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Antioxidant and Physical Properties of Dual-Networked Contact Lenses Containing Quercetin Using Chitosan and Alginate

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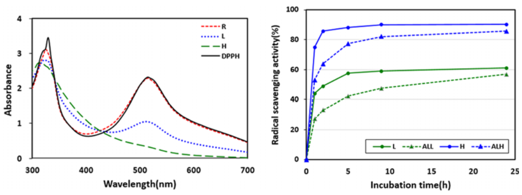
Abstract: The purpose of this study was to investigate the physical properties and antioxidant properties of dual networked contact lenses containing quercetin using chitosan and alginate. For sample preparation, the first step was to prepare contact lenses by adding quercetin, and the second step was to perform IPN (Interpenetrating Polymer Network) with 1% chitosan and alginate for 24 hours. The physical properties of contact lenses were evaluated in terms of light transmittance, water content, oxygen permeability, wettability, protein adsorption, and antibacterial properties. Antioxidant properties were evaluated by DPPH and ABTS assays. The higher the concentration of quercetin, the better the antioxidant properties. IPN-polymerized contact lenses with chitosan and alginate continuously released quercetin for more than 24 hours, extending the duration of antioxidant activity. Contact lenses containing quercetin showed lower physical properties compared to contact lenses without quercetin. However, contact lenses treated with IPN using alginate and chitosan had improved water content, oxygen permeability, and wettability. It was confirmed that quercetin improved the antioxidant properties of contact lenses, and alginate and chitosan further improved the antioxidant properties and physical properties of contact lenses.



Keywords: contact lens, quercetin, dual-network, alginate, chitosan, antioxidant, IPN.

1. Introduction

Hydrogels have high biocompatibility due to their high water content and physicochemical similarity with extracellular matrices.[1-3] Due to such properties, hydrogels are used in a range of areas including food additives, biomedical applications, tissue engineering and regenerative medicine, artificial skin, drug delivery systems, wound dressings, and contact lenses.[4-6]

In the past, hydrogel contact lenses were used for correcting refractive errors. In recent years, however, contact lenses have become high-value-added products that are more flexible and comfortable to wear. As such, they are now used in other areas, such as cosmetics, treatment by drug release, and ophthalmic diagnosis and prevention.[7] Contact lenses have different effects on the eyes depending on the material and design. As they are heavily involved in eye health, conditions such as physical and optical properties, biocompatibility, and non-toxicity need to be considered in manufacturing contact lenses.[8]

Wearing contact lenses for a long time can harm your eyes because the active oxygen generated near the lens starts to accumulate in the eyeball and destroy the cells around the macula. In addition, the increased use of various electronic devices such as tablets and smartphones with recent technological advancements results in excessive production and accumulation of reactive oxygen species (ROS), such as hydroxyl radicals, in the eye.[9] Exposure to high-energy UV rays from outdoor activities also increases the production of radicals in the eye and causes cell damage, which induces the apoptosis of human lens epithelial cells and causes various eye diseases, such as glaucoma and cataract.[10,11] ROS are unstable because they consist of atoms or molecules with an unpaired electron, and thus are prone to stealing electrons from other molecules. They damage membranes and blood vessels during this process, causing eye diseases.[12]

In recent years, a wide variety of studies have been conducted on antioxidant functional contact lenses containing natural antioxidants to prevent eye diseases caused by free radicals. These studies include manufacturing antioxidant contact lenses with dopamine (a natural antioxidant) on the hydrogel contact lens surface based on interpenetrating a hyaluronic acid network structure and hydrogel contact lenses combining gallic acid and chitosan.[13-15]

Quercetin is a natural antioxidant and plant polyphenol compound widely distributed in nature. It has high antioxidant and radical scavenging properties and a wide range of biological activities, such as anti-atherosclerosis effects, anti-allergy, antibacterial, anti-inflammatory, anti-viral, anti-cancer, anti-platelet aggregation, liver protective effects, and physiological activities, including lowering blood sugar. Research has been conducted on the application of quercetin to a multi-purpose solution for contact lenses to have antibacterial properties.[16]

Contact lenses can significantly affect your eyes because they lie directly on the ocular surface. This means that they can cause eye diseases, such as dry eyes and corneal edema, depending on the material and characteristics. Researchers have attempted

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to introduce natural polysaccharides to improve the biological stability and physical properties of contact lenses and reduce side effects with their physiological activities.[17,18] However, natural polysaccharides have a large molecular size, so they do not mix well with contact lens materials. For this reason, numerous studies have been performed on ways to form Interpenetrating polymer networks (IPNs) structures using natural polysaccharides in contact lenses.

IPN is a blend type of polymer and is a polymer containing two or more networks, and it is difficult to separate each network because polymer chains belonging to one network are physically entangled with other networks.[19] Application of these methods to the manufacture of contact lenses has been shown to significantly improve functions such as water content, wettability, oxygen permeability, protein adsorption and antibacterial properties. Contact lenses that went through the IPN process using natural polysaccharides such as alginate and chitosan showed various performance improvements such as wettability and oxygen permeability.[20,21]

Although many studies have been conducted on ophthalmic drug delivery systems,[22-24] there is a lack of studies on antioxidant contact lenses using natural substances. In addition, research and development have not yet been conducted on the duration of antioxidant activity related to the release rate of antioxidants in antioxidant contact lenses.

