Review Article

Application of bio-artificial cornea and its research progress

Lingqin Zeng, Gang Li, Wen Zeng, Chuhong Zhu

Department of Anatomy, National & Regional Engineering Laboratory of Tissue Engineering, Key Lab for Biomechanics and Tissue Engineering of Chongqing, Third Military Medical University, Chongqing 400038, China

Contributions: (I) Conception and design: LQ Zeng, W Zeng; (II) Administrative support: G Li; (III) Provision of study materials or patients: LQ Zeng; (IV) Collection and assembly of data: LQ Zeng, W Zeng; (V) Data analysis and interpretation: LQ Zeng; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Chuhong Zhu. Department of Anatomy, National & Regional Engineering Laboratory of Tissue Engineering, Key Lab for Biomechanics and Tissue Engineering of Chongqing, Third Military Medical University, Chongqing 400038, China. Email: zhuch99@yahoo.com.

Abstract: Cornea is a layer of transparent film which located in the forefront of the eye. In the world, more than 30% blindness patients were caused by corneal disease. Corneal transplantation is the only effective means of treatment of corneal blindness. Owing to a serious shortage of donor cornea, China's annual eye bank can only provide a small amount of surgery used cornea. Therefore, seeking a good equivalent corneal replacement is an important way to solve the problem of insufficient donor cornea. At present, in vitro reconstruction of the tissue engineering cornea can be used as a mild corneal equivalent substitute to solve the problem of insufficient donor cornea. We may be able to rebuild a good corneal replacement for corneal transplantation. And the corneal replacement can be acting as ideal grafts for corneal transplantation in patients with severe corneal injury in the future

Keywords: Cornea; tissue engineering; bio artificial

Received: 03 July 2017; Accepted: 27 July 2017; Published: 18 August 2017.

doi: 10.21037/aes.2017.08.01

View this article at: http://dx.doi.org/ 10.21037/aes.2017.08.01

Cornea is a layer of transparent film which located in the forefront of the eye. It was oval and covering the iris, pupil and anterior chamber. Most of the eye's refractive powers are supplied by the cornea. In the world, more than 30% blindness patients were caused by corneal disease. Corneal disease is the second largest incidence of eye blindness after cataract disease. The human body cannot repair or replace the corneal endothelial cells automatically. Therefore, corneal disease can only be solved by surgery which may replace corneas with a healthy cornea. Thus, there is a huge demand for the eye cornea. While in other countries and regions of the world, especially in some countries of Asia, coupled with some societal, cultural and religious reasons, the provision of corneas is extremely scarce and it is difficult to obtain a donor cornea. As a result, many patients cannot be cured owing to absence of implanted corneal donor. So, scientists began to seek to use the cornea replacement to help eye blindness patients to restore vision. It can be said that the history of artificial cornea development, that is, history of its materials constantly updated and developed in recent years. In recent years, it has been much progress in this area. This article made a review.

In 1771, French ophthalmologist Pellierde Quensey proposed implanting the glass to the rabbit cornea. In 1817, Weber first implanted an artificial cornea into the eyes of the blind patient. It was only six months left, but it is the first time that scientist replaces the corpuscles with a transparent object and let the patient see the light. Over the next few decades, ophthalmologists utilize different optical materials to produce artificial cornea, and ultimately failure because of the body's rejection and eventually the occurrence of infection or loss. In the true sense, the generation of corneal substitutes should be the emergence of tissue engineering artificial cornea (Tissue Engineering Cornea). The principle of tissue engineering cornea is use of biological materials (such as collagen) as living cell culture stent and build alternative donor corneal tissue. Culturing tissue engineering artificial corneas requires

suitable seed cells and carrier scaffolds and certain training techniques. On the stent, the seed cells can grow into the cornea which has the exact same or similar structure and function with the artificial cornea. In recent years, many researchers have tried to separate corneal cells from different corneal cell layers, and then put these cells into a culture dish. Or we can use a biomaterial as a scaffold to reconstruct corneal replacements which are very similar to human cornea. Now, corneal cells have been grown on biomaterials for three-dimensional culture. And scientist can reconstruct artificial corneas. This corneal replacement is very similar to the human cornea. Because it is built from the corneal cells, it can replace the host cornea and can do the same stimulation in the human cornea. Tissue engineering artificial cornea can not only be used to replace the cornea, but also has great application prospects in the field of medical research.

