa*. The results showed that green tea treated contact lenses had fewer bacteria, with a 41.9% inhibition rate when compared to the control. However, the results were not significant. Another experiment was done by exposing contact lenses to 10^6 CFU of *P. aeruginosa* first, and then treating them with 100 mg/mL of green tea to determine its bactericidal properties. The results showed that green tea significantly reduced the bacteria present on contact lenses ($p < 0.05$). In conclusion, green tea shows an inhibitory effect on *Pseudomonas aeruginosa* on contact lenses.

Keywords: Green tea, contact lenses, *Pseudomonas aeruginosa*

1. Introduction

Contact lenses are known to be susceptible to bacteria attachment and therefore result in infections such as corneal ulcers and microbial keratitis in the eyes of those who wear them.[1,2,3] The risk of infection is often due to poor personal hygiene in the handling of the lenses and the storage cases, which provide the ideal environment for the growth of bacteria.[4,5] Many lens cleansing solutions were evaluated for their antimicrobial properties; however, these solutions also risk being contaminated with the bacteria from the contact lens.[4,6,7,8] *Pseudomonas aeruginosa* is the leading cause of contact lens related ocular infection due to the nature of the bacteria's ability to survive in the eye, on the contact lens, and in the storage case.[9,10,11] It is an opportunistic pathogen in humans, causing infection in compromised tissue, and the bacteria can typically be found in a biofilm environment with some surface or substrate.[12] In addition, *P. aeruginosa* is able to produce protease for its invasion and attachment to the cornea of the eye and causes the microbial keratitis.[9,13,14]

Many studies were done by using organic and inorganic substances as coating for the contact lens, to prevent bacterial attachment. Concanavalin A, a lectin substance, was used in injured rabbit cornea in order to compete with *P. aeruginosa* for the binding of cornea cells. The results suggested that concanavalin A was able to reduce the number of bacteria found on the cornea.[15] However, concanavalin A is known for its ability for blood agglutination and is toxic in high amount.[16,17] Matthews et al. performed another study on the coating of contact lenses with selenium to inhibit the growth of *P. aeruginosa.*[18] The study consisted of testing prolonged wear of the selenium coated contact lenses *in vitro* and *in vivo* by having rabbits wear them. It was found that the coating allowed for extended-wear over a period of two months, and prevented *P. aeruginosa* colonization with no adverse effects on the cornea.[18] Selenium, however, is a powerful antioxidant that inhibits bacterial growth by forming "super-oxide" radicals. The problem with selenium is that it is very harmful if inhaled or if it comes in contact with the skin. It can cause neurotoxicity, cancer, and harm to an unborn child.[19]

Tea is also a very powerful antioxidant, and has been shown to have antibacterial, anti-inflammatory and anticancer properties.[20,21,22,23,24] Flayyih et al. conducted a study by obtaining *P. aeruginosa* isolated from the corneal scrapings of various eye infections, in which seventy-seven percent of these infections were due to the wearing of contact lens. Black tea was then used to test its bactericidal properties. The result showed that black tea was able to inhibit these bacterial isolates in the *in vitro* studies.[25]

Green tea, which comes from the same plant as black tea, *Camellia sinensis,* has also been associated with many medical properties, including anticancer properties, improvement in cardiac health, and the lowering of stress.[26,27] The difference between the two plants is that green tea is made from new leaves, while black tea is made from the aged leaves that are either fermented or oxidized.[24,28] However, their chemical composition is somehow different, and green tea contains more of the specific antioxidant polyphenols, catechins, than black tea.[24,29] Thus, it is not surprising that green tea is more potent in its antioxidant properties than black tea.[30,31,32] In addition, the catechins play an important role in the inhibition of bacterial growth.[33,34,35] Liu et al. performed a study and tested a specific catechin, epigallocatechin gallate (EGCG), the most abundant catechin in green tea polyphenols, on *P. aeruginosa*. They found that under neutral conditions, the green tea polyphenol effectively induced the bacteria stress-related genes and thus, inhibited the growth of the bacteria.[36]

