

**Tannic Acid Loaded pHEMA-Quaternary Chitosan Hydrogels for Use as Antimicrobial Contact
Lenses**

Rebekah Switzer

North Carolina School of Science and Mathematics - Durham

Abstract

Contact lenses are very popular for vision correction, with an estimated 140 million users globally. Contact lens usage drastically increases the risk of developing bacterial keratitis, an infection in the cornea caused by common bacteria when lenses are mishandled. Bacterial keratitis is typically treated with antibiotic-loaded eye drops, but this treatment is not ideal due to the potential development of antibiotic resistant bacteria. This project developed a hydrogel using hydroxyethyl methacrylate, crosslinking with poly(ethylene glycol) dimethacrylate, and incorporating quaternized chitosan through crosslinking and tannic acid through hydrogen bonding. This combination was decided on for improved antimicrobial activity while maintaining ideal mechanical properties and hydrophilicity. Data on water content, water contact angle, transmittance, tensile strength, and bacterial inhibition has been collected. Water content for all hydrogels fell within the lower limit of HEMA contact lenses, near the natural water content of HEMA; water contact angles showed the gels were very hydrophilic; transmittance showed some blocking of light that increased as tannic acid concentration increased and potential UVA blocking properties; tensile testing showed reasonable although slightly high Young's modulus scores; bacterial testing showed significant inhibition with higher concentrations of tannic acid. Further biocompatibility testing is the next step towards use as a contact lens material to prevent bacterial keratitis and cut down on unnecessary antibiotic usage.

Table of Contents

I. Introduction	1
1.1 Contact Lens Associated Bacterial Keratitis	1
1.2 Previous Solutions to Bacterial Keratitis	1
1.3 Contact Lens Materials and Properties	1
1.4 Quaternized Chitosan Properties	2
1.5 Tannic Acid Properties	3
1.6 Past Research Conducted	4
1.7 Hypothesis	4
II. Materials and Methods	5
2.1 Materials	5
2.2 Synthesis of pHEMA-QCS Hydrogels	5
2.3 Incorporation of Tannic Acid	5
2.4 Equilibrium Water Content	5
2.5 Water Contact Angle Test	5
2.6 Transmittance	6
2.7 Tensile Testing	6
2.8 Antimicrobial Activity	6
III. Results and Discussion	7
3.1 Evaluation of Equilibrium Water Content	7
3.2 Water Contact Angle Evaluation	8
3.3 Tensile Testing of Hydrogels	9
3.4 Analyzing Transmittance of Hydrogels	10
3.5 Bacterial Analysis	11
IV. Conclusions	12
V. Future Directions and Limitations	13
VI. Acknowledgments	13
VII. Works Cited	14

I. Introduction

1.1 Contact Lens Associated Bacterial Keratitis

Contact lenses are very popular for vision correction, with an estimated 140 million global contact lens users [1]. Wearing contact lenses drastically increases the risk of developing bacterial keratitis, an infection in the cornea—the clear dome on the surface of the eye. Contact lens-associated keratitis occurs in 2-4 out of 10,000 contact lens wearers, with 90% of the cases attributed to bacteria [2]. One million US health care visits for keratitis or other contact lens complications occur annually [3]. The infection is typically caused by *Pseudomonas aeruginosa* or *Staphylococcus aureus*, both very common bacteria which can easily come in contact with the eye if contact lenses are not cleaned and handled properly during and after each use. The most common symptoms of bacterial keratitis are pain and redness in the eye, but if left untreated can result in vision loss or even blindness [3].

1.2 Previous Solutions to Bacterial Keratitis

Typically, bacterial keratitis is treated with antibiotic-loaded eye drops. To help prevent the initial development of bacterial keratitis, improvements can be made directly to contact lenses to prevent bacteria from adhering to the lens and growing on the cornea. Multiple contact lens solutions with antimicrobial capabilities have been developed, decreasing bacterial adhesion or killing surface-level bacteria to prevent potentially harmful infections. These solutions include metal nanoparticles and direct release of antibiotics into the eye through drug-loaded contact lenses [4]. While both of these methods have proved effective, they also present significant risks. Metal nanoparticles can be toxic to the eye if they penetrate the epithelial barrier, causing stress and apoptotic damage [5]. Excess antibiotic usage can lead to the development of antibiotic-resistant bacteria and cause exaggerated inflammatory responses during treatment. Therefore, altering the contact hydrogel itself could provide a biocompatible option with lower risk to human health.

1.3 Contact Lens Materials and Properties

Hydroxyethyl methacrylate (HEMA) is an oxygen-permeable monomer with a naturally high water content due to polar properties due to the negatively charged hydroxyl group (see **Figure 1**). These characteristics allow for wetting of the surface of the hydrogel, improving comfortability on the surface of the eye [7]. HEMA is known as a biocompatible and hydrophilic monomer with ideal mechanical properties for contact lens production [8].

