# Synthesis and Characterization of Collagen-Chitosan-Sodium Hyaluronates as Artificial Cornea

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**Abstract.** As reported by Riset Kesehatan Dasar, Indonesia facing a serious problem of blindness with 966.326 cases of 2 million population in 2013. Gold standard such as keratoplasty couldn't completely heal corneal injury due to the recipient's rejection and limited donor. In order to overcome those concerns, artificial corneal tissue needs to be developed to produce an artificial cornea which is biocompatible, supports corneal function, and applicable. This research was conducted to provide an initial description of Collagen – Chitosan - Natrium Hyaluronate (Col-Chi-NaHA) as an artificial cornea through FTIR analysis, hydrophilicity and water absorption measurement. The artificial cornea was synthesized by forming ratio of collagen 20% w/v and chitosan 10% w/v, both solutions were dissolved in 0,1 M and 0,6 M acetic acid separately. Into the mixed solution, NaHA was dripped slowly with variation of 0%, 0,3%, and 0,6%. The homogenous solution was cast and heated for 24 hours at 35°C. The dry membranes were neutralized to obtain a pH around 7. From the analysis known that all of the characteristic bands of Col-Chi-NaHA could be observed in obtained membranes. Water absorption measurement demonstrated percentages over 90% on all membranes. The contact angle of obtained membranes was decreased by the addition of NaHA. The results showed that artificial cornea made from Col-Chi-NaHA successfully fabricated has hydrophilic properties to support cellular interaction and provide hydration to keep eye's clarity and permeability. Therefore, Col-Chi-NaHA could may be a suitable material for the tissue-engineered cornea.

## 1. Introduction

Corneal blindness still becomes a major problem with high prevalence in Indonesia. According to Riset Kesehatan Dasar by Indonesian Ministry of Health, there are about 966.329 cases of corneal blindness in 2013. This type of blindness is considered as the second most common blindness after cataract [1]. The cornea is a translucent protective layer for the eye. Cornea injury happened because of trachoma and lepra infection, insufficient nutrition to the cornea and trauma [2]. Gold standard such as keratoplasty couldn't completely heal corneal injury due to limited donor. On the other hand, the rejection rate of implant cornea is about 13,3% to 65% four months after keratoplasthy. This could lead to unwanted reactions from the patient because of the non-biocompatibility [3]. Indonesia Eye Bank in 2017 stated that there are only 5-10 percent of blindness patients performed corneal transplantation. To overcome those problems, tissue-engineered cornea which is biocompatible, supports corneal function, applicable and biodegradable needs to be developed.

Chen [3] succeeded in creating tissue-engineered cornea with collagen type 1 which is the main component of cornea. He discovered that collagen 20% + chitosan 10% + 0,5% sodium hyaluronate is better in the terms of biocompatibility than the one without collagen. It was found that collagen 1% + chitosan 2% + 0,5% sodium hyaluronate could transmit 95% of light. This result is in line with the ability of the human cornea to transmit UV light in about 30% to 75% (with wavelength 310 – 400 nm) and visible light in about 75% to 95% (with wavelength 400 – 700 nm). However, it is discovered that during in vivo study, the same concentration of sodium hyaluronate performed different transparency. Therefore, we need to perform other research to see the impact of sodium hyaluronate to corneal transparency.

To overcome those problems, this research is conducted to produce artificial cornea based on collagen with addition chitosan and sodium hyaluronate. Chitosan has a good bio adhesion, permeability, toxicity, and ocular tolerance properties. Addition of chitosan to collagen can improve the stability of collagen and maintain the integrity of the structure. Liang et al in 2011 stated that chitosan can accelerate corneal endothelial cells regeneration in rabbits. Chitosan has many amines and hydroxyl functional groups so it could bond with collagen. On the other hand, sodium hyaluronate can improve light transmittance [3].

## 2. Material and Method

### *2.1. Materials*

Materials used in this research are collagen (Col), chitosan (Chi), sodium hyaluronate (NaHA), chloric acid (HCl), acetic acid, hydroxypropyl methylcellulose (HPMC), phosphate buffer saline (PBS) and distilled water.

