Inovation Of Collagen Based Corneal Hydrogel with the Addition Of Glycopolymer As the Solution For Irreversible Blindness By Corneal Ulcers

Disca Sandyakala Purnama[1*], Hendita Nur Maulida[1], Rara Setya Angtika[1], Astriani Hendayanti[1], Anandhika Muhammad Satria[1]

[1]Program Studi S1 Teknobiomedik, Fakultas Sains dan Teknologi, Universitas Airlangga, Surabaya
*Corresponding author: discasandyakalapurnama@gmail.com

ABSTRACT

Indonesian Health Ministry Data showed that 5.3% of 100,000 population in Indonesia suffer from corneal ulcers. Corneal ulcer is one of the five leading causes of largest blindness morbidity and loss of vision. Corneal transplant being the only medicine accepted widely despite there are many post-surgery complications. The study was conducted to review the initials make a synthetic cornea from natural ingredients collagen type I composite with a glikopolimer from poly (1,8-octanediol-co-citrate) (POC) and poly (vynyl-alcohol) (PVA) hydrogels form to review increase the strength of mechanical and biodegradibilty as candidate synthetic cornea. The result of Fourier Transform Infra Red (FTIR) tin that strong cross Occurs on Force C = O stretching Which is a cluster formation of ester IN 1735 cm^{-1}. The result of tensile test showed that the value of elasticity modulus tends to decrease with increasing concentration of collagen with modulus of elasticity in a row for a review variation collagen 1%, 3%, and 5% is 13.26 MPa, 13.21 MPa, and 11.5 MPa. Moisture balance test results Phosphate Buffer Saline (PBS) for 7 days for a review sample of collagen Variation 1%, 3%, and 5% is 89%, 91% and 92%. Spectroscopic test results showed that all the various samples can continue light until appoximately 98% accordance to standart of cornea. The synthetic cornea from collagen type 1 and glycopolymer from poly(1,8-octanediol-co-citrate) (POC) and poly(vynyl alcohol) (PVA) have a potential to be solution for irreversible blindness caused by corneal ulcers.

Keywords: corneal ulcers, collagen type 1, glycopolimer, corneal hydrogel

INTRODUCTION

WHO data in 2014 shows that there are 45 million blindness sufferers in the world, of which one third of the sufferers taste in Southeast Asia, including Indonesia. WHO 2004 data indicate that corneal ulcer (corneal injury) is a major public health problem because it can cause prolonged morbidity, loss of vision (both 1 eye and 2 eyes). According to 1993 data, it was shown that the incidence of corneal ulcers occurred 5.3% per 100,000 Indonesian population, the cause of which was trauma, contact lens wear, and sometimes the unknown cause (Fandri, 2013). Corneal ulcers are also the number 2 cause of blindness in Indonesia (Wijaya in Fandri, 2013).

The formation of ulcers in the cornea is mostly found by the presence of collagenase formed by new epithelial cells and inflammatory cells. Two forms of ulcers in the cornea are known, namely central and marginal or peripheral. The causes of corneal ulcers are bacteria, fungi, and herpes simplex. Bacteria

that often cause ulcers include alpha hemolytic Streptococcus, beta hemolytic Streptococcus. Staphylococcus aureus, Moraxella likuefasiens, Pseudomonas aeruginosa, Nocardia asteroides, Alcaligenes sp, and several other bacteria (Ilyas, 2006). Objective symptoms of ulcers include ciliary injection, partial loss of corneal tissue, and the presence of infiltrates. In more severe cases of iritis accompanied by hypopyon to permanent blindness (Ilyas et al, 2002).

To date, transplantation using donor tissue has been the only widely accepted treatment for irreversible corneal blindness. However, donor transplant treatment has many shortcomings in postoperative complications such as host response (autoimmune), donor limitations, mismatches and length of recovery time (C. Deng et al, 2010). Therefore, synthetic corneas instead of donor tissue is an alternative solution that is widely used. Recently, the results of phase 1 clinical trials in humans have shown that corneal replacement material from crosslinked recombinant human collagen has successfully helped tissue regeneration including corneal and nerve cells (C. Deng et al, 2010).

