Clinical application of tissue-engineered keratoprosthesis

Chen Jia-jia, Wang Wen-zhan, Zhu Yu

Department of Ophthalmology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China

Abstract

BACKGROUND: Keratoprosthesis is a special kind of refractive apparatus made of alloplasm shaping material, which is designed to replace the turbid cornea tissue. The visual acuity may be obtained after surgical implantation into the affected eyes.

OBJECTIVE: To introduce the advance of keratoprosthesis research.

METHODS: A computer-based online retrieval was performed among Wanfang and Medline databases, by using the key words of "keratoprosthesis, corneal transplantation, biocompatibility, tissue engineering" in English and Chinese. The animal experiment and clinical progress of artificial cornea transplantation, implantation and biocompatibility, complications were discussed.

RESULTS AND CONCLUSION: A total of 204 articles were selected, according to the inclusion and exclusion criteria, 50 articles were included in this study. People have made great progress in the research and production of keratoprosthesis within recent years. There are many new ideas in material choosing, handling and designing ways of non-tissue-engineered keratoprosthesis. Meanwhile, the appearance of active tissue-engineered keratoprosthesis created a new method for the research and production of keratoprosthesis.

Subject headings: keratoprosthesis; corneal transplantation; biocompatibility; tissue engineering

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Chen Jia-jia, Master, Attending physician, Department of Ophthalmology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China

Corresponding author: Chen Jia-jia, Department of Ophthalmology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China

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INTRODUCTION

Keratoprosthesis (KPros) is a special kind of refractive apparatus made of alloplasm shaping material, which is designed to replace the turbid cornea tissue. KPros was first envisaged by a French ophthalmologist, Pellier de Quensey in 1771[1]. In 1859, Heusser first implemented the implantation of KPros[2]. The transparent glass implanted only stayed for 3 months. In 1905, Zirm successfully implemented human allokeratoplasty. The technique transferred the people's enthusiastics towards KPros. With the extensive development of allokeratoplasty, the existing problems gradually arise, such as severe xerophthalmia, cornea blindness damaged by corneal limbus stem cells, and turbid cornea caused by cornea new vessels. In addition, the source of allograft cornea is limited, which again arouses people's interest of KPros implantation surgery. In 1947, Stone first used polymethyl methacrylate (PMMA) in KPros experiment, which greatly promoted the development of KPros. Since 1960s, besides the exploration of KPros materials, scholars also modified the design and implanting methods of KPros. Furthermore, corresponding to polymer KPros, the tissue-engineered KPros with biological activity has entered a period of rapid development within recent years.

DATA AND METHODS

Literature retrieval

A computer-based online retrieval was performed by the first author among Wanfang and Medline databases, by using the key words of "keratoprosthesis, corneal transplantation, biocompatibility, tissue engineering" in English, Chinese and Russian.

Inclusion criteria

(1) Original research on animal studies and clinical experiments addressing the KPros, with reliable argument evidence. (2) Introductions of the modification of KPros materials and design. (3) Research on the histocompatibility of the improved KPros and the progress of tissue-engineered KPros.

Quality assessment

A total of 204 articles were screened out. According to the inclusion and exclusion criteria, 50 articles were included in the final analysis.

Data extraction

In cases of differences of opinion, agreement was achieved after discussion or a third researcher made a decision regarding inclusion of the article. The positive or negative results of clinical significance were preferred in information recording.

RESULTS

Baseline information of the involved articles

Among the involved 50 articles[1-50], 16 ones[1-3, 5, 8, 11, 15, 19, 22, 25-27, 29-30, 44, 49-50] are animal experiments and clinical trials about the KPros, 11 ones[7, 16-18, 20-21, 23-24, 28, 32-33] introduced the improvement on the KPros materials and design, and 22 ones[4, 6, 9-10, 12-13, 14, 31, 34-43, 45-48] investigated the histocompatibility of the improved KPros and the progress of tissue-engineered KPros.

Study characteristics of the involved articles

The choice of KPros materials

Inorganic materials

(1) Glass: Glass was the first applied material for KPros, but it only stays for 3 months[2]. At present, as a non-micropore and non-diosmosis material, glass can't meet the requirement of frame material, but as a optical center material, it still has good optical properties, stable physical and chemical properties, easy to be moistened by water, anti-megatemperature, easy to disinfect the merits. Because it is prone to fragmentation, difficult to process, and has other shortcomings, the glass has been replaced by PMMA.