For this reason, there is a need for research on the fabrication of high-functional antioxidant contact lenses using natural antioxidants, controlling drug release rates with IPN polymerization using natural polysaccharides, and extending the duration of antioxidant functions.

This study fabricated high-functional antioxidant contact lenses using quercetin, a natural antioxidant, and these contact lenses were double networked with natural polysaccharides (alginate and chitosan) to form IPN structures. The purpose was to improve the release rate of quercetin to check whether this led to extending the duration of antioxidant activity and enhanced contact lens properties such as water content, oxygen permeability, wettability, protein adsorption, and antibacterial properties.

2. Experimental

2.1. Reagents

The monomers used to prepare the hydrogel were 2-Hydroxy ethyl methacrylate (HEMA), N-Vinyl-2-pyrrolidone (NVP), Glycidyl Methacrylate (GMA), and styrene. Ethylene glycol dimethacrylate (EGDMA) was used as the crosslinking agent, and 2,2-Azobisisobutyronitrile (AIBN, Japan, JUNSEI) and Ammonium persulfate (APS) were the initiators. Quercetin was used as a natural antioxidant, and sodium alginate and chitosan was used as natural polysaccharide. Chitosan used low molecular weight water solubility. All the reagents used in this study were products of Sigma-Aldrich (USA), with the exception of AIBN.

2.2. Fabrication of contact lenses containing quercetin

The contact lenses were fabricated using hydrophilic monomers HEMA, NVP, and GMA, a thermal initiator (AIBN), and a crosslinking agent (EGDMA) (Table 1). Quercetin is poorly soluble in aqueous media, so it was dissolved in ethanol until the concentrations in samples reached 0.15% and 0.3%, respectively. The polymerization samples were pre-treated at 80 ℃ for 150 minutes after mixing all of them except for AIBN. After volatilizing the ethanol, the temperature was lowered to room temperature, and AIBN was added and stirred. The mold for fabricating the contact lenses was made of polypropylene material, thermally polymerized at 120 ℃ for 1 hour.

The fabricated contact lenses were labeled R for contact lenses without quercetin, L for those containing 0.15% quercetin, and H for contacts containing 0.3% quercetin.

2.3. Fabrication of double-network contact lenses using natural polysaccharides

Alginate and chitosan have poor miscibility with the monomers used to fabricate contact lenses. Therefore, these polysaccharides were not used in the initial polymerization, but a secondary network was made using the interpenetrating polymer network (IPN) method. This study used the semi-IPN method. Alginate and chitosan were added to distilled water at a 1% ratio, stirred for 2 hours using ultrasonic waves, then stirred for another 30 minutes by ultrasonication after adding 0.3% of APS, before removing the unreacted monomers. The R, L, and H contact lenses were incubated in 1% alginate, chitosan IPN solution at 37 ℃ for 24 hours.

A schematic diagram of the process of double-networking with alginate and chitosan in contact lenses is presented in Figure 1. Contact lenses polymerized using the IPN method were labeled by adding AL to the ones IPN-polymerized with alginate and CH for those with chitosan. For example, contact lenses R

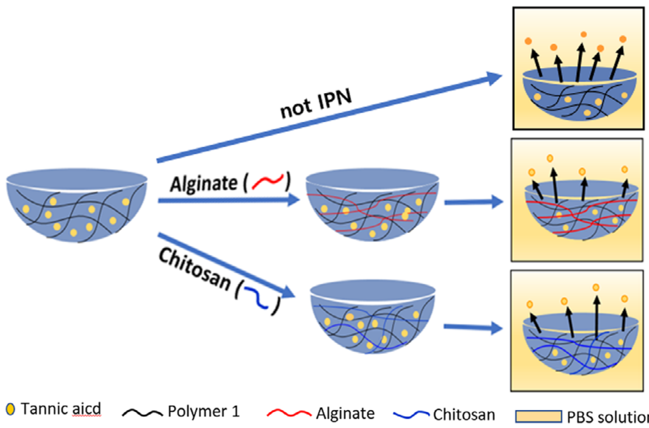

Figure 1. Schematic of quercetin release from contact lenses double networked with polysaccharides.

Table 1. Percent composition of hydrogel contact lens (%)

HEMA	NVP	GMA	EGDMA	1% AIBN	Quercetin (in ethanol)
42	10	5	3	10	30

networked with alginate were labeled ALR, and those networked with chitosan were labeled CHR. If the contact lenses were IPN-treated with alginate, they were labeled ALR, ALL, and ALH, respectively, by adding R, L, and H depending on the amount of polysaccharides.