Seed cell

Seed cells used for artificial cornea require: (I) high proliferation capacity; (II) has long-term and sustained ability for maintaining its physiological function and biological activity.

In the culture dish, normal corneal epithelial cells can only be monolayer growth and passage, but can not be cultured with a layer of squamous epithelial structure. In experiments with rabbit corneal stroma as a carrier to culture rabbit corneal epithelial cells, Friend found that stratified epithelial cells were in a position to appear. And they also detect the connective structure in the living cornea (1). In 1986, Schermer *et al*. (2) demonstrated that stem cells are present in the limbal epithelium. The characteristics of stem cell stem cells are: a low degree of differentiation, long cell life cycle, a high degree of differentiation and proliferation and self-renewal ability. Researchers found that only epithelial cell culture with corneal central tissue can only pass 2–3 times, and stem cell culture in limbal cells can pass to 12 generations. Therefore, the use of this feature oflimbal stem cells as a seed cell is the best choice for bioartificial cornea.

In general, corneal endothelial cells have only a very weak ability to proliferate. If cultivating human embryonic corneal endothelial cells in vitro, we can see cell division. The growth factor is critical to adult corneal endothelial cell culture and division. In 1990, at the place of Fuch corneal endothelial dystrophy, McLaughlin in inoculated with cultured human corneal endothelial cells to allow the cornea to be transparent (3). In 1991, Insler used two times of inoculating corneal endothelial cells to increase the density of endothelial cells (4). After 48 hours of re-culture, the corneal endothelial cells were transplanted on the recipient cornea. The results were satisfactory. At present, the industry recognized that the key to determine the transparency of the cornea is whether the function of the corneal endothelial cell is intact. It has been reported that about 50% of the cornea preserved in the eye bank cannot be transplanted due to endothelial dysfunction. Therefore, the successful transplantation of endothelial cells in vitro will successfully improve the success rate of most corneal transplantation.

We can extend the life of human skin fibroblasts and corneal endothelial cells through various methods. For example, the E6 E7 gene can enhance the cell differentiation potential by activating the negative regulatory genes of cell growth. And they cannot change its split time. Griffith *et al*. transfected cells by reverse transcription method. The E6 E7 gene recombined in human corneal epithelial cells, endothelial cells and fibroblasts. And the life span of the cells was prolonged. They obtained three kinds of cells which were comparable to corneal function (5).

Reconstruction of corneal tissue is achieved in several steps: separation of corneal cells, cell culture, and reconstruction of the epithelium, stroma and endothelium. The first step is to divide the main 3 layers of the cornea cells. Tegtmeyer *et al*. isolated three types of corneal cells from the bovine cornea (6). First, the cornea was placed in a steel container with the inner layer facing up and covering the insulin-EDTA solution for 7–8 h. Then the endothelial cells were separated with the knife and were placed into a Petri dish. Substrate corneal cells are obtained by removing the endothelium and the epithelium. The growth of stromal cell corneal cells was after 6–7 days. The epithelial cells are placed under the substrate layer for culture.

Yoichi Minami obtained corneal cells of each layer by enzymatic digestion. Corneal tissue was incubated at 37 ℃ for 60 min. Then the endothelium and epithelium were isolated under a microscope using a surgical scissors and inserts. Then they obtained cell stromal cells by removing epithelium and endothelial cells with enzymes digested. The isolated cells were cultured in tissue culture medium. Studies have shown that in the corneal cell culture, the cell growth and proliferation can be enhanced by adding serum in the culture medium.

Constructed carrier scaffolds using tissue engineering

The bio-artificial corneal carrier scaffold material is the basis for the reconstruction of the cornea in vitro. It provides the required environment for seed cells to grow. And at the same time they are progressively degraded in vivo during tissue renewal. Ideal corneal stent material should have the following characteristics: (I) good biocompatibility; (II) can be degraded in the body; (III) appropriate pore size and porosity; (IV) mechanical strength and plasticity; (V) appropriate curvature, transparency and oxygen permeability. At present, we mainly use natural biological materials, synthetic biodegradable polymer materials and these two cross-linked composite materials to build tissue engineering cornea.