(2) Hydroxyapatite: Hydroxyapatite is made of natural coral reef materials through inorganic processing and handling. Its main chemical ingredients are $Ca_{10}(PO_4)_6(OH)_2$, which is similar to the main inorganic elements of human bones and is stable in body fluid. Its pore structure is similar with Harvard's system of human bones, and has micropores connected with each other inside. Because of the ingredients and structure, hydroxyapatite has a high biocompatibility, non-noxious, and non-antigenicity. Fiber vascular tissue may grow in the inner micropores, improve the nutrition supply of the tissue before and after implantation, and increase the firm of contiguity between implant and receptor, thus reduce the chance of infection, necrosis, and injury. The nature is stable and light, which has small stimulus and pressure to the surrounding tissues. In 1997, León *et al* [3] first reported the animal experiment that applied natural coral reefs hydroxyapatite to KPros. The KPros is made in this way: they used a PMMA of 3 mm in diameter as the optical center spectacle-column, cut massive hydroxyapatite into frame parts with a inner curvature radius of 8 mm, outer curvature radius of 7 mm, diameter of 10 mm, insert the mirror-column into the frame, and then connect them with bone cement. After 12 months of implantation operation, there is no infection, water leakage, exodus of implants and other serious complication emerge. Pathological examination and the scan of technetium 99 bone both confirmed that the fiber vascular tissue grows in the micropores of the material. A large number of reports revealed that hydroxyapatite has a good application prospect[4-5].

(3) Bioglass ceramic: At present, the most successful application material of the porous hydroxyapatite is the natural coral reefs hydroxyapatite after processing, which is collected from the South Pacific. It is light, and has evenly distributed pores, concentrate pore size (about 0.2–0.5 mm). But its high cost and small number partly restrict the extensive material's application. Recently as a substitute for hydroxyapatite, bioglass ceramic in animal experiment shows a high extrusion rate (51%) after corneal implantation, and easily breaks after implantation (62%), thus it has an impact on the corneal metabolism, leading to the opacity of the corneal front board. A report from Zhongshan Ophthalmic Center shows that bioglass ceramic with aperture of 20–70 μm, porosity of 37–72% is not suitable for KPros frame material temporarily[6].

(4) Titanium: Linnola *et al* [7] used titanium and titanium coating on the active glass-ceramic as KPros material, and then implanted it into rabbit eyes. The experimental findings proved that titanium is an ideal material for KPros, and the titanium coating on the ceramics is more effective to prevent cells endogeny. In subsequent studies, scholars have changed bioglass ceramic as surrounding materials, and transplanted into rabbit eyes, but the test results are disappointing.

Organic materials

The application of organic materials, especially polymer materials, promotes the research on KPros materials. During the World War Two, people found that plexiglass can stay a long time in the pilot's cornea[8]. Later animal experiments gradually confirmed the discovery[9]. It aroused people's interest of the application of organic polymer materials.

(1) Silicone resin: The main ingredient of silicone resin is poly-methyl siloxane (methyl vinyl silicone) based on dimethyl vinyl silicone-oxygen, which is binded with carbon atoms into single chains at high temperature, and then into polymers. The main advantages of silicone resin are: light (1.0 share), good thermal stability, high transparency, stable molecular structure and good anti-aging properties. The main disadvantages is its poor anti-tension ability, dirt such as surface protein has large attachment and is difficult to handle, lower the transmissionrate.

(2) PMMA: After long-term clinical application, PMMA is an ideal optical material. It has a high transmission rate (92%), refractive index 1.491, stable performance, high resistance to aging and environmental changes, non-biodegradable, anti-acid, alkali and organic solvents, light, not easily broken, high plasticity and easy to process. As an ideal optical material, PMMA still has certain shortcomings. Its high hardness is not good for testing eye

pressure after KPros implantation. It is limited to tolerance YAG laser, and easy to be broken by YAG laser, so it is difficult to deal with the meta-memb after KPros implantation, and the single released after laser has biological toxicity. It can't be disinfected under high pressure or steam heating. Surface epithelial cell has poor adhesion and cannot form the continuous corneal epithelium. It has dangers such as latent infection, leakage, corneal dissolved after operation.

(3) Fluorocarbon polymer: Since 1980s, the research and application of porous material injected new vitality to the KPros material science. Fluorocarbon polymer is one of the organic materials with micropores which have been used widely. Researchers have high hopes for an in-depth study.