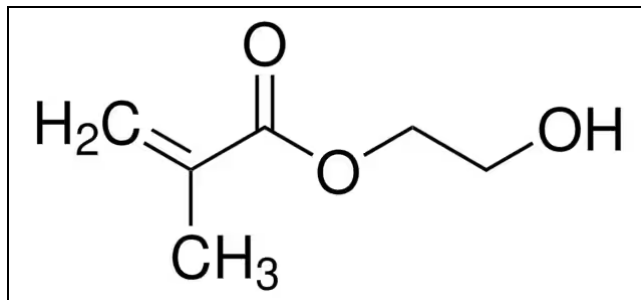


Figure 1. Chemical structure of 2-hydroxyethyl methacrylate.
Figure reprinted from Sigma-Aldrich, (Product No. 477028), Merck KGaA.
<https://www.sigmaaldrich.com/US/en/product/aldrich/477028>.

Polyacrylamide (PAM) is another monomer which has been used for contact lenses, but is not ideal due to risks associated with unreacted acrylamide monomers during synthesis, lower oxygen permeability, and lower hydrophilicity compared to HEMA [9]. Lenses need to be hydrophilic to remain wetted and comfortable in the eye and oxygen permeable to ensure the cornea receives needed oxygen to remain healthy with prolonged contact lens usage.

1.4 Quaternized Chitosan Properties

Chitosan is derived from chitin, which is naturally found in the cell walls of crustaceans, fungi, insects, and microorganisms [10]. Chitosan is a popular choice for medical applications due to its biocompatibility, biodegradability, and antimicrobial properties. For contact lens applications, chitosan's moisture retention would help to keep the lens hydrated and comfortable. Chitosan's antimicrobial activity comes from interacting with the negatively charged microbial cell membranes to cause structural damage and leakage of intracellular components [10]. Quaternary chitosan is made by introducing quaternary ammonium structures onto the chitosan macromolecule (see **Figure 2**).

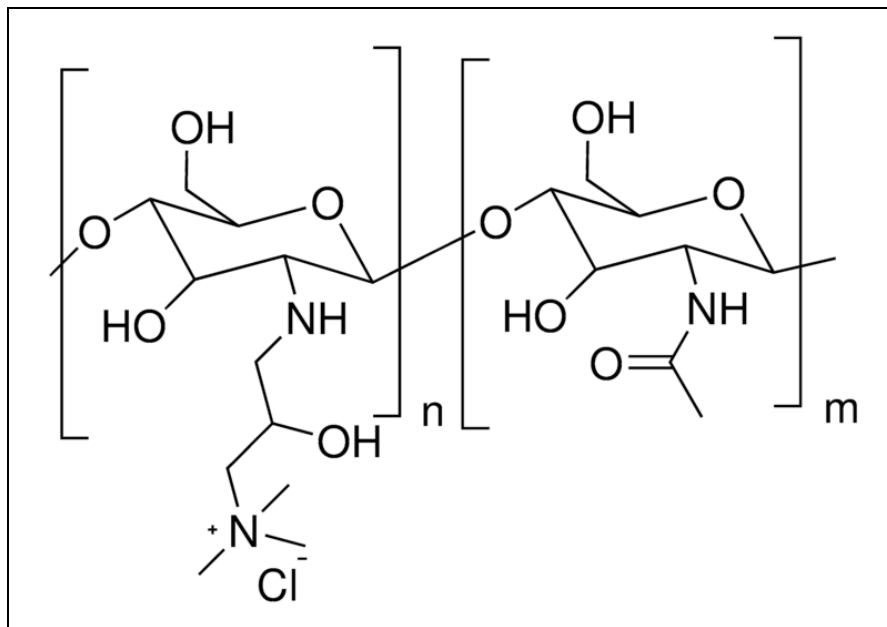


Figure 2. Chemical structure of quaternary chitosan ammonium salt.
 Figure reprinted from Sigma-Aldrich, (Product No. 933899), Merck KGaA.
<https://www.sigmaaldrich.com/US/en/product/aldrich/933899>.

This alteration improves solubility, effectiveness over a wide pH range, and biocompatibility, making it a better candidate for incorporation into a contact lens hydrogel [10].

1.5 Tannic Acid Properties

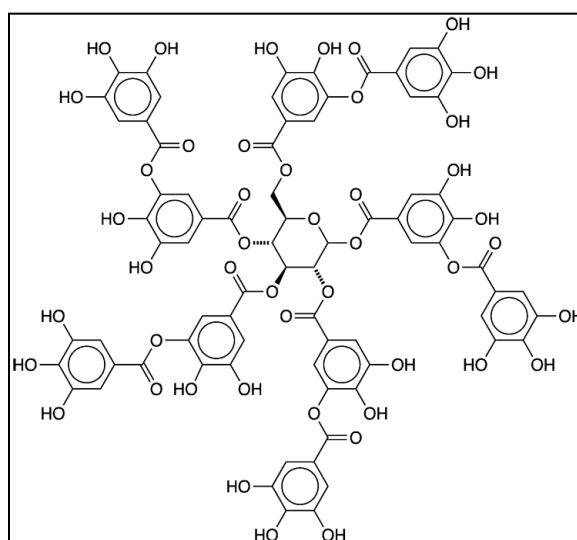


Figure 3. Chemical structure of tannic acid.
 Figure reprinted from "Tannic acid," ACS Molecule of the Week, January 16, 2018, American Chemical Society.
<https://www.acs.org/molecule-of-the-week/archive/t/tannic-acid.html>

Tannic acid is a natural polyphenol with promising antimicrobial and antioxidant properties. Antioxidant properties come from numerous hydroxyl groups in the chemical structure which can donate electrons to neutralize free radicals (**Figure 3**). Antimicrobial activity is carried out when the molecule interacts with and disrupts cell membranes of bacteria, fungi, and viruses, leading to cell lysis and death. These two properties allow bacteria growth to be inhibited without causing damage to the eye when tannic acid is released, as shown in Jiao, Z. et al., preventing bacterial infection and eliminating the need of antibiotics for treatment [9].