However, for clinical conditions where endothelium failure or excess collagenase is present, the mechanical strength of collagen needs to be increased by combining collagen with glycolopolymer material into hydrogel form. In this research, poly (vinyl alcohol) glycopolymers (PVA) are used as hydrogel forming agents and also have properties similar to body tissues, have good bicompability properties and also optical properties that can be adjusted as desired (K. Liu et al., 2008). Then added poly (1,8-octanediol) (POC) which has good mechanical properties, degradation and surface energy, where these properties are very important in controlling the biological response to the material to be implied (Richard et al, 2009). So by making composites from collagen added with glycopolymers (PVA and POC) can make cornea hydrogel which has good mechanical properties and can also support the regeneration of corneal and nerve tissue (C. Deng et al, 2010).

Based on that background, it is proposed to manufacture artificial corneal hydrogels from collagen and glycopolymers which are biodegradable and permanently able to restore vision. The cornea has a very high affinity for water. Corneal tissue placed in water or physiological fluids causes the tissue to swell or turgid, along with it, the stroma becomes less transparent (Hogan et al, 1971; 58). Hogan, et al. Added that a mechanical pressure of 60 mmHg is required for the corneal stroma that is submerged in physiological salt water to maintain ideal corneal transparency and hydration. The addition of glycopolymers to the anterior part is hydrophilic so it will keep the cornea moist with tear fluid. This cornea will be attached to the eye cells that will be supported by stitches at the edges.