Amnion

At present, the clinical application of amnion as a carrier to expand the corneal limbal stem cells in vitro, and the formation of corneal epithelial graft treatment of alkali burns, has achieved good results. Adds *et al*. (7) found that amnion preserved at 4 ℃ and at –80 ℃ *in vitro*, *in vivo* two kinds of preservation methods, the difference between two amnion corneal epithelialization is not significant.

Collagen

As the core component of the corneal extracellular matrix (ECM), collagen (collagen) plays a very important role to maintain the transparency of the cornea. It is a biocompatible, in vivo degradable, low immunogenically structural protein (8). So far, there are more than13 kinds of collagen have been identified. In natural tissue, collagen and other components are linked together. They need special treatment when utilize. In recent years, collagen has been extensively used in the field of tissue engineering cornea (9). Griffith and other obtain the morphological structure, physiological function and other corneas similar to the normal cornea. Their experimental methods were mainly inoculated with immortalized human corneal epithelium, matrix and endothelial cell lines in collagen-chondroit in carrier scaffolds and cultured in vitro (5).

In 2006, Liu *et al*. (10) cross-linked psoriasis to prepare tissue engineered corneal stroma and obtained artificial cornea similar with normal corneal stroma. The cornea has a high refractive index, good transparency, and corneal epithelial cells with superior biocompatibility and so on. The shortcomings of collagen as a stent are: they are still cannot large-scale production, soaring cost of commercialization collagen, fast degradation rate, poor mechanical strength, and the efficacy of infection is not significant. Therefore, it is necessary in order to further improve its physical and chemical properties and biological properties to prepare carrier scaffolds (11).

Gelatin

Gelatin can be prepared from the local hydrolyzate of collagen. Its solubility and biocompatibility are excellent. Gelatin has no antigenicity. Physical and chemical properties of the gelatin can be changed. It is the ideal scaffold material in the tissue engineering. In 1980, Jumblatt *et al*. (12) cross-linked gelatin and glutaraldehyde. Then they inoculated them into corneal endothelial cells. However, the resist enzymolysis ability of the structure is relatively poor and cannot be applied in surgical transplantation. In 2006, Hsiue *et al*. (13) prepared a gelatin-based carrier and inoculated human corneal endothelial cells in this stent. Then they transplanted the carrier in rabbit cornea. The cornea of the investigational eye was restored after surgery.

Chitosan

Chitosan deacetylation can produce chitosan (chitosan), which has the benefit of no immunogenicity, good biocompatibility, easy to degrade in the body and produce non-toxic substances, a wide range of sources, low cost. Chitosan is a skillful combination of collagen and cellulose. Rabbit corneal stromal cells were placed on scaffolds prepared by chitosan-hyaluronic acid. We can obtain well-grown rabbit corneal stromal cells (14). They used collagen-chitosan as scaffold material, rabbit corneal stromal cells and human corneal stromal cells as seed cells to reconstruct tissue engineered corneal stroma in vitro. And then they transplanted them into rabbit corneal stromal capsules to obtain normal cell morphology cornea. And the biocompatibility of the stent was safe, and the specific marker protein of seed cells was positive. Therefore, as a new research idea, chitosan derivatives can be invoked as carrier scaffolds for tissue engineering corneal stroma. The drawbacks are that the molecular size is inconsistent, the degradation rate is slow, it is difficult to form an effective pore structure, so it is also clinically limited (15).

Fibrin

As a native ECM component, fibrin can be polymerized from the monomer state into a network gel by the action of thrombin. It has good plasticity, good biocompatibility and degradability (16). In 2006, Alaminos *et al*. (17) produced a composite scaffold containing fibrin and agarose. They inoculated corneal epithelium, matrix and endothelial cells on the scaffold to obtain tissue engineering cornea. The morphological and function of the cornea are comparable to the normal cornea.

Talbot *et al*. (18) used fibrin as a scaffold (3T3 fibroblasts as trophoblast). They inoculated rabbit corneal limbal stem cells on them. After 2 weeks of in vitro culture, they obtained the ideal corneal epithelium of the layer for autologous transplantation. Chen *et al*. (19) inoculated fibrin gel on carrier scaffolds after reconstruction of rabbit corneal stromal cells in vitro. They obtained a highly transparent tissue engineering rabbit corneal stroma. They stromal cell grew in good condition by the followed experimental. The fiber-like cell connection structure grew on the scaffolds. They had good biodegradability. Fibrin-based matrix scaffolds can also support the growth and differentiation of corneal epithelial cells. The disadvantage of fibrinics is poor mechanical properties. They require further research and improvement.