1.6 Past Research Conducted

One method that has been developed to improve the hydrogel structure is incorporating quaternized chitosan salt, also known as hydroxypropyltrimethyl ammonium chloride chitosan (HACC), as a macromolecular crosslinker. Quaternized chitosan is used for enhanced antimicrobial properties, increased tear protein deposition resistance, and improvements in mechanical properties of the hydrogel. HACC was used rather than regular chitosan for increased water solubility through added quaternary ammonium structures [13]. Natural polyphenols, such as tannic acid or epigallocatechin gallate, have also been incorporated into contact lens hydrogels in previous research to improve antimicrobial properties [9][14]. There are multiple potential monomers that can be used to form contact lens hydrogels and could be combined with tannic acid and HACC, such as polyacrylamide (PAM) and 2-hydroxyethyl methacrylate (HEMA). HEMA is generally considered a better monomer for contact lenses than PAM due to its oxygen permeability, mechanical properties, and drug-releasing capabilities, but has not previously been used in conjugation with tannic acid in contact lens applications.

1.7 Hypothesis

This study aims to crosslink HEMA with quaternized chitosan and incorporate tannic acid in a hydrogel that could function as a contact lens material with improved antimicrobial activity while maintaining ideal hydrophilicity and mechanical properties for comfortability.

II. Materials and Methods

2.1 Materials

2,2'-azobis(2-methylpropionitrile), 2-Hydroxyethyl methacrylate, and poly(ethylene glycol)dimethacrylate were obtained from Sigma Aldrich. Quaternary chitosan ammonium salt was obtained from Mark Nature through Amazon. Tannic acid was obtained from Alfa Aesar. K-12 *E. coli* was obtained from Carolina Biological.

2.2 Synthesis of pHEMA-QCS Hydrogels

pHEMA-QCS hydrogels were synthesized by first dissolving 10 mg quaternary chitosan ammonium salt in 1240 μ L deionized water, then adding 6 mL HEMA, 120 μ L methacrylic anhydride, and 140 μ L PEGDMA and vortexing to mix for 3-5 minutes. After thoroughly combining, 7.3 mg of the thermal initiator, AIBN, was dissolved in methanol and vortexed into the solution. The hydrogels were then placed in the oven on a teflon sheet within a glass dish at 65 °C for 24 hours. After synthesis, the hydrogels were soaked in deionized water overnight to allow swelling to equilibrium.

2.3 Incorporation of Tannic Acid

After successful synthesis and initial hydration, hydrogels were dried in an oven at 65 °C for 24 hours and placed in solutions of varying concentrations of tannic acid (0, 5, 10, 15 g/L) to incorporate tannic acid into the hydrogel matrix through hydrogen bonding. Tannic acid was incorporated after initial synthesis to prevent disruption of initial crosslinking.

2.4 Equilibrium Water Content

Equilibrium water content was determined gravimetrically. Wet hydrogels were massed after removing surface water with a paper towel, and placed in an oven at 65 °C. Hydrogels were massed until constant mass was achieved, and equilibrium percent water content was calculated using the below formula:

$$\frac{(\text{wet mass} - \text{dry mass})}{\text{wet mass}} \times 100$$

2.5 Water Contact Angle Test

A 50 μ L drop of water was micropipetted onto a flat disk of hydrogel. Images were taken on a Moto G Power 5G smartphone, aligned parallel to the flat hydrogel surface to ensure consistent measurement of droplet angles. ImageJ was used to quantify the angle between the gel and the water droplet formed.

2.6 Transmittance

Transmittance was measured using a Vernier spectrophotometer (Spectro-Vis). The instrument was calibrated without a cuvette, and a strip of hydrogel between 1-2 mm thickness was suspended over the light source during data collection.

2.7 Tensile Testing

Hydrogels were initially cut into a dogbone shape, and dimensions (length, width, and thickness) were measured using a caliper. Force and displacement data was collected using a Vernier Structures & Materials Tester. This data was converted into a stress strain curve, using the equations below, and Young's modulus was calculated using the slope of the linear portion of the stress-strain graph.

$$\textit{Strain} = \frac{\textit{displacement}}{\textit{original length}} \quad \textit{Stress} = \frac{\textit{force}}{\textit{cross-sectional area}}$$

2.8 Antimicrobial Activity

Bacterial solution from *E. coli* suspended in LB broth was determined to have an absorbance at 600 nanometers less than 1.00. A bacterial solution (200 μL) was then micropipetted into dilution tubes and hydrogel samples of nearly equal masses were submerged in solutions, including a control with only bacterial solution, and then incubated in a shaker overnight at 37 $^{\circ}\text{C}$. After incubation, the samples were serially diluted to a concentration of 1:10⁶ and spread on agar plates to incubate for 15 hours at 35 $^{\circ}\text{C}$. Plates were imaged after incubation and colonies were counted using ImageJ software.

