In Vitro Assay of Cornea Artificial Properties

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Abstract

Blindness is still the crucial eye disorder. Many possibility of blindness cause, one of them is cornea ulcer. Cornea ulcer is characterized by the presence of suppurative infiltrate and partial loss of cornea surface due to tissue death. The tissue necrosis is caused by bacteria, fungi, and herpes simplex virus. In this decade, transplantation even from donor is the only treatment which could widely accepted for blindness. Hence, the cornea synthetic could be the alternative solution. However, treatment donor transplants have many shortcomings in post surgical complications such as immuno rejection, incompatibility and the length of time healing. As technology develops, there are many corneal substitutes based on natural sources derived from collagen or their derivatives because they promise better properties in biocompatibility. The aim of research are to conduct the synthesis and characterization of collagen- chitosan- glycerol - HPMC as artificial cornea such as water content, spectrophotometry and degradation value. Based on the spectrophotometric test, collagen-chitosan-glycerol sample has a higher absorbance value which is 3.29% while collagen-chitosan-glycerol-HPMC sample value is showed 1.57%. In the water content test, collagen-chitosan-glycerol sample has a higher water content when compared to collagen-chitosan-glycerol-HPMC. The water content results are showed the difference in the water content value of sample with collagen- chitosan- glycerol and sample with collagen- chitosan- glycerol- HPMC. The degradation percentage of collagen-chitosan-glycerol sample is 0.9469% while the value of collagen-chitosan-glycerol-HPMC sample is 0.6861%. Degradation study is showed that under simulated physiologic conditions for 3 days, the percentage of degradation were low and it was in accordance with the artificial corneal necessity.Biocomposite of collagen-chitosan-glycerol could be considered as artificial cornea due to the proximity with the corneal characteristics.

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Introduction

Blindness is especially global issues in the world due to its high prevalence and the great impact. Blindness have an impact to social life and influence productivity of people. There are 45 million blindness sufferers in the world, where one third from total sufferers are in Southeast Asia, including Indonesia.[1]

Indonesia has the second highest rate of blindness in the world, with around 1.5 percent of the population, or 3.5 million Indonesians.[2] According to WHO if a country has a blindness rate of more than 1%, this problem is a social problem. The main causes of blindness are cataract 0.78%, glaucoma 0.20%, refractive disorders 0.14%, retinal disorders 0.13% and corneal abnormalities 0.10%.[3]

Cornea consists of from multi-layered epithelium, Bowman membrane, stroma, Descemet membrane, and endothelium. Corneal endothelium cells (CECs) are attached with tightly in part under Descemet membrane, this cell is considered as origin of start peak nerve and it is formed monolayer from hexagonal cell. The function of corneal endothelium is to keep corneal transparency by hydration arrangement of stromal through barrier and pump function.[4] Human CECs can not regenerate and when the CECs are experienced derivation function,

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corneal transplantation would be urgently needed to correct vision. Various factors could be the causes of derivation function of CECs layer, including age, trauma, and disease. When total endothelium cells decreases below critical value, cornea lost clarity of optical characteristic due to edema which later can be lead to blindness.[5]

Corneal transplantion have various method start from replace all over part of cornea,until only replace damaged part of cells. The high invasive of the whole corneal transplantation has been done widely and it stimulate the development of slightly invasive methods. Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) is a partial thickness cornea transplant procedure that involves selective removal of the patient's Descemet membrane and endothelium, followed by transplantation of donor corneal endothelium in addition to donor corneal stroma. DSAEK is one of slightly invasive method which are developed nowadays. This method is eliminated disfunctional Descemet membrane and CECs by small slicing in the anterior part. Then, a thin layer of corneal endothelium donor is inserted to anterior partin order to replace CECs. This method is more favorable because thin layer only replaced through incision small, less possibility of infection, rapid healing time and visual rehabilitation.[6]

Various kind of material were explored for regeneration and potential implantation of CECs, such as collagen-based material, amniotic membrane, hyaluronic acid and silk fibroin. Collagen-based materials have low tensile strength compared with human cornea, while the amniotic membrane is available in limited number and has clarity difference level depend on the amnion taken time. Meanwhile clarity level clarity is an important indicator in cornea.Hyaluronic acid and silk fibroin have some weakness in implantation.

Chitosan is a natural polymer from glucosamine and N-acetyl glucodamine, which is widely used in pharmacy and tissue engineering because its biocompatibility, biodegradability, and anti-microbial properties. Chitosan has capability to support acceleration angiogenesis on network cornea and network skin.[7] Collagen was found in the vitreous body, the corneal epithelium, the notochord, the nucleus pulposus of intervertebral dises, and embryonic epyhelial-mesenchymal transitions.[8]

Fibronectin, collagens, vibronectin, and laminins constitute much of the extracellular matrix of the healing cornea epithelium basement membrane.[9]

Glycerol as dehydrating agent, has antimicrobial and antiprotease properties and maintains corneal structure, making it suitable for long-term storage of corneas for purposes not requiring viable cell layers.[10]

Hydroxypropyl methylcellulose (HPMC) containing ocular lubricants can help to maintain physiological corneal density and may be beneficial in the treatment of dry eye disease.[11] Based on the background above, collagen - chitosan-glycerol-HPMC biocomposite are the good combination for corneal artificial candidate.