Hyaluronic acid

As a natural acidic GAG polymer, hyaluronic acid is non-toxic, non-carcinogenic, biocompatible and has a controlled effect on the differentiation and penetration of certain cells (20). The lack of hyaluronic acid as a stent is easy to dissolve in water, quickly absorbable, only a short time to stay in the organization. It is difficult to attach and the mechanical is not strong. And thus inevitably they must chemically modify to obtain more stable solid materials.

Acellular corneal stroma

In recent years, the hot tissue scaffolds of tissue engineering cornea research are acellular corneal stroma. Because it can not only the natural cornea structure and mechanical properties, but also contains a specific growth factor, which is synthetic stent does not have. They can provide a suitable growth environment for cell growth. It is also possible to remove specific ingredients in the tissue that may cause immune rejection (21). There were a number of research results on the use of acellular methods to treat heterologous source cornea and the preparation of acellular corneal stroma. Porcine acellular corneal stroma scaffolds prepared by phospholipase A2 (22) have good biocompatibility, no antigenicity, good mechanical properties and transparency in rabbit corneal transplantation. After 12 months, they are still able to stay stable.

The tissue-engineered corneal lamina was obtained by the preparation of the acellular porcine corneal stroma using 0.5% SDS (23). The stent has good biocompatibility and transparency, useful mechanical properties. The cornea became normal for 1 month after transplanted. Scientists observed a tissue repair capacity.

Gonzalez-Andrades *et al*. prepared scaffolds in vitro and reconstructed the tissue engineering corneal stroma using the NaCl and SDS two decellularization methods (24). They found that NaCl-made stents were more conducive to the growth of human corneal stromal cells. Different corneal stents with different pore sizes and ECM structures can be obtained by using different cell methods. Thus, the selection of effective methods in a number of acellular methods is the key to the preparation of acellular porcine corneal stroma (19).

Autocrine ECM

The use of Cell-Based Approaches to assemble tissue engineered corneal stroma carrier stents is another hotspot in recent years. It is primarily to add certain ingredients in the culture medium to stimulate cells to secrete ECM. The cells themselves carry out autocrine ECM. Scientists can obtain carrier stent similar to the normal corneal morphology (25,26). Autologous on corneal fibroblasts, 36 m thick ECM structure was achieved after one month (26). The preparation of the stent must have a large number of non-autologous cells to secrete cells. It will take a long way to meet the need of clinical treatment requirements.

Polyglycolic acid

Polyglycolic acid (polyglycolic acid) degraded to glycolic acid in vivo. It is easy to be metabolized by the body. It is also often used as a stent due to the good biocompatibility feature. By changing its molecular weight, we can also get the desired strength, which is not exist in a lot of other materials.

Lin *et al*. used Polyglycolic acid - fiber as Carrier support

and rabbit corneal stromal cells as seed cells (27). After corneal transplantation, rabbits were examined restored transparent two months after cornea transplanted. The researchers found that neatly arranged collagen fiber structure in the transplant area.

After one week, they inoculated rabbit adipose-derived stem cells as seed cells on a polyglycolic acid scaffold (27). For the autologous corneal transplantation of experimental animals, it was found that adipose-derived stem cells could differentiate into fibroblast-like cells and had good growth status. The cornea could be gradually recovered and the stent could maintain the normal function of corneal epithelial cells and endothelial cells. However, the biggest drawback of polyglycolic acid is that its degradation products produce large amounts of acid. It may lead to seed cell poisoning and even death, or local severe inflammatory reactions which are not applicable in clinical (28).

Polylactic acid-polyglycolic acid copolymer

Polylactic acid and polyglycolic acid can polymerize in a certain proportion to form polylactic-co-glycolic acid polymer material. They are not only ideal for mechanical strength, but also biocompatible and degradable. In vitro, corneal stromal cells were able to bind to polylactic acid-polyglycolic acid copolymers. They have excellent biocompatibility (20). With the New Zealand white rabbits as the model, after the corneal stroma interbody transplanted, the grafts were observed to become transparent1 week after surgery. This suggests that, as a tissue engineering corneal stent, material polylactic acid-polyglycolic acid is non-toxic, non-antigenic and has good biocompatibility and degradability. The drawback of this material is that local acid will be produced in the degradation process. The presence of acid may have potential stimulating damages to new blood vessels.