P. aeruginosa is also known for its biofilm properties and its antibiotic resistance.[37,38] Abidi et al. studied the effect of four natural plant extracts as antimicrobial agents on the *P. aeruginosa*. They found that these extracts exhibited antimicrobial properties against the biofilm of the bacteria.[39] Radji et al. also performed studies of green tea on methicillin-resistant *S. aureus,* and multi-drug resistant *P. aeruginosa*. Their results showed that green tea can act as an antimicrobial agent, and can be incorporated into drug therapy to combat these antibiotic resistant bacteria.[40]

The goal of this research was to study the effect of green tea on inhibiting *P. aeruginosa* from attaching and growing on contact lenses. It was hypothesized that the use of green tea, through coating, would effectively inhibit *P. aeruginosa* from attaching and growing on contact lenses, and that the use of green tea would effectively reduce the amount of *P. aeruginosa* present on infected contact lenses.

2. Materials and Methods

2.1. Relationship Between The Optical Density And Cell Number Of *P. aeruginosa*

Seven 1:2 serial dilutions were done using a prepared culture of *P. aeruginosa* (Carolina Biological Supply Company, NC) in 0.1 M Phosphate Buffered Saline (PBS) to obtain the relationship between the optical density and cell number of bacteria. Bacteria were grown at 37 ºC for 24 hours, and the optical densities of the stock and each dilution (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128) were measured at a 600 nm wavelength using a DU 720 General-Purpose UV/Vis Spectrophotometer (Beckman Coulter, NJ). Each dilution was further diluted to obtain the countable numbers between 30-300 colony forming units (CFU), and 0.1 ml or 0.5 ml were plated on two nutrient agar plates (Carolina Biological Supply Company, NC). All plates were then incubated at 37ºC for 24 hours. The amount of bacteria that grew on the plates was then used to calculate the original amount of bacteria and was plotted against the optical density. Four trials were done, and a linear regression curve was plotted to obtain the amount of bacteria in which OD =1.

2.2. Preparation of green tea and black tea solution and disk diffusion assay

Four different concentrations of green tea and black tea solutions, 25 mg/mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL, were prepared with autoclaved water. An organic Green tea Matcha (Kiss Me Organics, WY) and organic Black tea Matcha (Pure Matcha, JP) were purchased and used to prepare 1 mL of each concentration, and the pH of each tea solution was then measured.

The 10^7 CFU of *P. aeruginosa* was used and plated on Mueller Hinton Agar plates (Difco Laboratories, MD). Sterile plain disks (Fisher Scientific, MA) were dipped into 25 mg/ mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL concentrations of prepared black and green tea respectively. The disks were dripped dried and placed in the center of each section of the plates and were grown at 37º C for 24 hours. The plates were then examined, and the diameter of the zone of inhibition for each concentration was measured. Four trials were done and the averages of the inhibition clear zones were calculated.

2.3. Testing The Coating Of Green Tea On Contact Lenses

Six new Acuve Moist Brand Contact Lenses (Johnson & Johnson, NJ) of -3.00 prescription strength were dripped dry from the original packaging using forceps sterilized in ethanol, and transferred to sterile vials. Three vials each containing 1 mL of PBS and three vials each containing 1 mL of 100 mg/mL of green tea were used. For each vial, one contact lenses was placed in the solution for one hour. After that, these contact lenses, and three more from the original packaging used for a positive control, were removed and placed in separate sterile vials containing 1 mL of 10^6 CFU of *P. aeruginosa*, and incubated for another hour. The contact lenses from the original packaging were also placed in a 1 mL solution of PBS to serve as a negative control. To recover the bacteria, the solutions from each treatment were diluted at a 1:10 dilution with PBS, and 0.1 mL of each was plated on 2 nutrient agar plates. The plates were incubated at 37ºC for 24 hours and the bacteria were enumerated. A total of seven trials were done.