2.4. Evaluating the antioxidant function of the contact lenses

The antioxidant properties of contact lenses were evaluated based on the radical scavenging rates over time using a UV-vis spectrophotometer (Agilent, Cary 60 UV-vis) with the DPPH and ABTS+ methods. Evaluation using the ABTS+ method was performed by mixing a 7 mM ABTS solution and 2.4 mM K-at the same ratio, reacting it in a dark room for 24 hours and measuring the change in absorbance using the formula below. The ABTS+ solution was diluted with distilled water so that the optical density (OD) of the absorbance at 734 nm did not exceed 1. Evaluation using the DPPH method was performed by placing each of the contact lens samples in 10 mL vials, adding 3 mL of 0.2 mM DPPH solution to each vial, and incubating at 37 ℃ after blocking light to calculate the radical scavenging rate.[25]

Radical scavenging ability =

$$\frac{\text{Absorbance of control - Absorbance of antioxidant samples}}{\text{Absorbance of control}} \quad (1)$$

2.5. Evaluating the physical properties of the contact lenses

The physical properties of the contact lenses were evaluated by measuring and averaging the light transmittance, water content, refractive index, oxygen permeability, and contact angle five times each.

The light transmittance was measured in the visible light spectrum (380 nm~780 nm) using the Cary 60 UV-vis spectrophotometer (Agilent). The water content was measured using the gravimetric method specified in ISO 18369-4:2006, Ophthalmic opticsContact lenses-Part 4: Physicochemical properties of contact lens materials and the refractive index was measured with an ABBE Refractometer (ATAGO DR-A1) in accordance with ISO 18369-4: 2006.

Oxygen permeability was measured using the polarographic method specified in ISO 18369-4:2006 with a 201T Permeometer (Rehder). The radius of curvature of the polarographic cell was 8.7 mm. The current was measured while maintaining a humidity of 98% and a temperature of 35℃ ± 0.5℃. The contact angle was measured using the sessile drop method.

2.6. Antimicrobial activity test

E-Coli (ATCC 10536) was used to investigate the antibacterial properties of the antioxidant contact lenses containing quercetin and the contact lenses IPN-treated with alginate and chitosan. The strain was purchased from the Korea Culture Center of Microorganisms. 1 μL of *E-Coli* was added to 20 mL of liquid medium and incubated at 37 ℃ for 12 hours before use. Each sample was placed into a vial containing 5 mL of liquid medium, and 1 μL of the cultured *E-Coli* was added and incubated at 37℃ for 3 hours, then diluted 10000 times with saline. 1 mL of the diluted solution was smeared on a dry film medium and incubated at 37℃ for 24 hours.[26] *E-coli* 3M Petrifilm™ was used as the film medium. The average CFU (Colony forming unit) was obtained by testing each sample three times.

2.7. Protein adsorption

For the protein, this study used Bovine Saline Albumin (BSA), which is similar in shape and physicochemical properties to human albumin. The molecular weight of the BSA from Sigma-Aldrich was 66.4 kDa.

Each sample was added to a solution in which 5 mg/mL BSA was dissolved in PBS, then incubated at 37 ℃ for 24 hours to absorb the protein. After removing the protein adsorbed samples and washing them with PBS, they were added to a 3% Sodium Dodecyl Sulfate (SDS) solution and heated at 95 ℃ for 15 minutes. The proteins attached to the samples were desorbed by shaking them slowly with an agitator for 3 minutes to measure the absorbance. Absorbance at 280 nm, which is the maximum absorption wavelength of the protein, was measured using a Cary 60 UV-vis spectrophotometer (Agilent). The molar extinction coefficient was calculated using $\varepsilon_{280\,nm} = 6.7\ cm^{-1}M^{-1}$ for 1 mg/mL, and the protein adsorption amount was calculated as three average values for each sample using: [27]

$$Q = \frac{VC}{m} \quad (2)$$

where,
Q is the protein adsorption amount
V is the volume of the solution
C is the protein concentration in the solution
m is the mass of the hydrated test specimens

3. Results and discussion

3.1. Antioxidant properties of the contact lenses containing quercetin

Contact lenses containing quercetin, which has high antioxidant action and radical scavenging ability, were fabricated. Figure 2 shows the absorbance of each sample measured to evaluate their antioxidant functions.

As shown in Figure 2(a), in terms of the absorbance measured by the DPPH method, R without quercetin showed little difference from the DPPH solution (0.2 mM) at 517 nm. On the other hand, L containing 0.15% quercetin decreased by about 50% compared to the DPPH solution, and H containing 0.3% quercetin exhibited an absorbance of about 0.3, much lower than that of R.

As shown in the results measured using the ABTS method in Figure 2(b), R showed an absorbance of 0.5 at 734 nm, about 40% lower than the ABTS solution (0.77), and lenses L and H containing quercetin showed almost no absorbance.

All contact lenses containing quercetin had antioxidant properties, and the higher the content of quercetin, the higher the antioxidant properties.