Composites

There is another idea of using composite materials. That is, If the natural materials and synthetic materials cross-linked and integrated through a certain proportion or specific method, physical and chemical properties and biological function of the tissue engineering corneal carrier scaffold can be improved. For example, a porous polylactic acid-polyglycolic acid sponge (29) can be prepared by remodeling in the presence of sodium chloride by removing the particles. The sponge is filled with a collagen solution, hyaluronic acid or Human amniotic membrane. Then they prepare a composite sponge by lyophilization. And the corneal epithelial cells and stromal fibroblasts were inoculated on these scaffolds to observe the growth of the cells. Compared with the untreated stents, the modified scaffolds can more easily promote cell adhesion and hyperplasia. Polyvinyl alcohol-collagen scaffolds were set by cross-linked with type I collagen and polyethylene (17). After inoculation of upper corneal epithelial cells, the results showed forming a composite structure. It has good compatibility.

The type I collagen extracted from calfskin and rat tail tendons (30) was able to mix with chondroitin sulfate. Then they prepared a composite scaffold with glutaraldehyde as a crosslinking agent. After adding corneal stromal cell suspension, tissue engineering corneal stroma can be reconstructed in vitro. The increase in mechanical strength of the stent is mainly the use of cross-linking agent. The improvement of transparency was mainly by chondroitin sulfate.

The reconstruction of the cornea

As the porcine corneal stroma has good biocompatibility, it is clinically used widely. In addition, pigs can also be economical feeding in a large number of disease-free environments. They are non-primates and mature fast. And ethical and security-related issues caused by pigs are much less than that caused by chimpanzees and baboons. Amano *et al*. (31) suggest that the porcine corneal stroma is expected to replace the human corneal stroma, which is a carrier of human corneal endothelial cell growth. Porcine cornea is widely available. The structure of porcine cornea comes closer to human corneal tissue. However, it has anti-source and pathogenic hazards.

Acellular porcine corneal stroma is an ideal carrier scaffold. It has a similar structure of the human cornea, a wide range of sources, low immunogenicity. The shortcomings are: containing porcine stromal cells and genetic material; cannot be directly applied in tissue engineering, must be removed in order to get a wide range of applications. A scientist (32) treated the porcine corneal stroma in a combination of several methods and trypsinase. In the post-transplant tissue, there is good biocompatibility and no inflammatory and immunological rejection. In order to further improve the strength, toughness and pore size of acellular porcine corneal stromal material as scaffolds, it is also useful to remove the cell components in the porcine

corneal stroma by repeated freezing and thawing. In the process of in vitro reconstruction, after incubation with rabbit corneal stromal cells, rabbit corneal stromal cells were found to be more likely to move into the scaffold material (33).

In short, acellular porcine corneal stroma is an ideal natural material scaffold. It has good biocompatibility. Choosing a different acellular approach, we can get a different organizational structure. How to choose a method of acellularization, it can be simple and effective and best to maintain its morphological structure, is the urgent need to solve the problem of utilizing porcine corneal stroma.

IV Comparison between tissue engineering bio-cornea and the same type of cornea.

Tissue engineering artificial corneal replacement consists of three layers of cell layers. Scientists have compared the reconstructed human cornea with the actual human cornea (34). The artificial cornea has significant similarity with the human cornea. No matter the expansion of the matrix, gene expression, tissue transparency and the physiological function and physiological activity. They showed greater sensitivity on the artificial cornea. In the case of light propagation, the artificial cornea exhibits a great deal of sensitivity to varying degrees of damage after in contact with the chemical. This is similar to the natural cornea including the appearance, morphology, transparency and matrix expansion. It has a meaningful response to varying degrees of damage; especially show great sensitivity for the stimulation of chemical substances. It is also one of the important functional characteristics of the human cornea. Artificial corneas reconstructed by Yoichi Minami are transparent. The cells can be observed under inverted microscopes. Its clarity does not achieve as high as the human cornea. It is enough to identify epithelial cells, stromal cells and endothelial cells. The reconstructed cornea is about 0.18–0.23 μm thickness, which is similar to the human cornea. Due to the high permeability of the artificial cornea, some factors secreted by cells and culture fluids can pass the cornea smoothly.