First of all, Lamberts *et al* [10] recommended using porous material polytef fluorocarbon polymer and vitreous carbon polymer as peripheral stents. This kind of material is hard, rough, wet, stable and easy to process. The porosity is of 70%–90%, and the aperture is of 100–500 μm. White *et al* [11] reported the animal experiment of penetrating KPros, which use Proplast as periphery, PMMA as optical center area. The KPros is stable after operation. At the early age, fiber cells grow in it and the longest case stayed for more than 3 years.

Another two kinds of soft fluorocarbon polymer materials, *i.e*. Impra and Gore-Tex[12]. They both belong to expanded polytet rafluoroethylene, and have the same physical and chemical properties, such as white, soft, smooth, porosity of 77%–82%, aperture of 18–22 μm, water absorption. The differences between them are the fiber courses and hardness. Impra fiber has one-way course, arranged in order, good for the growing of fiber cells. Gore-Tex fiber is arranged in disorder, which correspondingly reduces the effective pore area, and has an effect on the fiber cells growing.

Legeais *et al* [13] compared and observed the above three materials' histocompatibility after implanting the corneal layers. During the 4–8 months' observation period, they found that only Impra showed good histocompatibility and stability. Histological test showed fibroblasts grow in the micropores and secret collagen. In addition, after white Impra was implanted into rabbit eye's cornea, 1 month later, the material gradually became transparent.

The presumption is that after the collagen secreted by intercellular substance and fibroblast fills the micropores, the material has the same refractive index with the cornea, causing the reduction of light scattering and the increasing of transparency. It is regrettable that the transparency can't reach the requirement of optical center. In short, the properties of the material, including the thickness, hardness, micropore's aperture, the course of the fiber, affected its stability after implantation[13].

(4) Polyvinyl alcohol copolymer hydrogel: In 1960, Otto Wichterle and Drahoslov Lim in the Polymer Chemistry Institute (Prague, the Czech Republic) announced their research results, and issued a paper named "The Biological Application of Hydrogel", which opened the application of hydrogel in medical area. The main polymer they used is hydroxyl methacrylate, and then it appeared a variety of polymers, for example, poly(2-hydroxyethyl methacrylate) (PHEMA).

Such material is different from PMMA, it contains hydrophilic group–OH and has good hydrophilic property. Its moisture content is from 20% to 70%, and the water content has obvious impact on the physical properties. Hydrogel material has certain permeability, so gas, dielectric and glucose can pass. Its visible light transmission rate can reach as high as 97%. In ophthalmology area, hydrogel material is successfully applied to the production of soft contact lens and folded soft artificial lens.

In early 1990s, Chirila, a polymer chemist in Lion Eye Institute (Australia), and his research team[14-15] began to apply PHEMA to KPros. After seven years' concentrated research, they developed a new type of soft KPros, the Integration Type, which is called the "real" cornea by some scholars[16].

Chirila KPros looks like thicker soft contact lens, and has a diameter of 9 mm, thickness of 0.5 mm, surface curvature radius of 8 mm, post curvature radius of 8.5 mm. The structure is also made up of two parts, optical center and the surrounding. The materials of the two parts are the same. Both are hydrogel polymers, so they have the same chemical properties. But they have different water content, so they have their own physical properties, therefore, eliminating the long-term problems on material interface.

Optical center has a diameter of 7 mm, which expands the horizons and easy for ophthalmologists totally examining the inner eyes. In addition, optical center material has relatively high water content (about 38%), soft, smooth, no holes, and has the same refractive index with human cornea. The periphery has a diameter of 2 mm, soft, network space structure. It can be directly sutured into corneal bed-sik and reduce the corneal trauma and complications caused by complex operation.

Under the function of divinyl glycol, the center and surroundings polymerized as one. The interface area formed a penetration polymer mesh transitional zone, which is 0.5 mm wide, smooth, soft and has high anti-tension strength. The soft and smooth surface makes it possible to measure eye pressure after operation. In addition, PHEMA material cells have poor adhesion, which can avoid post-memb formation after operation. Soft PHEMA material has small gravity with the tissues inside eyes, which helps maintain blood-aqueous barrier.

Biological materials

Many experiments and clinical research proved that the imcompatibility of alloplasm material and tissues is the main reason for KPros prolapsing. So many scholars[17-18] recommended using auto-materials as KPros stents, in which teeth, ear cartilage and cricoid are applied more and have the highest success rate. The KPros that the implant stayed for the longest used auto-teeth as surrounding material (Osteo-odonto-keratoprosthesis, OOKP). The operation was designed and first applied by Strampelli [17]. Ricci *et al* [19] reported the longest holding time is more than 20 years. Tan *et al* [49] reported 15 cases in which the patients received OOKP, including severe Stevens Johnson syndrome, chemical burns and thermal burns, an average follow-up of 19.1 months, 11 patients achieved the best corrected visual acuity 0.5.