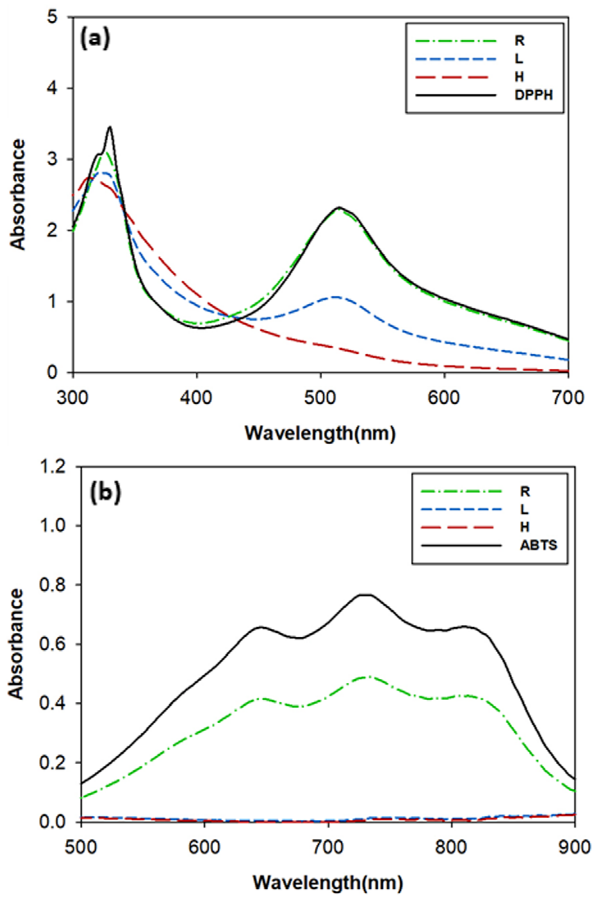

Figure 2. Absorbance of contact lens with quercetin through DPPH(a) and ABTS method (b).

The radical scavenging rate of each sample was obtained to investigate the free radical scavenging activity of the antioxidant contact lenses. Figure 3 shows the results. Since the absorbance at 517 nm cannot be 0 because of the high peak in the 300 nm-350 nm region in Figure 2, a control group was set at 100% to calculate the radical scavenging rate. For the control group, 9 μL of 1% quercetin stock solution was added to 3 mL of DPPH or ABTS solution, and the absorbance after 2 hours was measured using the radical scavenging rate equation. The calculated value was set to 100% to obtain the radical scavenging rates of the remaining samples. The content of the quercetin stock solution was the same as that of H containing 0.3% quercetin.

R (without quercetin) showed a very low radical scavenging rate (1.6%) in the DPPH assay, but H (containing 0.3% quercetin) showed a high radical scavenging rate of 91.7%. L (containing 0.15% quercetin) showed a moderate radical scavenging rate (52.3%) between that of R and H.

In the ABTS assay, R (without quercetin) showed a radical scavenging rate of 49%. On the other hand, L and H both showed 100%.

3.2. Antioxidant properties of the IPN-polymerized contact lenses with natural polysaccharides

IPN polymerization was performed using natural polysaccharides to maintain the antioxidant function of contact lenses for a longer time and improve their physical properties. The antioxidant function of contact lenses containing quercetin dual-networked with chitosan and alginate was evaluated to examine changes in antioxidant function depending on IPN polymerization. In addition, the duration of antioxidant activities of L and H without IPN and IPN-treated ALL, ALH, CHL, and CHH were compared.

Figure 4 shows the radical scavenging rates of the IPN-treated samples with alginate and the non-IPN-treated samples. After 24 hours reaction time between the samples and the DPPH solution, the radical scavenging rates of H (with high quercetin content) and L (with low quercetin content) were 90.2% and 61.0%, respectively. On the other hand, the radical scavenging rate of R (without quercetin) was 18.9%, which was significantly lower compared to the L and H. Among the samples IPN-treated with alginate, ALH had the highest radical scavenging rate.

In terms of the duration of antioxidant activity, L and H showed an equilibrium state at about 5 hours and 2 hours, indicating that quercetin was released only for 5 hours and 2 hours, respectively. On the other hand, the radical scavenging rates of ALL and ALH networked with 1% alginate improved for more than 8 hours.

ALL and ALH containing quercetin had slightly lower antioxidant properties than non-networked L and H. However, the

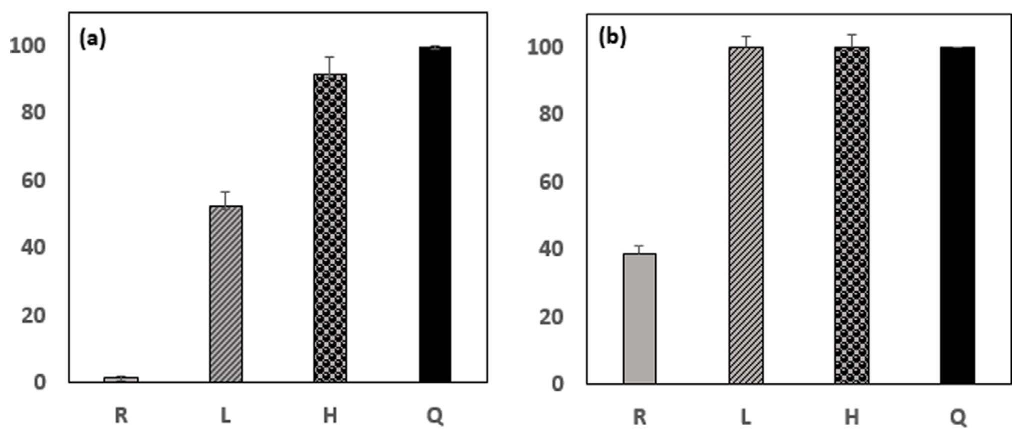

Figure 3. Relative radical scavenging capacities of lenses with quercetin through DPPH (a) and ABTS method (b).