2.4. The Inhibitory Properties Of Green Tea On *P. aeruginosa* Treated Contact Lenses

Another six new -3.0 prescription contact lenses were dripped dry from the original packaging using sterilized forceps, and transferred to separate sterilized vials containing 1 mL of 10^6 CFU/mL of *P. aeruginosa* and incubated on an orbital shaker rotator (Model KJ-201BD, Laboratory Sky, CN) for one hour. After that, the contact lenses were then dripped dried and transferred to sterile vials with three containing 1 mL of autoclaved water, and three containing 1 mL of 100 mg/mL of green tea. The contact lenses were incubated in these solutions for one hour at room temperature. All these treated contact lenses, along with three more from the original packaging used for a negative control, were removed and placed in sterile vials containing 1 mL of PBS to recover the bacteria. The solutions were diluted at a 1:10 dilution with PBS, and 0.1 mL of each solution was plated on 2 nutrient agar plates. The plates were incubated at 37ºC for 24 hours and the bacteria were enumerated. A total of seven trials were done.

2.5. Statistical Analysis

A one-way ANOVA with a post-hoc Tukey test was run on vassarstats.net. The test was used to determine the difference between the treatment groups within each experiment. The significance was set as $p < 0.05$. Figures were created using Microsoft excel with values shown as mean and Standard Error of Mean (SEM) for clarity.

3. Results

3.1. Optical Density And Cell Number

The relationship between optical density at 600 nm and the concentration of *P. aeruginosa* in CFU/ mL is shown in Figure 1. The resulting equation of the curve was $y= 9x10^8X – 3x10^7$. It was then determined the concentration of the *P. aeruginosa* was $8.7x10^8$ CFU/mL for when OD_{600} nm is equal to 1.

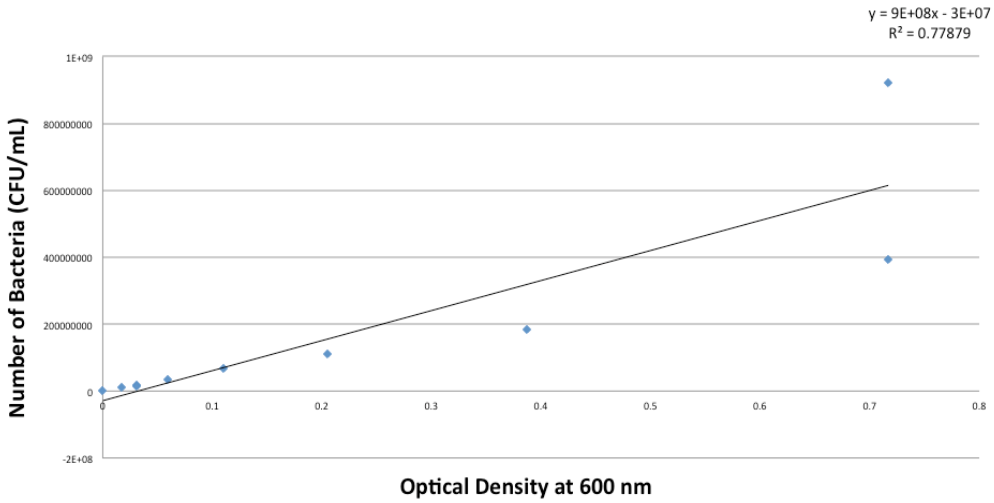

Figure 1: The correlation of the number of *P. aeruginosa* (CFU/ml) versus optical density

3.2. Disk Diffusion Assay

The pH of both black tea and green tea were found to be equal to 7. Both black tea and green tea were found to have an inhibitory effect on *P. aeruginosa* in the disc diffusion assay, however, green tea demonstrated a stronger effect than black tea (Figure 2). Green tea produced larger diameters of the zones of inhibition when compared to those of black tea in the same concentration. The differences between the green and black tea at the concentrations of 50 mg/ml and 100 mg/ml were significant ($p<0.05$). However, the differences between the concentrations 25 mg/ml and 200 mg/ml in both teas were considered to be marginally significant ($p=0.06$). The 100 mg/ml concentration depicts the most significant difference between the teas, with an average of a 2.05 cm diameter clear zone from the green tea. In addition, the 100 mg/ml of green tea produced the best soluble solution and would be used for the subsequent contact lenses experiments (data not shown).