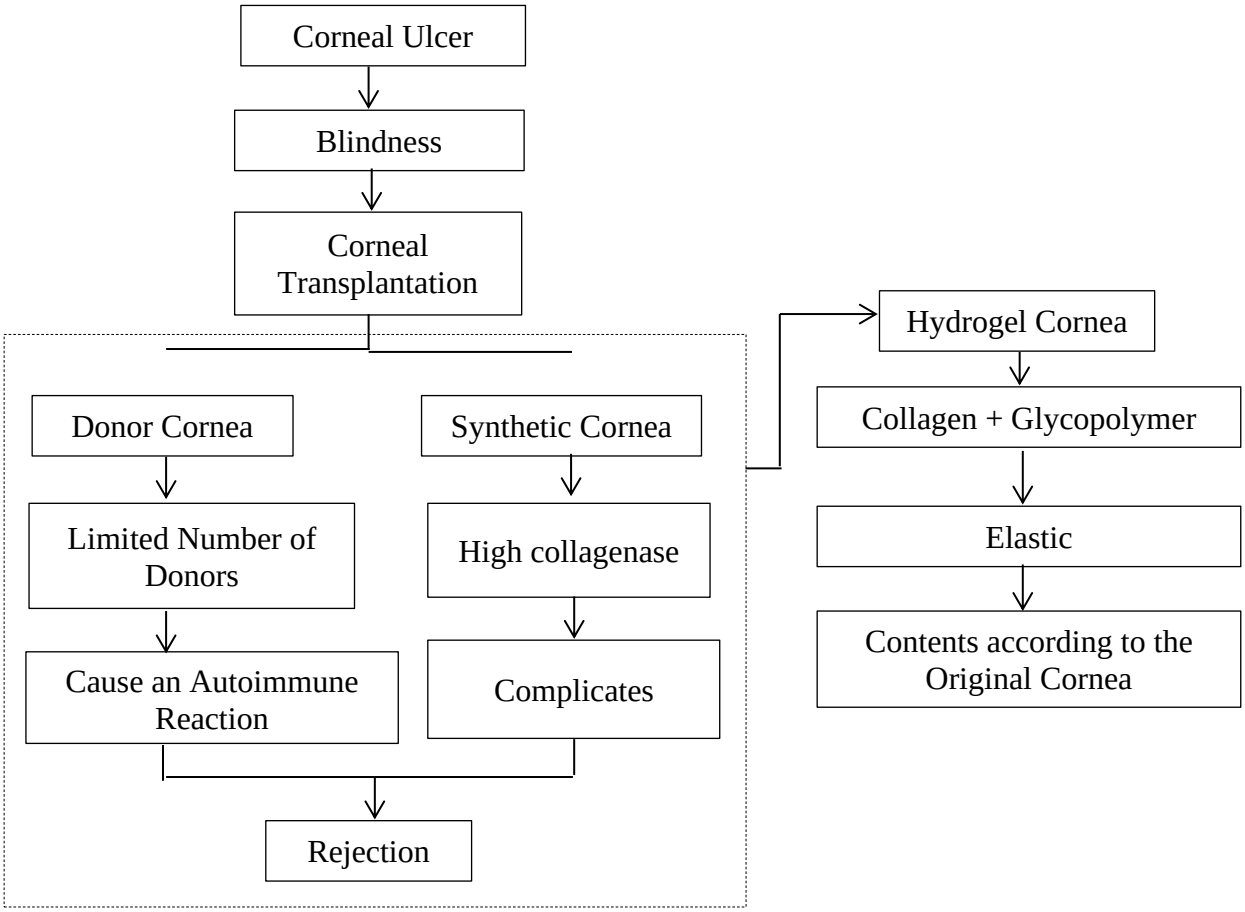


Figure 1. Schematic Conceptual Research

MATERIALS AND METHODS

Materials

The tools used are Mettler Toledo digital scales, Yellow MAG HS 7 magnetic stirrer, freezer, freeze dryer, Perkin Elmer Fourier Transform Infra Red (FTIR), IMADA autograph, UV-Vis spectrophotometer. While the material used is collagen type I from red snapper produced by BATAN, poly (vinyl alcohol) from Sigma Aldrich, poly (1,8-octadienol) from Sigma Aldrich and citric acid and Phosphate Body Saline (PBS).

Method

Glycopolymer Synthesis

Poly(vinyl alcohol) 40% w / v dissolved in distilled water. Poly (1,8-octadienol) -co-citric-acid made poly (1,8-octadienol) and citric acid were stirred at 150 ° C for 20 minutes and then changed at 140 ° C for 1 hour. Then add 98% toluene the amount of weight produced from the previous solution and then stir until the solution becomes homogeneous. Then mix the solution of Poly (vinyl alcohol) and Poly (1,8-octadienol)-co-citric-acid and stir until homogeneous.

Synthesis of Collagen-Glycopolymer Hydrogel

Collagen of 2 grams is dissolved in 2% citric acid solution. Then some variations of collagen are made namely 1% v/v collagen, 3% v/v collagen, and 5% v/v collagen, then mix each collagen solution with the glycopolymer solution that has been made before and stir until homogeneous. Then print and dry using the freeze drier.

Fourier Transform Infra Red (FTIR) Analysis

FTIR is used to determine the functional groups of the material used and new functional groups obtained from the synthesis carried out in the sample. A sufficient sample is then added KBr powder which is then compacted with a hydraulic clamp and then placed in the specimen place and irradiated with infrared with wave numbers 4000-450 cm-1 (Smith, 2011).

Test the water content using (Phosphate Body Saline) PBS

The water content test is carried out to find out how much water content can be absorbed by the hydrogel, determined by calculating the weight of the hydrogel when after being put into PBS divided by the weight of the hydrogel before being put into PBS and expressed in %.