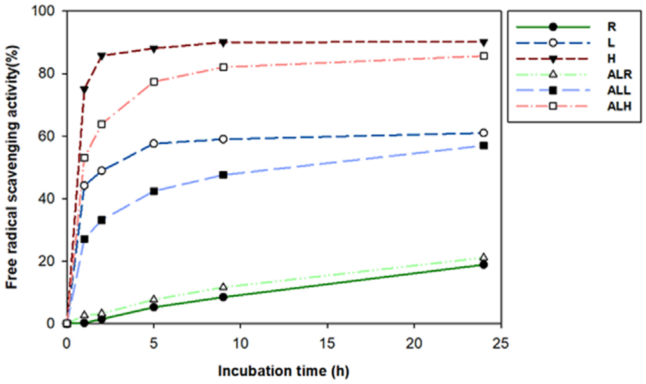

Figure 4. Relative radical scavenging capacities of interpenetrating alginate network contact lenses.

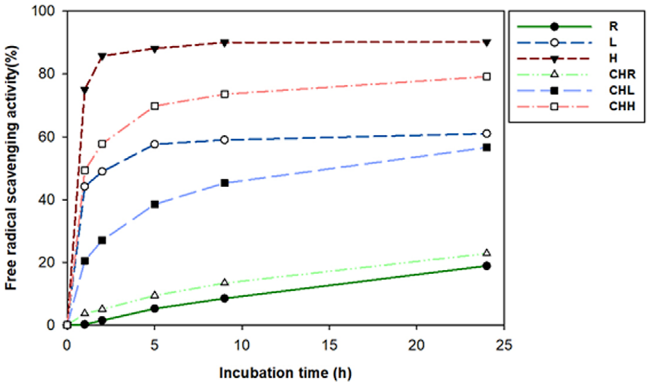

Figure 5. Relative radical scavenging capacities of interpenetrating chitosan network contact lenses.

duration of antioxidant activity improved after being networked with alginate, as the release of quercetin continued slowly.

Figure 5 shows the radical scavenging rates of the contact lenses containing quercetin IPN-treated with chitosan.

The lenses containing quercetin had much higher radical scavenging rates compared to those without quercetin. The higher the concentration of quercetin, the higher the radical scavenging rate. The radical scavenging rates of R and CHR without quercetin were similarly low. CHL and CHH networked with chitosan showed lower radical scavenging rates than L and H without chitosan. Although the antioxidant activities of L and H lasted only 5 hours and 2 hours, respectively, CHL and CHH networked with chitosan showed radical scavenging activities for more than 8 hours.

The effects of alginate and chitosan on antioxidant function and quercetin release were compared.

First, in terms of the effects of alginate and chitosan on antioxidant function, the radical scavenging rates of ALR and CHR networked with alginate and chitosan were almost the same as R without quercetin, indicating no significant difference in antioxidant function. However, among the contact lenses containing quercetin, the lenses IPN-treated with alginate had a 2%-7% higher radical scavenging rate than those IPN-treated with chitosan.

Both alginate and chitosan are natural antioxidants with antioxidant physiological activity. Therefore, this study investigated how the use of alginate and chitosan in IPN polymerization affect antioxidant function and quercetin release.

ALL and ALH networked with alginate released quercetin faster than CHL and CHH networked with chitosan. In addition, the antioxidant activity of contact lenses IPN-polymerized with natural polysaccharides lasted longer than those that were not. Natural polysaccharides alginate and chitosan form networks with the polymer of contact lenses and slow down the release of quercetin. Therefore, IPN by natural polysaccharides helps to maintain the release of antioxidants in contact lenses for a longer time.

The contact lenses without quercetin had similar antioxidant functions to ALR and CHR, which were IPN-polymerized with alginate and chitosan, respectively. Through comparing the antioxidant functions of the contact lenses containing quercetin with ALL and ALH (IPN-polymerized using alginate) and CHL and CHH (IPN-polymerized using chitosan), ALL and ALH were found to show more improved antioxidant properties than those IPN-polymerized with chitosan. This phenomenon was confirmed by Chavda *et al.*,[28] which reported that as the concentration of chitosan in the hydrogel increases, the swelling slows down and the polymer chain's flexibility is significantly limited through entanglement with the cross-linked chitosan network.