The innovative design of KPros

At present, three types of KPros clinical application are reported most[19-24], namely Dohlman, Strampelli and Cardona. The three all include fixing a rigid PMMA-optical scope-column, penetrating cornea, and anchored by surrounding tissues. The front surface of the scope-column is exposed outside the tissues and covers the surrounding tissues. Soft KPros is more effective at dealing with some high-risk cases than allogeneic keratoplasty.

Dohlman KPros

Dohlman designed a one-step-perforate KPros, also known as the Boston KPros, which is a “Link-deduction” type of PMMA, but the initial clinical trial results were disappointing. After the surgery, corneal stroma dissolving led to complications, such as the water leakage, discarding of implants and falling of retina. At the same time, protein enzymes produced by the epithelial tissue may lead to ulcers.

Recently, Dohlman *et al* [24-25] reported the same kind of KPros transplantation surgery of another group of patients, and made a three-year follow-up. Among them, 11 cases of dry eye patients received KPros’ scope-column came in the front surface of cornea, using eyelid to cover the KPros and cutting two months later. Five patients formed a KPros post-proliferation memb, which is the major complication. Nine patients retained the implants until the end of the track, six of whom significantly improved their vision. During 1990 and 1995, 48 patients received Dohlman KPros surgery, of whom 44 retained the KPros.

Strampelli KPros

Perforate KPros has better vision recovering effect than early penetrating KPros. People tried all kinds of materials to wrap the central optical scope-column, in order to increase the stability and tissue compatibility of KPros. Strampelli[17] used auto-teeth as surrounding material, and creatively put forward the “bone-teeth” KPros (OOKP) technology. Although the technology needs rather complex phased operation, but some reports say some patients’ KPros may retain in the body for more than 20 years.

Cardona KPros

The initial KPros is a plate-penetrating combination kind, used transparent penetration central scope-column and a plate implanted into the layer. The experiment proved that the discarding rate of this KPros is about 20%. Later he designed “nut-screw”, a fixed KPros. Its front surface looks like contact lens, has better cosmetic results, less water evaporation, thicker support issues, and doesn’t need donor cornea.

Aquavella *et al* [26] reported 31 cases of Cardona penetrating KPros implantation, covering periosteum, and prominent out of the eyelid. Follow-up after surgery is up to 84 months (35 months on average). 18 eyes formed KPros post-proliferation memb, 7 eyes had vitreous inflammation, 8 eyes had aversion or falling of KPros, 5 eyes needed another surgery.

MICOF KPros

MICOF is produced by eye surgery center in Moscow, Russia[27-28]. The operation is carried out in two phases. In the first phase, they implanted titanium stent into the corneal layer. Several months later, in the second phase, they implanted the thread PMMA scope-column into stent center, and cut off the crystallina, iris, and front vitreous at the same time. The eliminating blindness rate is above 90%. Corneal dissolution rate is 20%, usually needs surgical treatment, including autologous periosteum, autologous ear cartilage and allogeneic cornea implant repairing and conjunctiva covering. Reports show that hydroxyapatite KPros after surface modification of titanium stents in rabbit corneal layer[48], can make its both hydroxyapatite bioactive and metal strong mechanical properties, which provides a new method for clinical improvement MICOF KPros.

AlphaCor KPros

AlphaCor KPros was once called Chirils KPros, which has curved surface and flexible plate, transparent central part. Just as cornea, the edge is cavernous, but different from the past, it’s no need to cut off iris, lens and front vitreous when implanting. AlphaCor KPros has two types. Type One has a diameter of 9 mm, just as PK surgery when implanting, using chirurg to suture the sponge edge directly to implanting bed, then Type Two open a hole in the central conjunctiva. Type Two is a litter smaller than Type One. To make corneal board pocket from the outside of the corneal limbus, a small hole of 2.0–3.0 mm in diameter was drilled from central corneal post board. KPros is placed in the corneal layers, covered with conjunctiva. Four months later, a hole was drilled in central conjunctiva and front cornea. Hicks *et al* [29] reported the animal experiment result of Chirila KPros. The surgery is similar to penetrating keratoplasty, which can directly suture integration type KPros into corneal bed-sik.

