In Vivo Biomaterial Study of Collagen – Chitosan - Sodium Hyaluronate Composite as Artificial Cornea

Prihartini Widiyanti1,2*, Reni Prastyani3, Novi Dwi Widya Rini1, Marsya Nilam Kirana1, Tri Astutik1, Marcellino Rudyanto4

1. Biomedical Engineering Study Program, Department of Physics, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.
2. Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.
3. Department of Ophthalmology, Faculty of Medicine, Universitas Airlangga - General Hospital Dr. Soetomo, Surabaya, Indonesia.
4. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia.

Abstract
The cornea has a function as a protective layer of the eye and transmits light to the eye thus it has the potential to suffer damage. Damage to the cornea can cause blindness besides cataracts. Handling of corneal damage that is executed a lot has a high rate of rejection reaction from the patient's body and the limited number of donors. An alternative effort is created to replace the cornea by creating artificial corneas using different concentrations of sodium hyaluronate added to collagen and chitosan. To find out the results of artificial corneal cytotoxicity test and in vivo characterization in New Zealand rabbits (Oryctolagus cuniculus) with different concentrations of sodium hyaluronate. Corneal artificially made from sodium hyaluronate with a concentration variation of 0%; 0.3%; 0.6% w/v in collagen 20% w/v and chitosan 10% w/v. Artificial corneas that provide the best results through cytotoxicity testing were implanted in New Zealand rabbits (Oryctolagus cuniculus). Two tails were randomly selected which were divided into 2 groups: negative or untreated groups (G0) and positive groups or implanted cornea (G1 and G2) of the best study results in rabbit eyes. G0 sample (coll-chi without Sodium Hyaluronate) has a cell viability percentage of 93.02632% in the cytotoxicity test, while G1 sample (coll-chi +NaHa 0.3%w/v) has a cell viability level of 74.3342% and a G2 (coll-chi +NaHa 0.6%w/v) sample has 100% cell viability level. In the in vivo test by implanting G2 samples into rabbit eyes, showed no inflammatory results, there was no exudation in the anterior chamber and the centre of vision was clear. However, microscopically, the cornea is artificially the same thickness as the control cornea, producing a suitable stroma structure, and well-developed epithelial cells. All three samples are showed the percentage of cell viability above 70% with the G2 sample (coll-chi +NaHa 0.6%w/v) having the highest percentage in the cytotoxicity test. The G2 (coll-chi +NaHa 0.6%w/v) sample is implied in the rabbit's eye also shows that this corneal is biocompatible through the condition without inflammation and exudation, the corneal implant has same thickness with the control and the epithelial and stroma structure could grow normally same as in control.

Keywords: Artificial cornea, collagen, chitosan, sodium hyaluronate, in vivo.

Received date: 05 April 2020
Accept date: 10 May 2020

Introduction
Cornea is the front of the eye and functions as a protective layer in the eye that is translucent thus it is susceptible to damage. Corneal damage is caused due to the lack of nutrition, infection, and trauma.1 The damage of cornea can be the second leading cause of blindness in the world after cataracts. There are as many as 285 million people who experience blindness in the world.2 The prevalence of blindness in Indonesia is 3 million people (1.5% of the population). Every minute 1 person goes blind in Indonesia. The most common causes of blindness in Indonesia are Cataracts (0.78%); Glaucoma (0.20%); Refraction Disorders (0.14%); Retinal Disorders...
(0.13%) (Mainly Diabetic Retinopathy) and Corneal Abnormalities (0.10%) (Especially Xerophthalmia).³

Until now, the gold standard for handling corneal damage cases is keratoplasty which is transplanting the donor's cornea to the patient. However, Guilbert et al reported an average keratoplasty-to-rejection time of 19.8 ± 20.4 months (among 299 patients who experienced a rejection episode). The progression from rejection to failure was 49%.⁴ A global survey on corneal transplantation showed there were a total of 184576 corneal transplantations conducted in 116 countries and 284000 corneas procured in 2012. It also reported a substantial lack of tissue with only one cornea available for every 70 patients worldwide.⁵ Many patients who experience blindness need corneal transplant but only limited number of donors available. Hence, it is very needed an alternative effort to replace the cornea.

Study on corneal tissue has been conducted by Chen et al.⁶ by using collagen type I as the main component of the cornea. The study showed that collagen 20% w/v + chitosan 10% w/v and sodium hyaluronate 0.5% w/v had better biocompatibility than without collagen. It was also found that 1% w/v + chitosan 2% w/v collagen and the addition of 0.5% w/v sodium hyaluronate were able to transmit light by 95%. In vivo test showed that the qualitative transmittance of the three samples with sodium hyaluronate concentrations showed different results.⁶

This study was conducted to analyze the utilization of corneal replacement biomaterials with collagen-based ingredients added with chitosan and sodium hyaluronate. Collagen in study conducted by Kim⁷ is showed the ability to improve the hydrophilic nature of biomaterials. Collagen added with chitosan is able to maintain the form integrity of the biomaterial. However, sodium hyaluronate was also added which showed the ability to increase the percentage of light transmittance.⁶

Materials and methods

The material was prepared by dissolving 20% w/v collagen in 0.1 M acetic acid and 10% w/v chitosan in 0.6 M acetic acid. Collagen solution that has been crosslinked with HPMC was mixed and sterilized with chitosan solution for 60 minutes. Then, NaHA with 0% concentration variation; 0.3%; and 0.6% was added to the Col + Chi solution and stirred for 30 minutes. The concentration ratio of collagen, chitosan, and sodium hyaluronate can be seen in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collagen (w/v)</th>
<th>Chitosan (w/v)</th>
<th>Sodium Hyaluronate (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0</td>
<td>20%</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>K1</td>
<td>20%</td>
<td>10%</td>
<td>0.3%</td>
</tr>
<tr>
<td>K2</td>
<td>20%</td>
<td>10%</td>
<td>0.6%</td>
</tr>
</tbody>
</table>

Table 1. Concentration Ratio of Collagen, Chitosan, and Sodium Hyaluronate.