3.3. Evaluating the physical properties of the antioxidant contact lenses

Physical properties such as light transmittance, water content, oxygen permeability, and wettability of the fabricated antioxidant contact lenses were evaluated.

3.3.1. Light transmittance

Figure 6 shows the light transmittance of the contact lenses containing quercetin networked with seaweed polysaccharides.

Lenses R, ALR, and CHR without quercetin showed a high light transmittance of above 99%. However, as the content of quercetin increased, the light transmittance gradually decreased. CHH (IPN-treated with chitosan) showed no significant difference in light transmittance even when the quercetin content was high.

The IPN-polymerized contact lenses using alginate showed

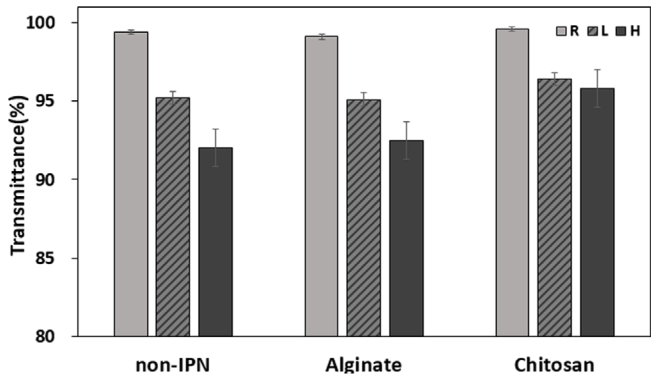

Figure 6. Optical transmittance of contact lenses containing quercetin networked with alginate and chitosan.

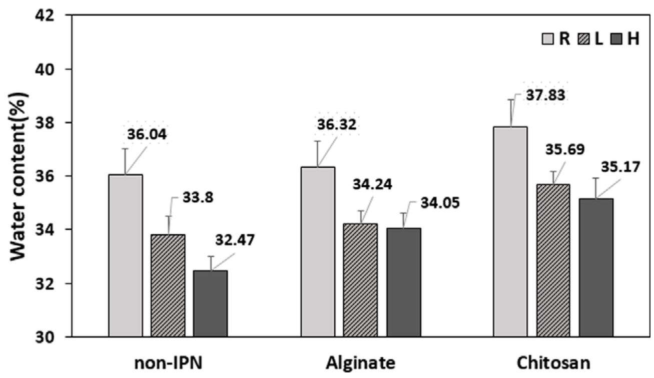

Figure 7. Water content of contact lenses containing quercetin networked with alginate and chitosan.

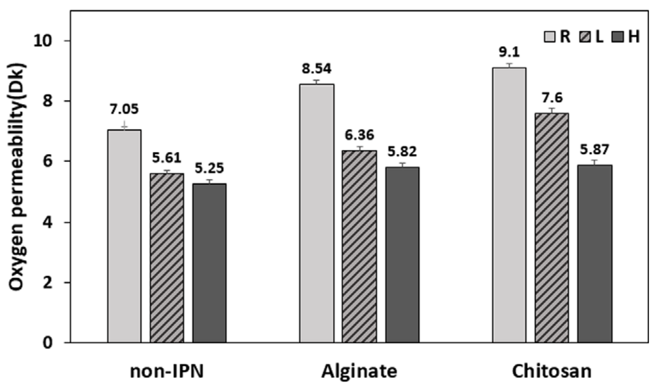

Figure 8. Oxygen permeability of contact lenses containing quercetin networked with alginate and chitosan.

no significant difference in light transmittance compared to the non-IPN-polymerized contact lenses. In terms of IPN-polymerized contact lenses with chitosan, R was similar to non-IPN-polymerized contact lenses, but L and H showed improved light transmittance.

Regardless of the quercetin content or IPN polymerization, the light transmittance of all contact lenses was more than 90%, indicating high transparency.

3.3.2. Water content

Figure 7 shows the water content of the contact lenses containing quercetin networked with alginate and chitosan.

The water content of the lenses networked with seaweed polysaccharides was generally high, and the ones IPN-treated with chitosan had a higher water content than those IPN-treated with alginate. Additionally, the higher the quercetin content, the lower the water content. Quercetin is a hydrophobic compound, so water content is affected both by whether the samples contain quercetin, and by the content of quercetin. In terms of comparing the water content according to whether IPN was performed and the IPN material, the water content of IPN-treated samples was high, and the water content of the lenses networked with chitosan was higher than those networked with alginate. Alginate does not show a significant difference in the water content because it has high gel and low water adsorption properties. On the other hand, chitosan has relatively low viscosity, so the water content was increased compared to the samples IPN-treated with alginate.

3.3.3. Oxygen permeability

Figure 8 shows the findings related to oxygen permeability, a significant factor in corneal metabolism.

In terms of oxygen permeability according to the concentration of quercetin, all contact lenses containing quercetin have reduced oxygen permeability compared to those without quercetin. The higher the content of quercetin, the lower the oxygen permeability. Quercetin is a hydrophobic active compound, so the higher the content, the more hydrophobic the manufactured contact lenses, resulting in reduced oxygen permeability.