Pull Test Using Autograph

Tensile testing is used to determine the elasticity of the material when implanted and to determine the mechanical response when the material interacts with body tissue, the sample is formed into a dog bone shape with a length of 63.5 mm and a width of 10 mm and 5 mm in each variation of the sample according to the American Society for Testing Materials (ASTM D 1822 L). Samples were given a 50N loading with a speed of 10mm / minute. The results obtained are the maximum stress value (MPa) and the amount of strain and elongation (Purwanti, 2010). The formula used to describe the relationship of stress and strain is:

$$E = \sigma / \varepsilon \quad (1)$$

where : Voltage: $\sigma = F / A$, F = force, A = cross-sectional area
Strain: $\varepsilon = \Delta L / L$, ΔL; change in length, L: length initially

Spectrophotometry Test

The sphrophotometry test is a test to determine the absorbance of a substance, where this test can be called the UV-Vis Spectrophotometer test, which means that the spectrophotometer is used for measurements in the UV and visible light areas. Absorbance itself is the amount of light absorbed from the total light emitted. The results of this test are in the form of a graph showing the absorbance relationship with the wavelength which later with the formula A = -log(I / Io), the value of the light absorbed will be obtained (Arrohmah, 2007).

RESULTS AND DISCUSSION

The results of this study are the cornea hydrogel where the hydrogel is formed through the freeze dry method in which the sample shape in all variations is made with a thickness of 0.5 mm with a diameter of 11-12 mm in accordance with the original cornea (Riordan-Eva, 2010). Then the corneal hydrogel produced is tested to match the parameters needed to be applied as a cornea through FTIR testing, tensile testing, moisture content and spectroscopy.

This research has resulted in the synthesis of a polymer of Poly (1,8-octanediol-co-citrate) (POC) with a treatment temperature of 140-160ºC for 1 hour. The temperature treatment is intended to occur cross linkages between the basic ingredients of polymer production namely citric acid and 1.8 octanediol, because based on product data that the boiling point of citric acid is 153ºC and boiling point is 1.8 octanediol 57-61ºC. Citric acid is binding on other chemical chains. Based on Fourier Transform Infra Red (FTIR) data, it is found that crosslinking occurs in the C = O stretch group which is an ester group formation at 1731 cm-1. According to Yang, et al, 2004, the presence of an ester group identified from the FTIR results indicates that the synthesis was successful.

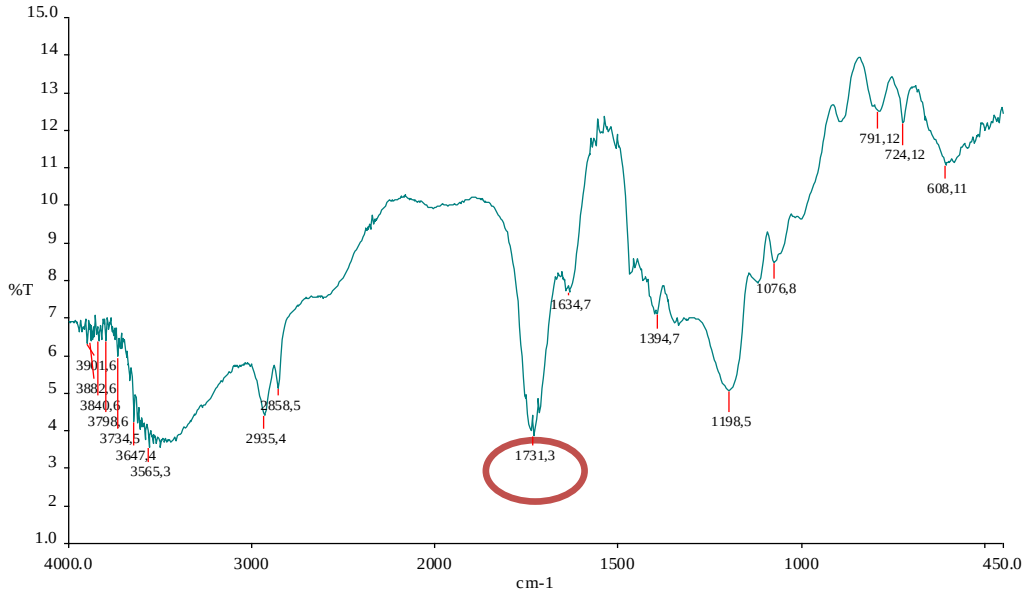
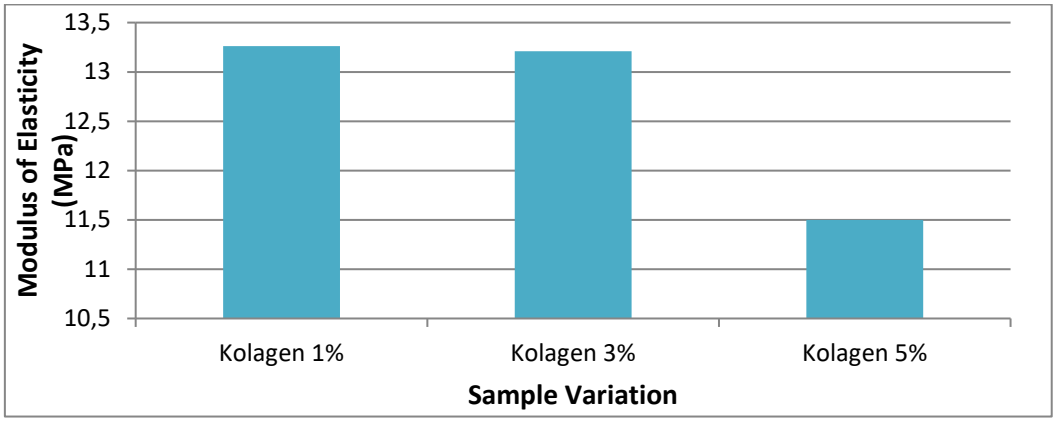