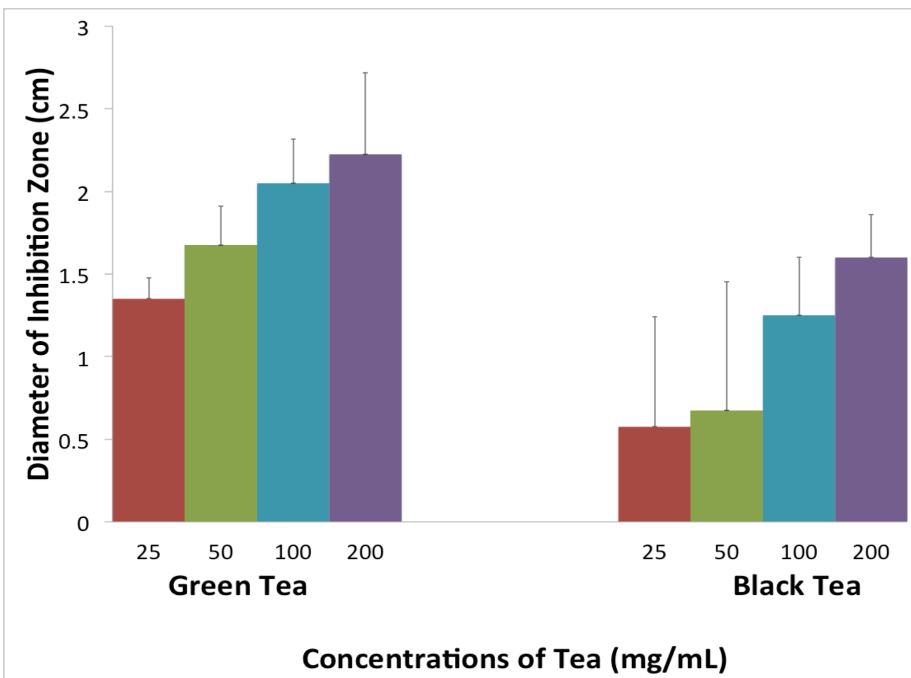

Figure 2: The average diameter (+SEM) (cm) of the clear zones when 10^7 *P. aeruginosa* was treated with green tea and black tea disc at different concentrations

3.3. The Inhibitory Properties Of Green Tea On Contact Lenses

Contact lenses were treated with different solutions followed by the incubation of 1 mL of 10^6 CFU of *P. aeruginosa* for an hour. Contact lenses that were treated with 100 mg/ml green tea had recovered 5645.45 ± 2399.7 CFU of *P. aeruginosa* when compared to the control with PBS that yielded 8690 ± 5232.05 CFU (Table 1). The original package of contact lenses that were incubated with an equal amount of *P. aeruginosa* had recovered 9722.2 ± 6287.8 CFU of the bacteria (Table 1). The green tea treated contact lenses had a 41.9 % inhibition rate when compared to the control that was not treated ($p= 0.062$). There was no significant difference in inhibiting bacteria when comparing contact lenses treated with green tea to contact lenses treated with PBS ($p>0.05$).

Table 1: The average number (± SEM) in CFU of *P. aeruginosa* recovered from contact lenses when treated with different solutions, followed by the incubation with 10^6 *P. aeruginosa* for an hour. The bacterial inhibition rate in percentage was calculated by the difference between the number of bacteria recovered from the original contact lenses without any treatment and the number of bacteria recovered from the treated contact lenses by PBS or green tea, divided by the bacteria recovered from the original contact lenses without any treatment

Contact lenses treatment	**Average number of *P. aeruginosa* (± SEM*) recovered (CFU)**	**Bacterial inhibition rate (%)**
Original Packaging (No bacteria)	0.083 ± 0.287	N/A**
Original Packaging (No treatment)	9722.2 ± 6287.8	N/A**
PBS	8690 ± 5232.05	10.6 %
100 mg/ mL of Green Tea	5645.45 ± 2399.7	41.9%

*SEM= Standard Error of Mean
**N/A= Not applicable

3.4. Testing Of Green Tea On Bacteria Treated Contact Lenses

The contact lenses that were incubated with 10^6 CFU of *P. aeruginosa* for an hour and treated with green tea afterwards were found to have significantly less bacteria (2887 ± 1441.18 CFU) when compared to contact lenses with equal amount of bacteria and treated with autoclaved water (61500 ± 3535.53 CFU) (Table 2) ($p<0.05$). The control with the original contact lenses had no bacteria recovered.