Results

The cytotoxicity values which are represented from percentage of cell viability of control (coll-chi without Sodium Hyaluronate) sample and artificial cornea G1(coll-chi +NaHa 0.3%w/v) and G2 (coll-chi +NaHa 0.6%w/v) can be seen in Figure 1.

In vivo test was performed with only implanted G2 sample in New Zealand rabbit eyes. The results appeared that artificially implanted corneas were not inflamed, there was no exudation in the anterior chamber and the visual centre looks clear as shown in Figure 2.⁸

While microscopically, artificial corneas were compared to the control cornea (the normal cornea taken from the other eye in one rabbit). It appears in Figure 3 that artificial cornea G2 (coll-chi +NaHa 0.6%w/v) have the same thickness as the control corneas.
Figure 2. Clinical Appearance Macroscopically after 8 weeks Implantation of Artificial Cornea G2 (coll-chi +NaHa 0.6%w/v).

Figure 3. Histophatology Anatomy Result of thickness with 40x enlargement; A. Control cornea or without implantation; B. artificial cornea G2 (coll-chi +NaHa 0.6%w/v).

Figure 4 below shows that artificial cornea G2 (coll-chi +NaHa 0.6%w/v) produce similar stroma structure compare with the control cornea.

Figure 5 below shows that in artificial corneas, the epithelial cells grow well and quite similar with the condition in the control cornea.

Figure 5. In Vivo Result Test Microscopically by Histophatology Anatomy with 40x enlargement to see epithel cell; A. Control cornea or without treatment; B. Artificial cornea G2 (coll-chi +NaHa 0.6%w/v).

Discussion

Cytotoxicity tests are used to determine the toxic effects of artificial corneas directly on tissue culture. One method used in this study is using the MTT Assay test. Three sample groups show the percentage of cell viability above 70% with the G2 sample having the highest percentage of cell viability compared to other samples. This shows that three samples match the characteristics of the cornea which is not toxic. Samples have a toxic potential if the cell viability of the percentage is below 70%. The samples are not toxic because all of the component of composite are biocompatible.

Collagen is a biocompatible and bioactive non specific polymer that, either alone or in combination with other materials, occupies a foremost position in the field of tissue engineering. Collagen type I is potential as a biomedical material. Collagen has well-documented structural, physical, chemical and immunological properties, is biodegradable, biocompatible, non-cytotoxic, with an ability to support cellular growth, and can be processed into a variety of forms. Chitosan exhibit outstanding biodegradable and biocompatible properties. Chitosan and its derivatives exhibit biodegradable, biocompatible, antimicrobial activity and low immunogenicity which are advantageous for development as biomaterials. Hyaluronic acid is a widely available, biocompatible, polysaccharide with distinguishing physiochemical properties which inspire its application throughout several fields of medicine.
Samples with the highest percentage of cell viability, G2 (collagen-chitosan +NaHa 0,6%w/v), were conducted in vivo characterization by implanting artificial corneas in New Zealand rabbits. In vivo characterization is observed macroscopically and microscopically. On macroscopic observation for 8 weeks after implantation, it appears that artificial corneas that have been implanted do not experience inflammation, there is no exudation in the anterior chamber and the centre of vision is clear. However, the results of microscopic observations will be compared to the corneal rabbit’s eye without implantation. Histopathology anatomy result of artificial cornea after implanted for 8 weeks was showed the same thickness as control cornea. Artificial corneae also were produced stroma structures that in accordance with the control cornea and epithelial cells have grown well on artificial corneas similar with in control cornea. These results indicate that the artificial cornea in this study are biocompatible. One of possibility cause from the material such as chitosan is biocompatible. Because it is not cause the inflammation and produce exudate. This result is in accordance with the research by Lagali which stated that transplant showing by three month the stroma cells were present in the construct, and the morphology and cellularity appeared normal. Collagen also has supporting effect due to its protective characteristic to skin by inhibiting the absorption of toxins and pathogens. It provides important role in biological functions of cell (cell survival, proliferation and differentiation), supports healing process and maintains structural integrity. Therefore, this artificial cornea has the potency to be observed by clinical trial based on the compatibility on in vitro and in vivo assay (physical, chemical, mechanical and biological assay) and can be considered as candidate for cornea replacement biomaterials in treating corneal damage.

Conclusions

All three samples in this study showed the percentage of cell viability above 70% while collagen - chitosan – natrium hyaluronat 0,6%w/v sample (G2) having the highest percentage in the cytotoxicity test. G2 sample implied in New Zealand rabbit eyes also showed that this artificial cornea is biocompatible due to the compatibility of thickness, stroma structure, epithelial growth and the absence of inflammation and exudate.

Acknowledgements

The author would like to deliver gratitude to Nurul Fitri Shabrina, Arantrinita for the assistance in corneal implantation to the experimental animals, to Ministry of Education Indonesia for the funding of this research and also to Institute of Tropical Disease and Veterinary Hospital Universitas Airlangga for the facilities and supports.

Declaration of Interest

There is no conflict of interest.

References