Materials and methods

Materials which are used in this research are collagen and chitosan from Biochitosan, hydroxypropyl methylcellulose (HPMC) from Sigma Adrich , SIP glycerol pro analysis, Phosphate Buffer Saline (PBS), Collagenase, acid acetate and distilled water. We use the Fourier Transform Infra Red (FTIR) machine, spectrophotometry Uv -Vis Flatron, ELISA Reader.

Synthesis of Collagen- Chitosan-Glycerol- HPMC

The synthesis steps was started by making collagen and chitosan solution in acetic acid. The solution is stirred for 7 hours. After that, each solution is added with 5 ml mixtureof glycerol and stirred until homogeneous. Once homogeneous, the solution is poured in a petri dish to dry at room temperature. In extraction methods, the sample is soaked in NaOH first, then removed and rinsed using DI water. We made 2 type of samples. Sample A is collagen-chitosan-glycerol mixture and sample B is collagen-chitosan-glycerol-HPMC.

Uv-Vis Spectrophotometry Test

In the Uv-Vis spectrophotometry test the membrane was cut in half size of the cuvette and then affixed to the cuvette layer for inclusion in the U-Vis spectrophotometry instrument. The blank solution used is acetic acid. Visible light spectrophotometry or UV-Vis is the measurement of light energy by a chemical system at a certain wavelength. In this spectro test 200-700 nm wavelength is used. The detector on this instrument will provide a radiation response at

various wavelengths. The process of irradiating the Uv rays on the sample coming out through the column and a detector on the opposite side, will get a large direct reading of the absorbed light.

Water Content Test

Water content is the quantity of water in material. We are used Phosphate Buffer Saline (PBS). The sample is cut into a square with a size of 1x1 cm and then put in a bottle that contains PBS solution. After soaking for 3 days, the sample is taken to measure the wet weight (W) then the sample is dried to a constant weight (Wo). From these data, to determine the percentage of water content is calculated using the following formula.[7]

$$Wt = [W0) / W] \times 100\%,$$

Where Wo is dry sample weight and W is wet sample weight.

Degradation Test

The degradation test was carried out in vitro by simulating corneal samples under physiological conditions using a solution of Phosphate Buffer Saline (PBS) and collagenase. The degradation media was consisted of 5 ml PBS and 0.05 gram collagenase. Each sample is weighed at 0.08 grams as the initial weight (Wo). Then the sample is put in a bottle containing PBS and collagenase solution. For 1 week, sample weighing and replacement of degradation media were carried out. The data obtained is calculated and analyzed using equation below :

$$\% \text{ Degradation} = Wt/Wo$$

Where Wo is dry sample weight and W is wet sample weight.

Results

Uv-Vis Spectrophotometry Test

In measuring Uv-Vis spectrophotometry, wavelengths of 200-700 nm are used. Absorption absorbance values in sample A (collagen-chitosan- glycerol) was 3.29% and in sample B (collagen- chitosan- glycerol- HPMC) was 1.57%. The results of the absorption graph are shown in Figure 1.

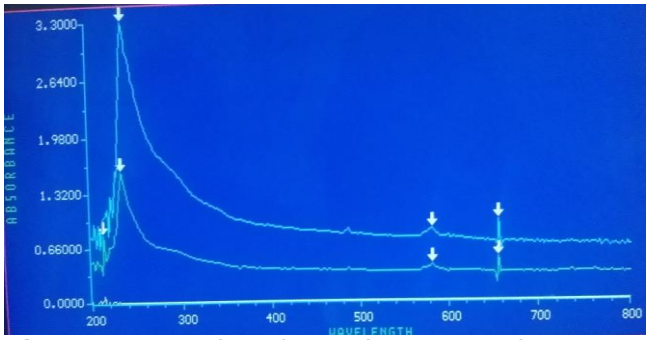

Figure 1. Results of Uv-Vis Spectrophotometry Test.

Water Content Test

The water content in sample A (collagen-chitosan- glycerol) was 13.7% and sample B (collagen- chitosan- glycerol - HPMC) was 6.76% as shown in Figure 2.

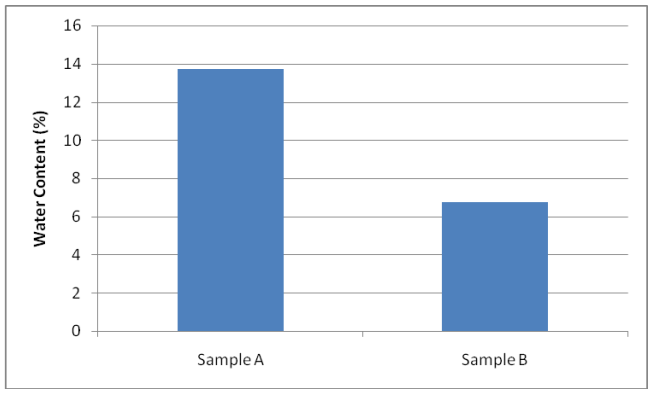

Figure 2. Water Content Test Results.