Table 2: The average number (±SEM) (CFU) of *P. aeruginosa* recovered from contact lenses when treated with different solutions for an hour after the contact lenses were incubated with 10^6 *P. aeruginosa* for an hour

Contact Lenses Treatment	**Average Number of *P. aeruginosa* (± SEM*) Recovered (CFU)**
Original Packaging (No bacteria)	0
Autoclaved Water	61500 ± 3535.53
100 mg/ mL of Green Tea	2887.78 ± 1441.18

*SEM= Standard Error of Mean

4. Discussion

In our *in vitro* studies, the green tea was used in an attempt to inhibit the *P. aeruginosa* from growing on contact lens. The Kirby Bauer disk diffusion assay was done to confirm the bactericidal properties of the green tea. Our results showed that green tea produced a larger diameter of the zone of inhibition when compared to the same concentration of black tea. In the previous studies, the minimum inhibitory concentration (MIC) of black tea alcohol extract was found to be 400 mg/mL on *P. aeruginosa* isolates with a 20 mm clear zone on the agar gel diffusion experiment.[25] This coincides with our findings with the use of the green tea on *P. aeruginosa* in the similar experiment, which produced a 20.5 mm sized clear zone, however, the concentration of green tea used was only 100 mg/mL. Several attempts to obtain the MIC of the green tea used in our studies were failed because of the dark color of the tea interfering the optical density reading (data not shown). However, this again proves green tea is more potent than black tea in inhibiting bacteria.[41] The stronger antioxidant properties of the catechins in green tea may attribute to its stronger antibacterial power. A previous study confirmed the antibacterial properties of the catechin was correlated to its antioxidant capacity on a phospholipid membrane model.[42]

In the *in vitro* contact lens studies, the green tea was used for the attachment of the contact lens before the treatment with *P. aeruginosa* for 1 hour. Our results showed that green tea does not effectively prevent *P. aeruginosa* from attaching and growing on contact lenses. Another experiment was performed by incubating contact lenses first with 10^6 CFU of *P. aeruginosa* for 1 hour, and then placed them in the treatment of 100 mg/ml of green tea for another hour. The bacteria recovered from the contact lenses treated with green tea showed a significant difference when compared to the control with autoclaved water. From our studies, it can be concluded that green tea showed a significant inhibitory property on the *P. aeruginosa* treated contact lenses, however, it was not able to remove all the bacteria from the contact lenses. This may be due to the high inoculum of bacteria (10^6) used in the experiment and the short treatment time of an hour. In a previous *in vitro* study, maximum numbers of *P. aeruginosa* were found to adhere on the contact surface within an hour, however, it would take generally 24 hours for the biofilm to be formed.[43] In addition, different isolates of *P. aeruginosa* may affect the ability of their attachment to the contact lenses.[44] The strain used in this experiment was for teaching use and not a clinical isolate, and this may affect our results, as well as the number of bacteria that attach on the contact lens during the one-hour time period. The Etafilcon A type of contact lenses of Acuve Moist with a high water content of 58% with ionic polymers were chosen to use in our study. The nature of the contact lens material also affects the attachment of the bacteria.[43,45] Different strains of *P. aeruginosa* were found to have less adhesion on the lens composed of ionic polymers than non-ionic polymers.[45] The pH environment also affects the bacteria attachment to the contact lens[45], and in our studies, the pH of the green tea was neutral.

Lastly, green tea's active ingredient, EGCG, has been shown to have the greatest antioxidant and antibacterial properties.[46,47,48] Further studies can be done testing this active ingredient against the *P. aeruginosa* on contact lens. In addition, the green tea we used is a type of matcha powdered form, and other types of green tea can be used for the future experiments. Overall, our results indicate the potential of using green tea as an alternate organic solution on the removal of the bacteria from the contact lenses.

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