Figure 2. FTIR POC spectrum

Then the tensile strength test is performed to determine the elasticity of the material. The results of the tensile test of the material show that the modulus of elasticity tends to decrease with the increase in collagen concentration.

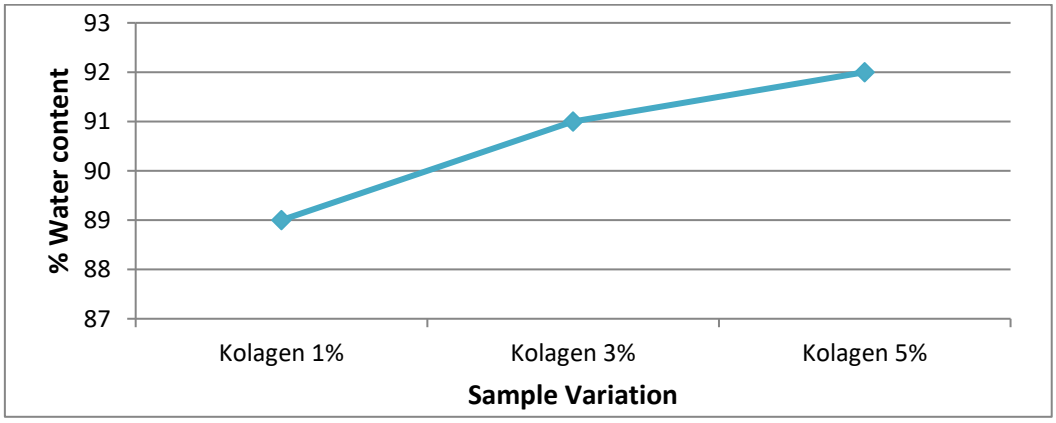


Graph 1. Composite Tensile Test Results

The highest point is produced by composites with a concentration of 1% collagen which is 13.26 MPa (Graph 2). The results of the tensile test data showed that the modulus of elasticity were successively varying at 1% collagen, 3% collagen and 5% collagen namely 13.26 MPa, 13.21 MPa, and 11.5 MPa. In general, the modulus of elasticity decreases with increasing collagen concentration because collagen provides brittle properties in the composite.