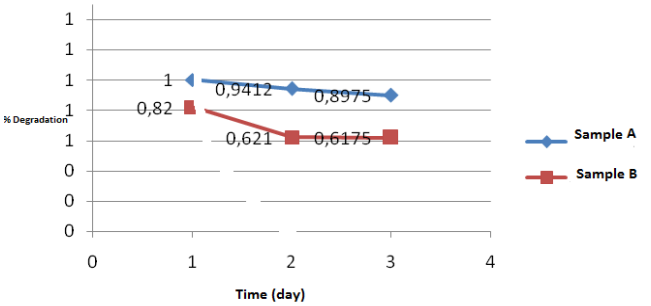

Figure 3. Degradation Test Results.

Degradation Results

In Figure 3, it can be seen in sample A (collagen- chitosan- glycerol) that the percentage of degradation is faster when compared to sample B (collagen- chitosan- glycerol- HPMC) . Sample A has an average degradation value of 0.9469% and sample B is 0.6861%. The addition of HPMC as stabilizer to the collagen-chitosan will cause more stable degradation value as seen in sample B.

Discussion

Uv-Vis spectrophotometry test is one method for analyzing elements with low levels both quantitatively and qualitatively based on the interaction between material and light. One of technique to measure corneal transparency is spectrophotometry.[12] There was quite little transmission of UV through cornea because the epithelium and stroma contain special proteins and vitamins that are considered to absorb much of this radiation and thus protect cornea and the inner content of the eye. Most Uv absorbance happens in the anterior corneal layers.[13]

The test results are showed that the absorbance of sample A is 3.29 % and the sample B is 1.57 % as shown in Figure 7. The absorbance of sample A (collagen- chitosan- glycerol) has higher value compared to sample B (collagen- chitosan- glycerol- HPMC). This is because the solution in sample A has a higher concentration level compared with sample B. Determination of the solution absorbance will yield the absorbance value very high in too concentrated solution due to many molecules that interact with light.[14]

Quantitative measurement of corneal light transmission requires passing a defined beam of light through the tissue and detecting how much is transmitted without absorption or scattering. The important thing is to avoid effects of refraction and reflection which can be achieved by surrounding tissue in medium with similar refractive index of cornea. When conducting transmission measurement, it is important to remove cornea from the globe to ensure that cornea is under tension. When the tension due to intraocular pressure is released, it would lead to increased light scatter.[15] The crucial thing is to ensure that the detector has small acceptance angle so as not to record any forward scattered light.[16]

Water is a chemical substance composed of two hydrogen atoms which are covalently bonded to an oxygen atom. Water content in a material greatly affects the quality of the material. In cornea water content determine the transparency. The corneal transparency and the light transmission ability is regulated by corneal thickness. Corneal thickness is influenced by water content. The water content measurement was performed and the result is showed the difference in the water content value of sample with collagen- chitosan- glycerol and sample with collagen- chitosan- glycerol- HPMC.The water content in sample A (collagen- chitosan- glycerol) is 13.7 % and sample B (collagen- chitosan- glycerol- HPMC) is 6.76%. The difference in the water content value of sample A and sample B is quite large, where in sample B has a lower water content than the sample A. The low water content in sample B might be due to the addition of HPMC as an emulsiferous substance. Emulsifers or emulsifying substances are substances to maintain water stability. Cornea covering several layers, part of the largest is stroma, which is 90 % of the total thickness cornea and consists of water, collagen, proteoglycans and keratocytes. Hydration cornea, defined as ratio weight wet to weight dry, around H = 3.2 for all species for levels physiological. Cornea must fulfilled certain water content level because ability cornea to perform its function to trasmit light was very influenced by corneal thickness. Corneal thickness is very depends on corneal moisture content.[17]

The implant was almost degraded at 7 months postoperatively without causing adverse inflammatory or immune reactions. Some keratocytes were observed to grow into superficial lamellae of the implant during 5-7 months postoperatively, showing that the new corneal tissue regeneration might be happened as degradation takes place. Based on the corneal implantation results by Long Y et al[18], collagen – HPMC membrane have excellent ability to promote new stromal keratocytes. The result of degradation study is showed that under simulated physiologic conditions for 1 weeks incubation, little dissolution after 1 week of exposure to a degradation solution and very little mass loss was found. Collagen has many hydrophilic groups which have a tendency to chain the surrounding solution when soaked. This phenomenon is advantageous for collagen-chitosan composite which can improve scaffold hydrophilicity. It is essential for tissue engineering scaffold.[19] Biomaterial based on collagen-chitosan and HPMC is showed characteristics which are in accordance with the biomaterial for keratoprothesis.[20]

Conclusions

Biocomposite collagen – chitosan – glycerol with addition of HPMC material based

on physicochemical characteristics tend to close to be good artificial cornea has been fabricated. The further research of physical and biological characteristics need to be conduct. The in vivo study must be considered to provide the ideal illustration of the corneal artificial characteristics.

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Declaration of Interest

The authors report no conflict of interest.

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