### *2.2. Sample Preparation*

The artificial cornea was made by dissolving 20% of collagen in 0,1 M acetic acid and 10% chitosan in 0,6 M acetic acid. Collagen solution that has cross-linked with HPMC then added and mixed with chitosan solution for 60 minutes. Then, NaHA with variance concentration of 0%, 0,3%, and 0,6% was added to the col-chi solution and stirred for 30 minutes. Concentration ratio of collagen, chitosan and sodium hyaluronate can be seen in Table 3.1. The homogenous col-chi-NaHA solution was casted on Perspex plate and heated with an incubator for about 24 hours and temperature 35℃ until the membrane become dry. Then, the dry membrane was dipped into PBS until the pH becomes normal.

### *2.3. Characterization Procedures*

*2.3.1. FTIR Test.* Dry membranes were cut in 1 cm x 1 cm and evaluated using Perkin Elmer Spectrum One Fourier Transform Infrared (FT-IR) Spectrometer over the range 4000 $cm^{-1}$ – 650 $cm^{-1}$.

*2.3.2. Hydrophilicity Test.* One droplet of water was dropped on dry membranes, then images were taken by a digital camera perpendicular to the sample. Contact angle from the images was evaluated using ImageJ software to create a graph of the contact angle vs time. From the results, we can obtain surface hydrophilicity value inversely proportional to contact angle.

*2.3.3. Water Absorption Test.* Dry membranes were cut in 1 cm x 1 cm, then immersed in distilled water for 24 hours. Water content rate ($R_1$) was assayed by weighing dry membranes in hydrated form film ($G_1$) and dry form (G) [3]

$$R_1 = \frac{G_1 - G}{G_1} \, x \, 100\% \quad (1)$$

## 3. Result and Discussion

### *3.1. FTIR Test*

Col-Chi-NaHA solution went through FTIR test to see the functional groups and any chemical interaction. FTIR results explained in Figure 1. NaHA spectral showed N-H and C=O groups at wavenumber 1650-1500 $cm^{-1}$ and 1700-1725 $cm^{-1}$ respectively. Collagen itself has shown similar functional groups with NaHA indicated by overlapping between spectral. The widening of the absorption band showed that NaHA functional groups are present.

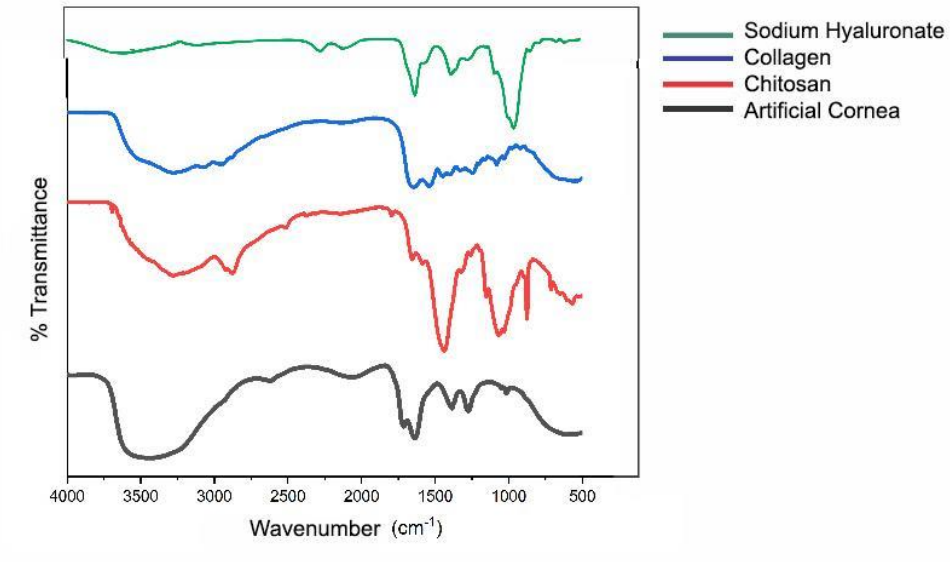


**Figure 1.** FTIR Spectra

### *3.2. Hydrophilicity Test*

Hydrophilicity test was done by measuring the contact angle of the artificial cornea. Forch (2009) stated that hydrophilic material has a degree of contact angle below 90°, meanwhile contact angle >90° considered as hydrophobic material. Figure 2 showed the degree of contact angle of the artificial cornea is below 90°. Therefore artificial cornea is hydrophilic and supports cellular interaction [4].