In accordance with the literature (Crabb, et al, 2006) that the modulus of elasticity for the human cornea is 3-13MPa, this indicates that all variations of the sample are still in the range in accordance with the functional standards of the human cornea.

Furthermore, to test the water content of the sample immersed in PBS solution for 7 days and then calculated the weight before and after immersion, from the water content test data found that the water content that can be absorbed by the cornea hydrogel with a variation of 1% collagen by 89%, collagen 3% by 91%, and 5% collagen by 92%.



Graph 2. Moisture Test Results

Injectability Test

IBS paste viscosity values were measured using viscotester show number of 120 dPa.s and applicable in terms of injectability test results as shown in Figure 2. From the test results, it can be concluded that the four samples of IBS has a very good ability in terms of the percentage results approaching 100%. Best percentage of injectability owned by the sample C with HA-gelatin ratio at 65:35 (w/w) is equal to 97,74% ± 0,19%.

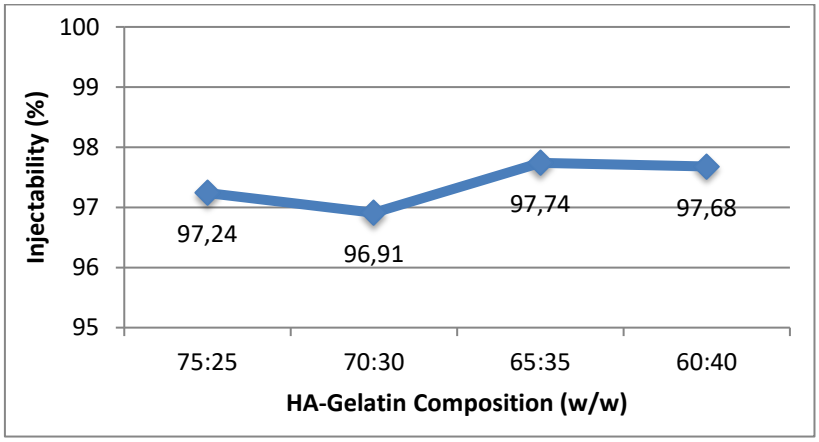
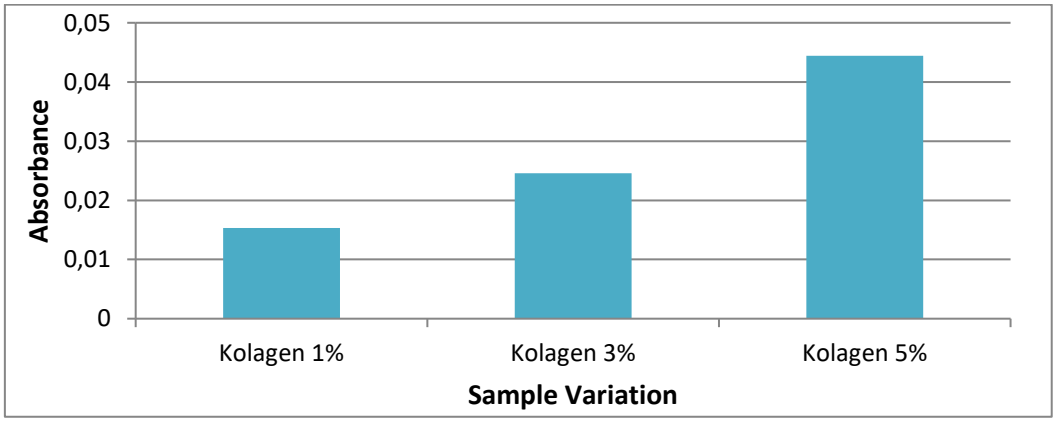


Figure 2. Injectability Testing Results of IBS Paste

From the result data it is found that the more collagen content makes the hydrogel's ability to absorb water even greater because collagen forms a crosslink that makes the hydrogel's strength in absorbing water higher. According to the literature (Righe, 1992) where in the human cornea has a water content of 81%. So that the sample variation is almost close to the standard, namely samples with 1% collagen which has a moisture content of 89%.