In terms of the oxygen permeability of the IPN-polymerized contact lenses with alginate and chitosan, the oxygen permeability of all contact lenses was increased compared to the non-IPN-polymerized contacts. Alginate and chitosan have wettability and contain abundant hydroxyl groups (-OH), so performing IPN polymerization with such natural polysaccharides increases oxygen permeability.[29,30] In terms of the correlation between oxygen permeability and water content, oxygen permeability increases as the water content increases, and decreases with lower water content.[31,32] This is because oxygen (O_2) increases as the water content contained in the contact lens increases. In terms of the measured water content and oxygen permeability, IPN polymerization with chitosan rather than alginate increased the water content slightly more than the non-IPN-polymerized contact lenses. Therefore, performing IPN polymerization with chitosan rather than alginate resulted in better oxygen permeability.

3.3.4. Wettability

Figure 9 shows the contact angles to evaluate the wettability of the contact lenses. The contact angle of the lens without using IPN polymerization was over 80°.

The contact angles of the contact lenses in which IPN structures were formed with alginate and chitosan were lowered by about 10° compared to the non-IPN-polymerized contacts, showing improved wettability. These results are attributed to the high wettability of alginate and chitosan, penetrating the contact

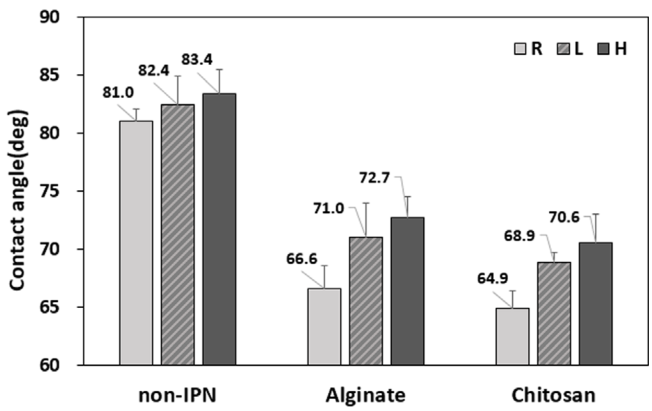

Figure 9. Contact angle of contact lenses containing quercetin networked with alginate and chitosan.

lenses and forming networks.

In terms of wettability according to the quercetin content, as the amount of quercetin increased, the contact angle increased and wettability decreased regardless of whether IPN was performed. Since quercetin is hydrophobic, wettability decreases as the content of quercetin increases.

The comparison of the contact angles of contact lenses IPN-treated with alginate and chitosan showed no significant difference, but using chitosan produced slightly lower angles.

Contact lenses with high water content levels may feel more comfortable, but they often worsen dry eye symptoms. Therefore, contact lens wearers who experience dry eyes due to exposure to dry environments should wear contact lenses with high wettability rather than high water content. The antioxidant contact lenses prepared in this study have low water content and high wettability, making them suitable for people with dry eyes.

3.4. Evaluating the biochemical properties of the antioxidant contact lenses

3.4.1. Protein adsorption

Figure 10 shows the protein adsorption of the fabricated contact lenses. The protein adsorption amount of R (without IPN polymerization and quercetin) was 0.541 mg/g. In the case of L and H containing quercetin, the amounts were 0.725 mg/g and 0.809 mg/g, respectively. The protein adsorption amount of the contact lenses containing quercetin was increased compared to those without hydrophobic quercetin. In addition, the protein adsorption amount increased more in lenses with high quercetin content than in those with low quercetin content.

For the contact lenses using IPN polymerization, all contact lenses containing quercetin showed increased protein adsorption compared to ALR and CHR. The protein adsorption amount of the contact lenses networked with seaweed polysaccharides decreased by about 0.3 mg/g-0.4 mg/g compared to those without seaweed polysaccharides, reducing protein adsorption significantly.

Wettability is strongly correlated with protein adsorption. As the wettability of the contact lens surface increases, the amount of protein adsorbed decreasesAlginate is a natural polysaccharide with high wettability, so it is widely used in medical supplies that require wettability, such as wound dressings.[33] Performing IPN polymerization with alginate reduces protein adsorption more than using chitosan, so IPN polymerization with alginate further improves protein adsorption. Another reason is that alginate is an anionic polymer due to the presence of carboxyl groups (-COOH) and chitosan is cationic, so protein adsorption decreases because of the negatively charged BSA and repulsion of charges.

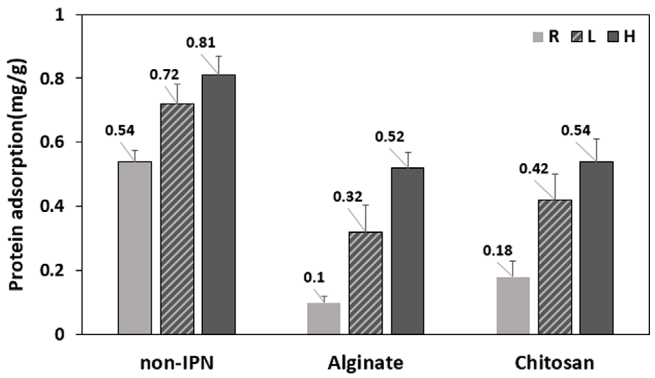

Figure 10. Protein adsorption of contact lenses containing quercetin networked with alginate and chitosan.