III. Results and Discussion

3.1 Evaluation of Equilibrium Water Content

The recorded equilibrium water content for all hydrogels tested, including HEMA hydrogels crosslinked with PEGDMA only, those crosslinked with quaternary chitosan (QCS) in addition to PEGDMA, and gels with various concentrations of tannic acid of both versions, fell near the water content of pure HEMA of 38%—typical for HEMA-based hydrogels (see **Figure 4**).

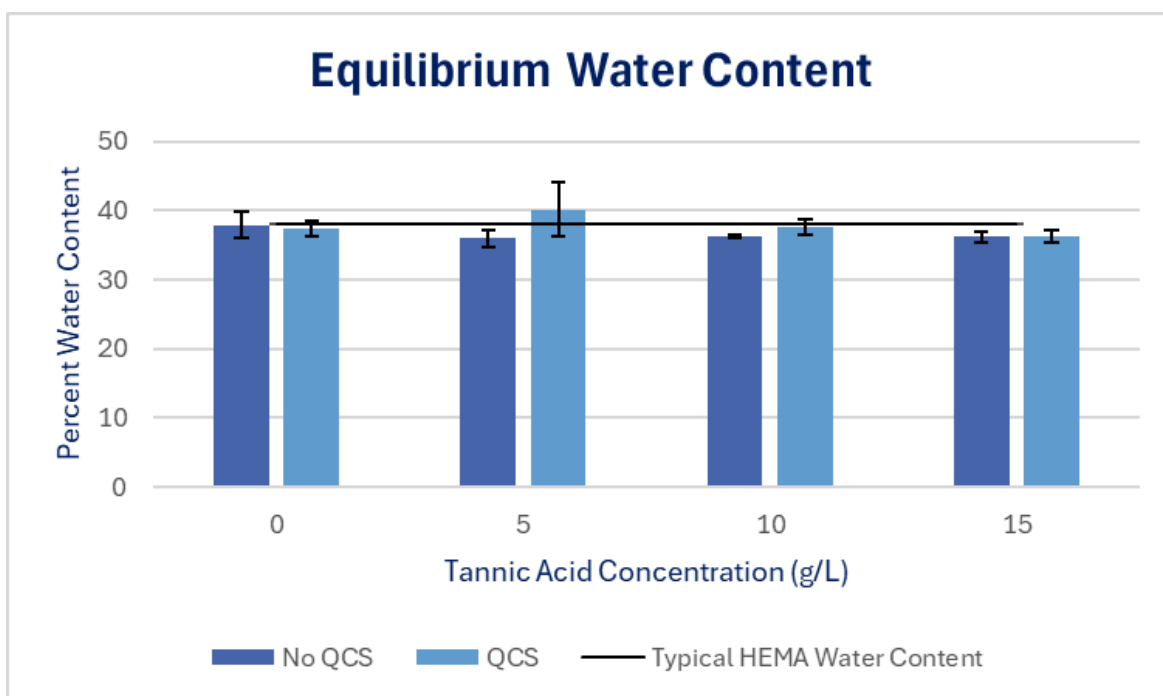


Figure 4. Hydrogels were massed before and after drying at 65 °C to determine initial percent water content after soaking in DI water until equilibrium.
Graph created by student researcher using Excel, 2025.

Both crosslinking quaternary chitosan and incorporating tannic acid had no apparent impact on water content. These hydrogels would be classified as low water contact lenses, indicating less water drawn from the eye to hydrate the hydrogels [15].

3.2 Water Contact Angle Evaluation

Water contact angles of a droplet of water on gel were measured to determine hydrophilicity. Angles were quantified using ImageJ.