Spectroscopy test using UV-Vis spectroscopy method aims to determine the amount of light absorbed or transmitted by the sample, where when light waves are passed on the sample then some of the light will be absorbed and also transmitted by the sample. The magnitude of the ability of solute molecules to absorb light is called absorbance (A) (Hermanto, 2009).



Graph 3. UV-Vis Spectroscopy Test Results

The spectroscopic test results showed that the absorbance value at 1% collagen variation was 0.0153, collagen 3% was 0.0246 and collagen 5% was 0.0444. The results show that the smallest absorbance value obtained by 1% collagen variation indicates that the sample absorbs only a little light and transmits 98% of the light passing through it.

CONCLUSION

1. Synthesis of Collagen-Glycopolymer is obtained by making glycopolymer first by mixing a 40% w/v PVA solution with 1.46 ml of POC solution and then mixing with 2% w/v collagen and then printed with the freeze dried method.
2. FTIR test results show that the sample contains an ester group at wave number 1753 cm-1, this shows that the synthesis results were successful. The tensile test results found that the sample has a modulus of elasticity in the range of 11.5-13.26 MPa. The results of the moisture content test found that the sample has the ability to hold water content of 89-92%. Spectroscopic test results found that the sample can continue the light by 98%.

REFERENCES

Arrohmah. 2007. *Studi Karakteristik Klorofil Pada Daun Sebaia Material Photodetector Organic*. Surakarta : Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Sebelas Maret.

C. Deng, et al. 2010. *Collagen and Glycopolymer Based Hydrogel For Corneal Application*. Elsevier : Acta Biomaterialia.

Crabb, et al. 2006. *Biomechanical and Microstructural Characteristics of A Collagen Film-Based Corneal Stroma Equivalent.* Tissue Engineering;12(6);1565-75.

Fandri, MY. 2013. *Penatalaksanaan Pada Pasien Ulkus Kornea Dengan Prolaps Iris Oculi Sinistra*. Medula, Volume 1, Nomor 1.

Hermanto, Sandra. 2009. *Teknik Analisa Kromatografi Soektroskopi*.

Ilyas S., Maylangkay B. H. H., Ulkus Kornea. 2002. *Ilmu Penyakit mata Untuk Dokter Umum dan Mahasiswa Kedokteran*. Edisi Ke-2. Jakarta : Sagung Seto. Hal : 131-134.

Ilyas S., Ulkus Kornea. 2006. *Ilmu Penyakit Mata*. Edisi ke-3. Jakarta : Fakultas Kedokteran Universitas Indonesia. Hal : 159- 160.

Liu K, et al. 2008. *Graphite/Poly (Vinyl Alcohol)Hydrogel Composite As Porous Ringy Skirt For Artificial Cornea*.Elsevier: Materials Science and Engineering C.

Purwanti, Ani. 2010. *Analisis Kuat Tarik dan Elongasi Plastik Kitosan Terplastisasi Sorbitol.* Teknik Kimia, Institut Sains dan Teknologi AKPRIND Yogyakarta. Jurnal Teknologi, Volume 3 Nomor 2, Desember 2010, 99-106.

Richard T.T, et al. 2009. *Recent Development on Citric Acid Derived Biodegradable Elastomers*. Bentham Science Publicers Ltd.

Righe, B. 1992. *Eye Contact*. Chem Br ; 28:241-4.

Riordan, Eva P . 2010. *Anatomi & Embriologi Mata In:Vaughan ,Asbury* .Oftalmologi Umum Edisi 17. Jakarta: EGC

Smith, Brian C. 2011. *Fundamentals of Fourier Transform Infrared Spectroscopy*, Washington: CRC Press.

Yang, J, et al. 2006. *Synthesis and Evaluation of Poly(diol citrate) Biodegradable Elastomers.* Biomedical Engineering Department, Northwestern University, Evanston, IL 60208, USA.