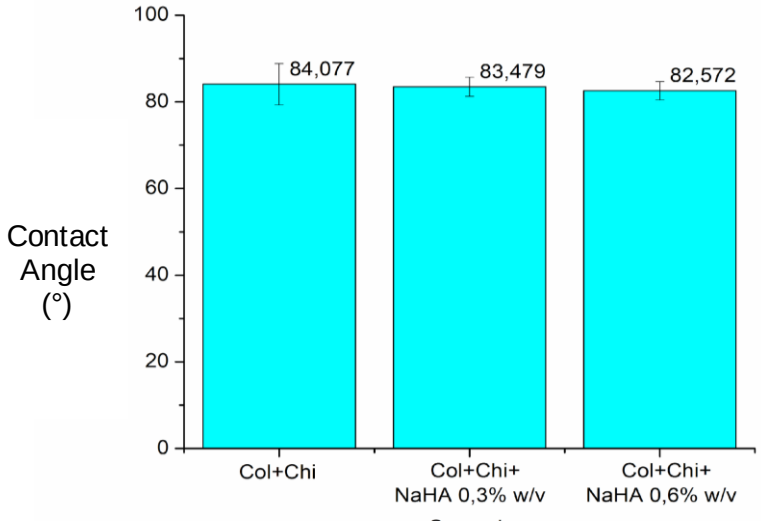


**Figure 2.** Degree of Contact Angle

The lower the degree of contact angle, the higher the hydrophilicity [4]. In this study it was found that the addition of NaHA was able to reduce the value of the degree of contact angle, in other words, the addition of NaHA was able to increase the hydrophilicity of the artificial cornea.

In previous studies, it was also known that the degree of contact angle of the collagen membrane was 90° [5]. In this study, all three samples of the artificial cornea had lower contact angles than collagen-based sample. It means that the hydrophilicity of artificial cornea is better than samples which used only collagen material.

### *3.3. Water Absorption Test*

Water absorption test was done to determine the ability of artificial cornea provides hydration to the eye to maintain the clarity of the eye. Figure 3 showed that artificial cornea has percentages of water absorption higher than 77%. This result corresponds to the characteristic of the human cornea where the epithelium and endothelium must absorb water at least 77% per weight of the cornea [6].

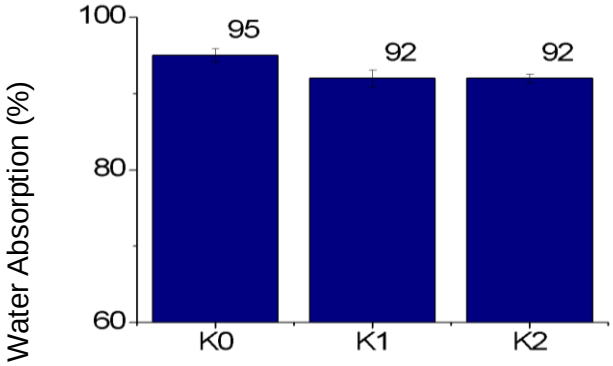


**Figure 3.** Percentages of Water Absorption

## 4. Conclusion

Artificial cornea based on Collagen – Chitosan – NaHA showed no changes in chemical interaction which indicates that those three materials were successfully fabricated. Artificial cornea had hydrophilic properties because the degree of contact angle was below 90°. The artificial cornea also has percentages of water absorption at 90%. Those properties support cellular interaction and provide hydration to keep eye's clarity and permeability. Therefore, Col-Chi-NaHA could be a suitable material for the tissue-engineered cornea.

## Acknowledgments

The authors would like to thank Indonesia's Ministry of Research for Higher Education and Technology for partially funding the research through Students Creativity Program 2019 and Universitas Airlangga in Surabaya for the knowledge and opportunities given throughout the writer's academic experience in the institution.

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