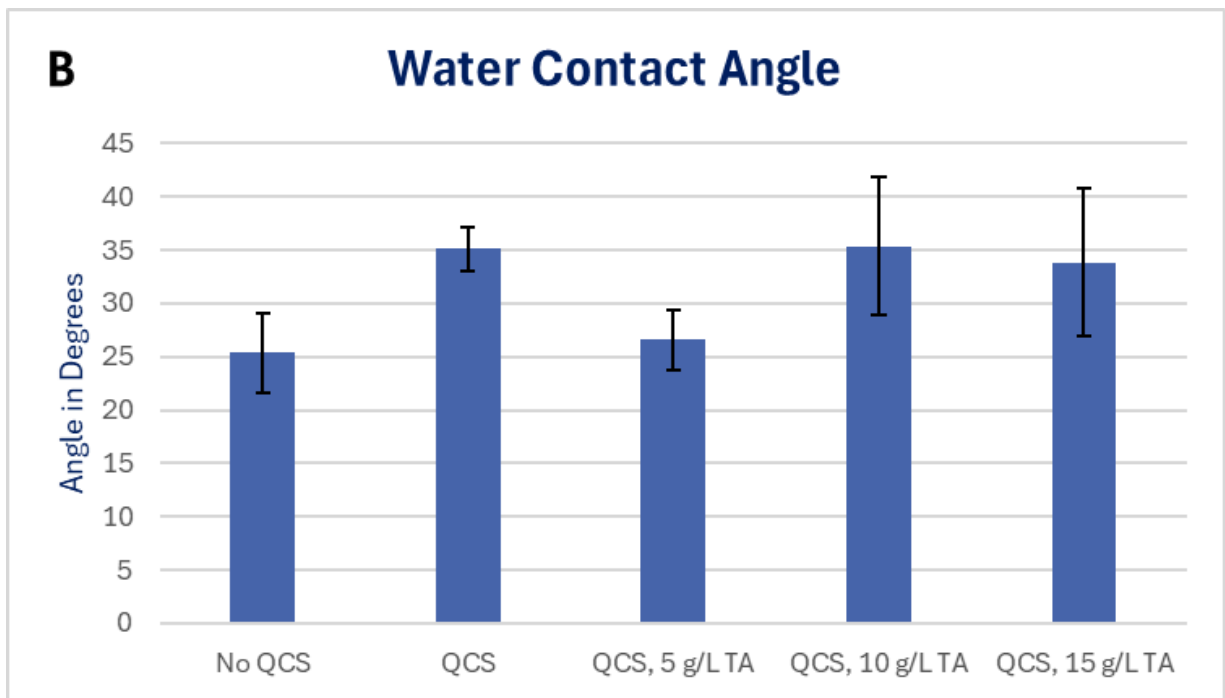
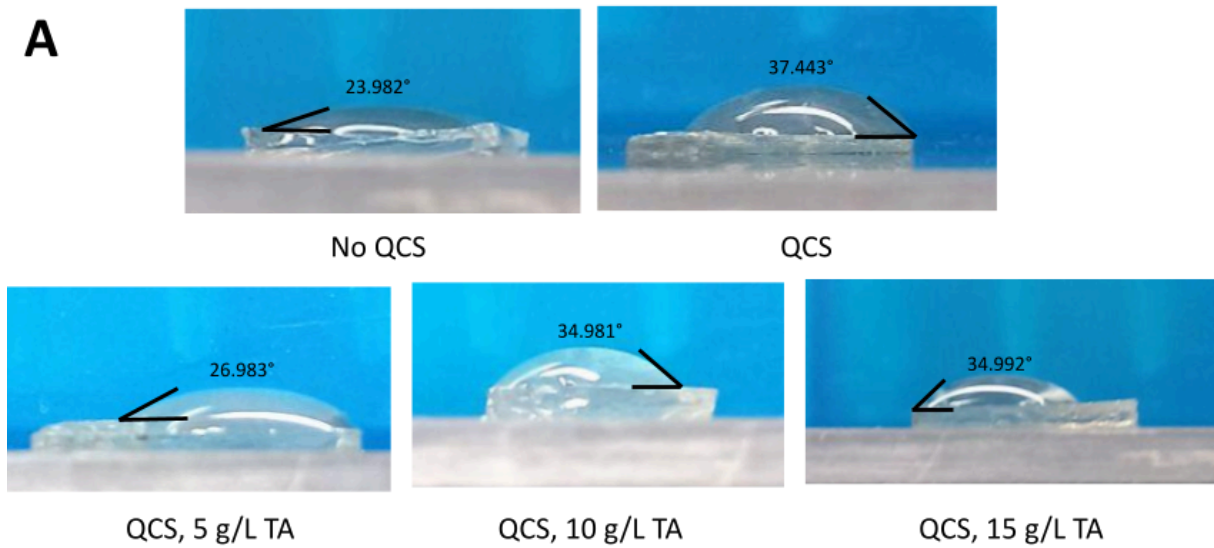


Figure 5

A) Water contact angle visuals resulting from a drop of water deposited on a flat gel sample. Angles were determined using ImageJ. Images taken by student researcher, 2025. B) Average water contact angles (n=3) for each all gels. Graph created by student researcher using Excel, 2025.

All water contact angles recorded fell between 20-40 degrees (**Figure 5**). This shows all hydrogels were highly hydrophilic due to angles significantly smaller than 90 degrees. Crosslinking quaternary chitosan showed an increase in water contact angle, indicating decreased hydrophilicity, from an angle around 25

degrees for the gel with no quaternary chitosan to 35 degrees with chitosan incorporated. Increasing tannic acid concentration had no clear impact on water contact angle.

3.3 Tensile Testing of Hydrogels

In addition to interactions with water, flexibility of hydrogels was tested. Young's modulus of elasticity is a measure of the rigidity of a material, with a higher modulus indicating a stiffer material and a lower modulus indicating more flexibility but decreased strength. The modulus of a contact lens ideally falls within the range of 0.2 to 1.5 MPa, indicating a low likelihood of breaking while moving in the eye without feeling too rigid [16].