The ideal artificial cornea should have the following characteristics: (I) excellent optical properties, stable physical and chemical properties; (II) able to long-term coexistence of autologous corneal tissue, and closely integration; (III) no adverse reactions, fewer complications; (IV) simple operation, easy to produce, affordable. The artificial cornea can bring the hope of rehabilitation for the majority of blind patients in the near future.

The biggest problems of artificial cornea are long-term fixation of artificial cornea in the human eye and artificial corneal surface epithelial activation. There are some major clinical complications, such as: (I) corneal opacity; (II) corneal aseptic necrosis or ulcer formation; (III) artificial cornea after the membrane; (IV) secondary glaucoma; (V) corneal shedding, stent fracture and so on. And this is also the largest gap between the bio-artificial cornea and the natural cornea.

Conclusions

In summary, corneal transplantation is the only effective means of treatment of corneal blindness. Owing to a serious shortage of donor cornea, China's annual eye bank can only provide a small amount of surgery used cornea. Therefore, seeking a good equivalent corneal replacement is an important way to solve the problem of insufficient donor cornea. With the continuous advancement of tissue engineering research, it is possible to obtain biologically active tissue engineering corneas through in vitro reconstruction. The biggest difficulties of artificial cornea are: how to make the artificial cornea long-term fixed in the human eye; artificial corneal surface epithelial activation; artificial cornea is not tough enough; the cornea has a damaging immune response after transplantation. At present, in vitro reconstruction of the tissue engineering cornea can be used as a mild corneal equivalent substitute to solve the problem of insufficient donor cornea. We may be able to rebuild a good corneal replacement for corneal transplantation. And the corneal replacement can be acting as ideal grafts for corneal transplantation in patients with severe corneal injury in the future.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editors (Weiyun Shi and Jin Yuan) for the series "Bioengineering Cornea" published in *Annals of Eye Science*. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/

10.21037/aes.2017.08.01). The series "Bioengineering Cornea" was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Friend J, Kinoshita S, Thoft RA, et al. Corneal epithelial cell cultures on stromal carriers. Invest Ophthalmol Vis Sci 1982;23:41-9.
2. Schermer A, Galvin S, Sun TT, et al. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. J Cell Biol 1986;103:49-62.
3. McLaughlin R, Schoessler J. Corneal endothelial response to refitting polymethyl methacrylate wearers with rigid gas-permeable lenses. Optom Vis Sci 1990;67:346-51.
4. Insler MS, Lopez JG. Heterologous transplantation versus enhancement of human corneal endothelium. Cornea 1991;10:136-48.
5. Griffith M, Osborne R, Munger R, et al. Functional human corneal equivalents constructed from cell lines. Science 1999;286:2169-72.
6. Tegtmeyer S, Papantoniou I, Christerl C. Reconstruction of an in vitro cornea and its use for drug permeation studies from different formulations containing pilocarpinc hydrochloride. Eur J Pharm Biopharm 2001;51:119-25.
7. Adds PJ, Hunt CJ, and Dart JK, et al. Amniotic membrane grafts, "fresh" or frozen? A clinical and in vitro comparison. Br J Ophthalmol 2001;85:905-7.
8. Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol 2002;13:377-83.
9. Liu Y, Wang X, Jin Y, et al. Can bone marrow cells give rise to cornea epithelial cells? Med Hypotheses 2008;71:411-3.
10. Liu Y, Gan L, Carlsson DJ, et al. A simple, cross-linked collagen tissue substitute for corneal implantation. Invest Ophthalmol Vis Sci 2006;47:1869-75.
11. Koizumi N, Sakamoto Y, Okumura N, et al. Cultivated corneal endothelial cell sheet transplantation in a primate model. Invest Ophthalmol Vis Sci 2007;48:4519-26.
12. Jumblatt MM, Maurice DM, Schwartz BD, et al. A gelatin membrane substrate for the transplantation of tissue cultured cells. Transplantation 1980;29:498-9.
13. Hsiue GH, Lai JY, Chen KH, et al. A novel strategy for corneal endothelial reconstruction with a bioengineered cell sheet. Transplantation 2006;81:473-6.
14. Vázquez N, Chacón M, Meana Á, et al. Keratin-chitosan membranes as scaffold for tissue engineering of human cornea. Histol Histopathol 2015;30:813-21.
15. Gao X, Liu W, Han B, et al. Preparation and properties of a chitosan-based carrier of corneal endothelial cells. J Mater Sci Mater Med 2008;19:3611-9.
16. Rama P, Bonini S, Lambiase A, et al. Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. Transplantation 2001;72:1478-85.
17. Alaminos M, Del Carmen Sánchez-Quevedo M, Muñoz-Avila JI, et al. Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold. Invest Ophthalmol Vis Sci 2006;47:3311-7.
18. Talbot M, Carrier P, Giasson CJ, et al. Autologous transplantation of rabbit limbal epithelia cultured on fibrin gels for ocular surface reconstruction. Mol Vis 2006;12:65-75.
19. Chen JS, Liang XD,She GR, et al. Study on fibrin glue's cohesive effect with rabbit conjunctival epithelium. Journal of Jinan University (Medicine edition) 2007;28:152-6.
20. Ma XY, Bao HJ, Cui L, et al. The graft of autologous adipose-derived stem cells in the corneal stromal after mechanic damage. PLoS One 2013;8:e76103.
21. Guo H, Wu X, Yu FS, et al. Toll-like receptor 2 mediates the induction of IL-10 in corneal fibroblasts in response to Fusarium solu. Immunol Cell Biol 2008;86:271-6.
22. Wu Y, Chu R, Zhou X, et al. Determination of the nerve growth factor level in the central cornea after LASIK and Epi-LASIK treatment in a rabbit model system. Cornea 2009;28:1144-8.
23. Pang K, Du L, Wu X. A rabbit anterior cornea replacement derived from acellular porcine cornea matrix, epithelial cells and keratocytes. Biomaterials 2010;31:7257-65.