3.4.2. Antibacterial properties

Figure 11 shows the antibacterial properties measured according to quercetin concentration and IPN polymerization using natural polysaccharides. R (without quercetin and IPN polymerization) had no antibacterial properties due to the growth of Too Numerous to Count (TNTC) bacteria. On the other hand, as shown in the figure, the incidence of *E. Coli* reduced significantly in L and H containing quercetin compared to R without quercetin. In terms of the number of bacteria, L (low quercetin content) was 56.33 CFU/ml and H was 16 CFU/mL, so the higher the quercetin content, the higher the antibacterial properties. The contact lenses IPN-treated with chitosan had significantly fewer bacteria than those IPN-treated with alginate.

The contact lenses that formed IPN structures with alginate and chitosan had stronger antibacterial properties than those without IPN polymerization. In the contact lenses IPN-polymerized with alginate, the number of bacteria in ALR, ALL, and ALH were 22.67 CFU/mL, 6.33 CFU/mL, and 1.33 CFU/mL, respectively. All contact lenses that formed IPN structures with chitosan did not grow bacteria.

As the content of quercetin increased, the number of bacteria decreased, and chitosan had higher antibacterial activity

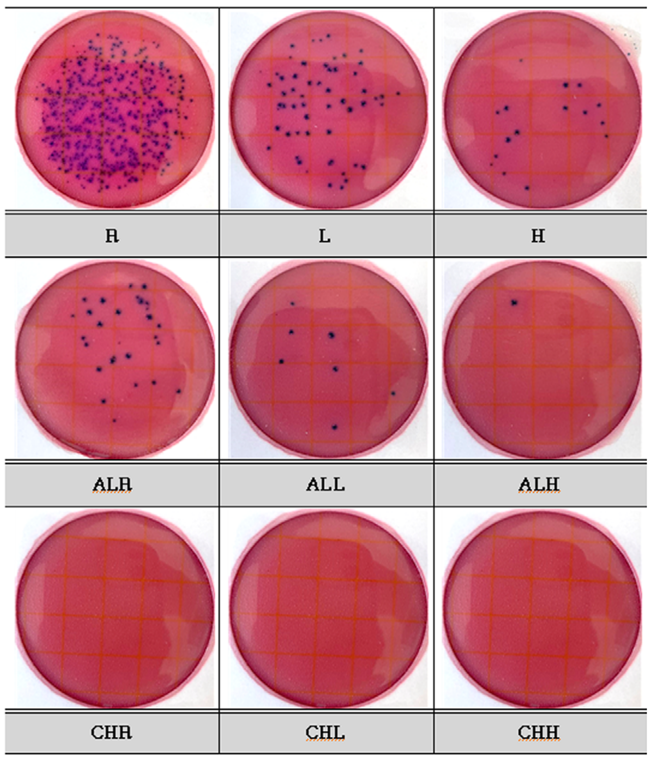

Figure 11. *E-coli* 3M Petrifilm™ photograph of contact lenses containing quercetin networked with alginate and chitosan.

than alginate. Alginate and chitosan are natural antimicrobial polymers, so their antibacterial activities increased further. Alginate has been proven to have antibacterial effects against *S.aureus* and *E. Coli* due to the antibacterial properties generated by anionic carboxyl groups (-COO).[34,35] In particular, chitosan is well known for its excellent antibacterial properties.[36,37] The D-glucosamine of chitosan exhibits immune activity by increasing the activity of Natural Killer cells involved in immune defense, and positively charged chitosan oligosaccharide molecules only destroy bacterial cell membranes without destroying neutral normal cell membranes due to their electrostatic attraction to negatively charged bacterial membranes.[38] Therefore, the contact lenses containing chitosan did not grow bacteria regardless of whether they contained quercetin, and had excellent antibacterial properties.

4. Conclusion

Contact lenses containing quercetin were prepared and double networked with alginate and chitosan, and then the antioxidant properties, physical properties, and biochemical properties of the contact lenses were analyzed. Contact lenses containing quercetin had antioxidant properties, and the higher the content of quercetin, the greater the antioxidant effect.

The dissolution rate of quercetin was reduced in the double networked contact lenses compared to the non-networked contact lenses, and the dissolution rate was continued slowly and continuously.

Although the physical properties of contact lenses containing quercetin were somewhat reduced, the moisture content, oxygen permeability, wettability, and protein adsorption properties of the contact lenses were greatly improved by going through the IPN process with polysaccharides. In addition, it was confirmed that the antioxidant duration could be increased by networking contact lenses containing quercetin with natural polysaccharides.

Conflict of interest: The authors declare that there is no conflict of interest.

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