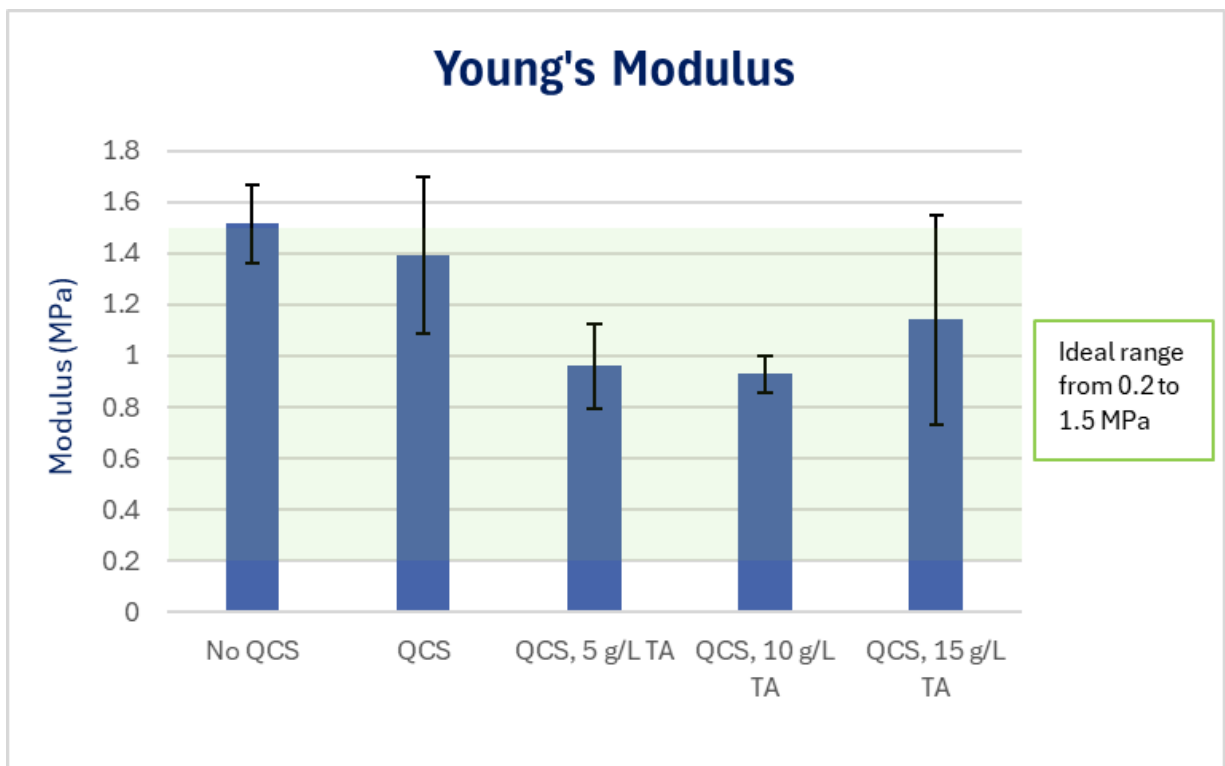


Figure 6. Young's modulus values calculated using the slope of the best-fit line of the linear portion of stress vs strain curves. Graph created by student researcher using Excel, 2025.

Nearly all tested hydrogels fell within the ideal range, with the average modulus of the control containing no quaternary chitosan or tannic acid falling slightly outside of this ideal range at 1.516 MPa (**Figure 6**). Incorporating tannic acid had the largest impact on the modulus, showing decreased values for all concentrations when compared to pHEMA-QCS hydrogels, indicating more flexibility for all concentrations.

3.4 Analyzing Transmittance of Hydrogels

Transmittance was tested to determine visibility when using contact lenses.

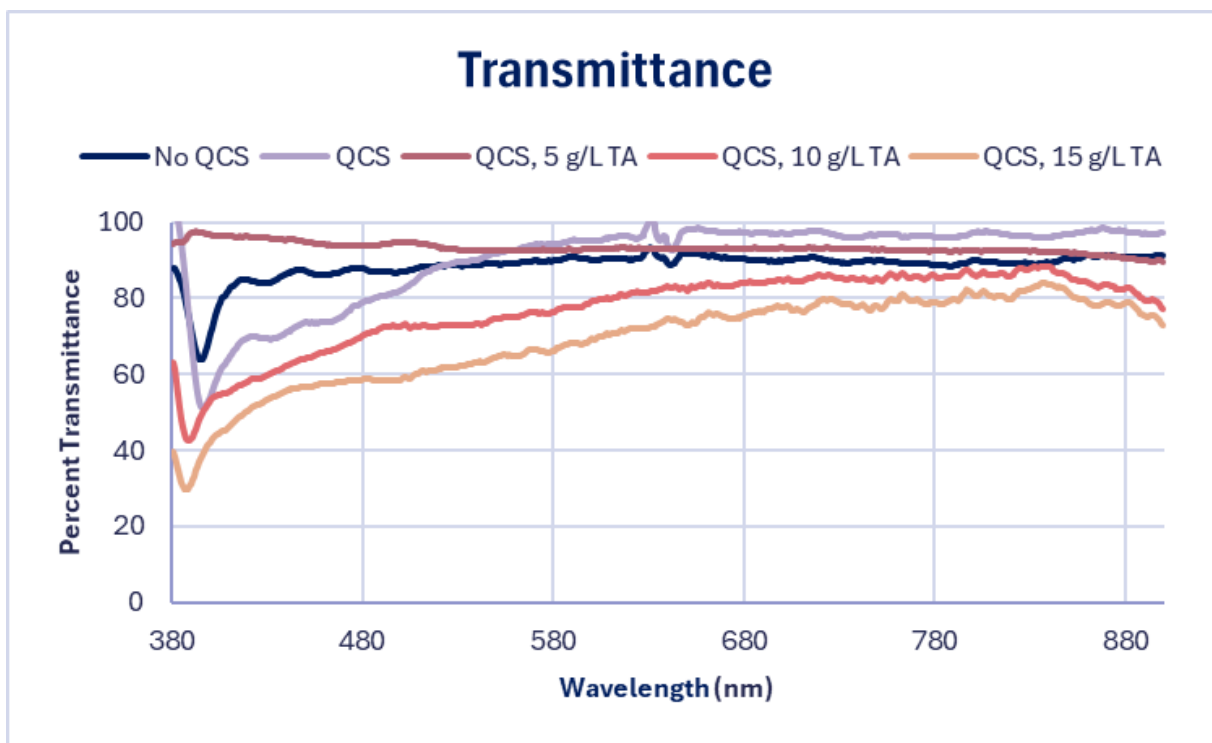


Figure 7. Percent transmittance of all gels was determined through spectroscopy.
Graph created by student researcher using Excel, 2025.