24. Gonzalez-Andrades M, de la Cruz Cardona J, Ionescu AM, et al. Generation of bioengineered corneas with decellularized xenografts and human keratocytes. Invest Ophthalmol Vis Sci 2011;52:215-22.
25. Du Y, Sundarraj N, Funderburgh ML, et al. Secretion and organization of a cornea-like tissue in vitro by stem cells from human corneal stroma. Invest Ophthalmol Vis Sci 2007;48:5038-45.
26. Carrier P, Deschambeault A, Talbot M, et al. Characterization of wound reepithelialization using a new human tissue-engineered corneal wound healing model. Invest Ophthalmol Vis Sci 2008;49:1376-85.
27. Lin YY, Carrel H, Wang IJ, et al. Effect of tear film break-up on higher order aberrations of the anterior cornea in normal, dry, and post-LASIK eyes. J Refract Surg 2005;21:S525-9.
28. Henriquez AS, Robertson DM, and Rosen DA. Tolerance of the cornea and eyelid to polyglycolic acid and rat-tail tendon sutures. An experimental study. Can J Ophthalmol 1974;9:89-103.
29. Lin PY, Kao SC, Hsueh KF, et al. Localized amyloidosis of the cornea secondary to trichiasis:clinical course and pathogenesis. Cornea 2003;22:491-4.
30. Doillon CJ, Watsky MA, Hakim M, et al. A collagen-based scaffold for a tissue engineered human cornea:physical and physiological properties. Int J Artif Organs 2003;26:764-73.
31. Amano S. Transplantation of cultured human corneal endothelial cells. Cornea 2003;22:S66-74.
32. Spoerl E, Wollensak G, Seiler T. Increased resistance of crosslinked cornea against enzymatic digestion. Curr Eye Res 2004;29:35-40.
33. Oh JY, Kim MK, Lee HJ, et al. Comparative observation of freeze-thaw-induced damage in pig, rabbit, and human corneal stroma. Vet Ophthalmol 2009;12 Suppl 1:50-6.
34. Polisetti N, Islam MM, Griffith M. The artificial cornea. Methods Mol Biol 2013;1014:45-52.

doi: 10.21037/aes.2017.08.01
Cite this article as: Zeng L, Li G, Zeng W, Zhu C. Application of bio-artificial cornea and its research progress. Ann Eye Sci 2017;2:62.