Nearly all hydrogels had an absorbance peak near 380 nanometers, with overall transmittance decreasing as tannic acid concentration increased (**Figure 7**). Transmittance values are likely lower than the transmittance of a contact lens made out of the corresponding material would be due to inability to standardize synthesis. Gel pieces were much thicker than a contact lens would be, ranging from thicknesses of 1-2 mm compared to the typical contact lens thickness of 0.2 mm [17]. Increased thickness would cause increased absorbance in samples tested when compared to a theoretical contact lens made from the material. There also appears to be some absorbance of UVA radiation, which could be beneficial in a contact lens to prevent this radiation from damaging the eyes. Further testing would need to be completed on absorbance over UV wavelengths to quantify.

3.5 Bacterial Analysis

Tannic acid was added to hydrogel after initial synthesis to improve bacterial keratitis prevention. Antimicrobial activity of all hydrogels was assessed by incubating gel samples in bacterial solution

overnight. After serially dilution to a factor of $1:10^6$, the solutions were spread on agar plated and incubated overnight.

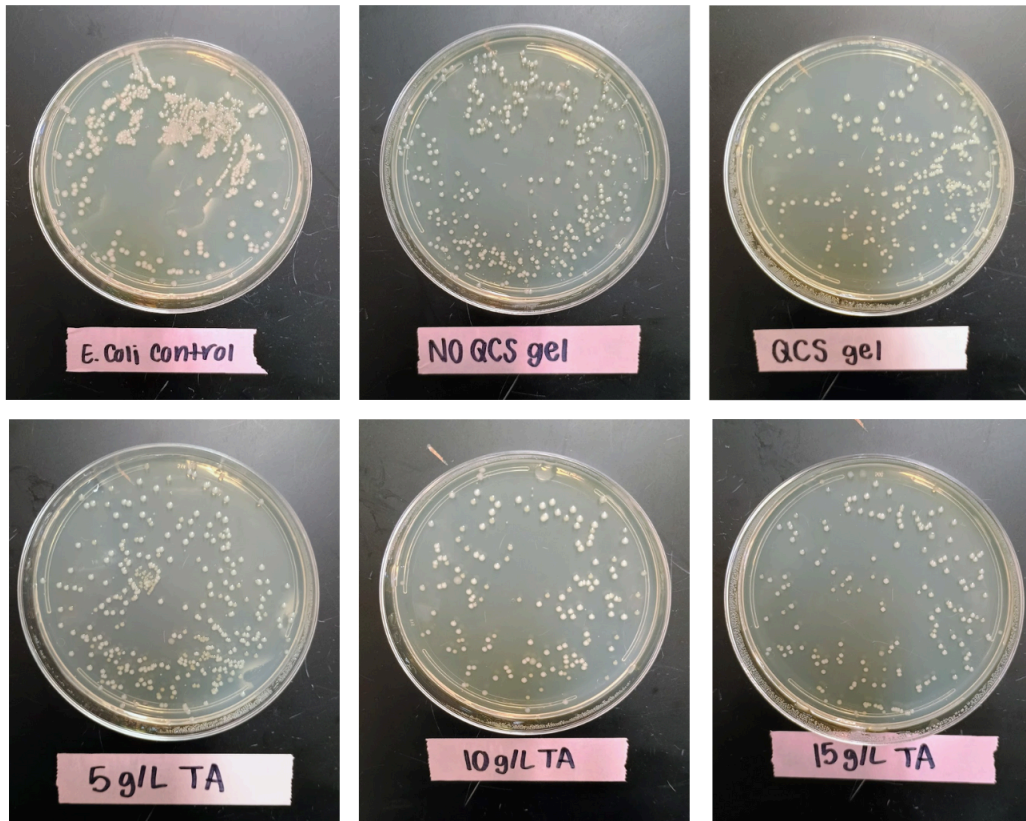
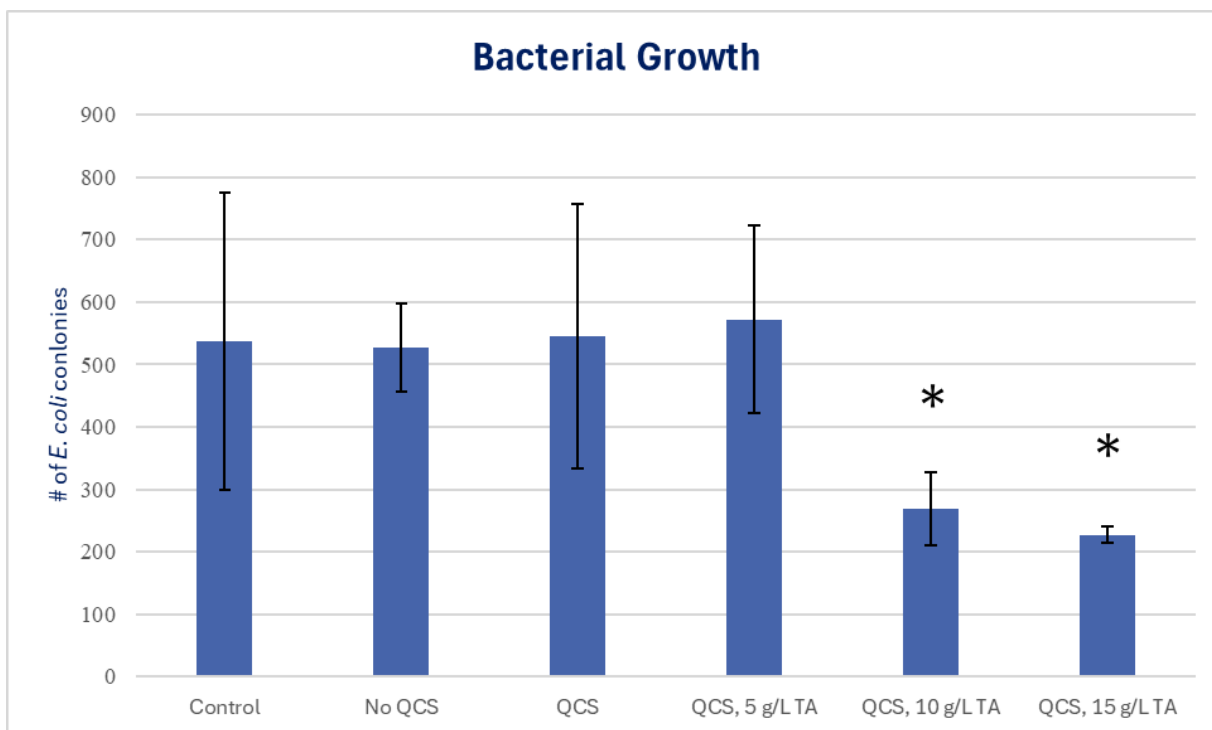


Figure 8. Images analyzed of bacterial colony growth. All plates labeled with tannic acid concentrations also contain quaternary chitosan. *Images taken by student researcher, 2025.*



* indicates $p < 0.05$ when compared to control

Figure 9. Colony counts of bacterial solution incubated overnight with gel samples, determined using ImageJ software. Graph created by student researcher using Excel, 2025.

Gels soaked in tannic acid solutions of 10 g/L and 15 g/L showed substantial inhibition of bacteria, with an average of 269 and 227 colonies, respectively, compared to a control of 538 colonies (**Figure 9**). These gels also showed less deviation from the mean over multiple trials, showing statistical significance compared to control with a Student T test. All other gels tested had relatively similar numbers of colonies grown, and no statistical significance when compared to the control. QCS on its own did not appear to improve antimicrobial activity, whereas tannic acid had a much larger impact.

IV. Conclusions

pHEMA-QCS hydrogels were successfully synthesized, incorporating tannic acid after initial synthesis. The addition of quaternary chitosan (QCS) to hydrogels was shown to have no clear impact on water content and cause a slight increase in water contact angle. Tannic acid had no clear impact on water contact angle or equilibrium water content. All data values remained near the typical water content of HEMA of 38% and all water contact angles showed strong hydrophilicity, ideal for comfortable contact lenses, demonstrating that addition of tannic acid does not affect the hydrophilicity of the pHEMA-QCS. Stress vs strain data yielded Young's modulus values within the reasonable range for contact lenses, from

0.2 to 1.5 MPa, with QCS and tannic acid both resulting in lower modulus values. Transmittance data indicated a substantial amount of light absorbed, increasing as tannic acid concentration increased, with highest absorbance at the lowest wavelengths of visible light. This is not ideal to provide contact lenses with high visibility, but may be due to the increased thickness of gel samples tested compared to contact lens thickness. Gels tested ranged from 1-2 mm thickness, while contact lenses typically fall around a thickness of 0.2 mm. Higher absorbance at lower wavelengths could also indicate a protective effect from UVA radiation. Bacterial data collected after incubating with gel samples indicates tannic acid is an effective solution to preventing bacterial growth of *E. coli* at high enough concentrations. Taken together, all data collected indicates the hydrogels synthesized are a promising contact lens material due to interactions with water observed through water content and water contact angle, tensile strength, and successful bacterial inhibition.

V. Future Directions and Limitations

With improved lab equipment, synthesis of hydrogels could be better standardized to improve consistency in the thickness of samples and achieve thinner samples more similar to contact lenses. Lenses would also be freeze dried before incorporating tannic acid if this equipment was available. Freeze drying creates a porous structure in the hydrogel, allowing better incorporation of tannic acid when soaking in tannic acid solutions. Future testing would include a bacterial assay with positively charged *S. aureus* to ensure inhibition over positively and negatively charged bacteria. Biocompatibility and cytotoxicity assays using cells would also be performed to ensure hydrogels are safe for biological applications. Cell testing was not possible in this setting due to lack of access to a sterile room for controlled growth.

VI. Acknowledgments

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