The postoperative complications were significantly lower than previous reports. Except for three cases in which the

graft surrounding and planting beds didn't heal well, 20 cases had no common complications. Histological examination showed: one month after surgery, fibroblast grew in the surrounding micropores, and synthesis collagen III. Phase two clinical experiment showed AlphaCor is safe and can enhance visual acuity[30], but there was calcification or pigmentation[31].

Seoul-based KPros

South Korea designed a double fixed KPros, "Seoul-based"[32]. It consists of three parts: in the middle, there is a long columnar optical center, which is formed from PMAA, diameter of 4 mm, length of 4 mm. The surrounding fixing part includes two sections, front and post slices. The front slice looks like a mushroom and fixed on the cornea. The post slice is a couple of U-like antennae stent fixed on the sclera. With the help of double fixing, and amniosis reconstruction the ocular surface, they found that the KPros combined with amniotic membrane grafting increased the stability of the KPros. There are two cases in which the patients received this kind of KPros. One case is Stevens-Johnson syndrome, receiving the keratoplasty after the failing of penetrating cornea transplanting. In the 18 months follow-up, KPros is in the right position, retina returns to normal, and vision is up to 0.2. Another corneal acid burnt patient, after 8 months follow-up, the KPros is in the right position, and vision is 0.4. Kim *et al* [50] implanted Seoul-based KPros into human cornea, in 62.8 months follow-up, the results were satisfactory.

Aachen KPros

Von Fischern *et al* [33] used silicone-gel material and solved the problem that PMMA cannot measure intraocular pressure as a KPros optical centeand vision limited. The silicone-gel KPros can improve the cell adhesion ability after surface modification, and increase its stability with issues. Silicone-gel KPros has an optical center diameter of 11 mm, thickness of 0.3 mm. The stent includes a sclera circle and eight branches fixed with sclera, which can strengthen the connections with surrounding tissues, lower the prolapsing rate of KPros, and measure eye pressure.

Enhancing the histocompatibility of KPros materials

Low-temperature plasma

Plasma is made up of neutral atoms or molecules, excited state of atoms or molecules, free radical, electron or anion, positive ion and radiation photon. In laboratory, people often use the gas radio frequency under the pressure of 0.1–100 Pa to discharge and get plasma. As the temperature of the particles, such as ion, free radical neutral atoms or molecules is close to or slightly below the room temperature, they're called low-temperature plasma. Polymer materials after low-temperature modification have the following characteristics: the molecules on the surface of the material arouse, ionize, and bond broken, but the material won't pyrolysis. The depth of modification is limited from a few dozen to several thousand angstroms on the surface, so the material won't lose its own characteristics. Sipehia[34] used anhydrous ammonia to deal with the memb of and polytetrafluoroethylene (PTFE) by plasma processing, and found it can promote the adhesion growing of bovine endothelial cells.

Latkany *et al* [35] used argon plasma to process polyethylene hydrogel, and found it can improve the adhesion growing of rabbit's corneal epithelial cells on the surface material. Two weeks after the plasma processing, polyethylene hydrogel formed the multistoryand smooth epithelial surface, which solved the problem that KPros material surface epithlializes quickly. Wu *et al* [36] used three materials (porous materials polymerized by 80% polypropylene and 20% polybutene, polyester porous materials, and expanded PTFE), which are processed through argon plasma, to implant into rabbit cornea, and found rabbit corneal edema reduced significantly after material processed, new angiogenesis delays and the scope is smaller, less infiltration of inflammatory cell.

Surface modification method

Surface modification is to fix some anchoring factors on the surface of the material, such as laminin, fibronectin, Type I collagen, Type II collagen, poly-L-lysine, lecithin or collagen protein fixed on the materials[37-40, 45]. The function of cells and stroma materials is the result of specificity identification cells adhering to protein molecules. Pettit *et al* [37] found the material is conductive to corneal epithelial cell's adhesion and growing after fibronectin modification. Merrett *et al* [45] thought that YIGSR peptides had more potential on cell's adhesion. Kobayashi *et al* [38] found the hydrogel fixed by Type I collagen supported the growing and adhesion of epithelial cells. They used the cell cultivated materials to make rabbit corneal layers transplant, which can block the infection of hydrogel implanted into the rabbit's eye. Aucoin *et al* [40] found peptide, which is modified appropriately on the surface is more conductive to cell adhesion and growth than a single peptide modification.

Chemical reshaping method

Chemical reshaping means to change the composition of materials through copolymerization, graft, and other methods. For example, lactose has strong hydrophobicity, slow degradation and not conductive to cell's adhesion and growth. By using the hydrophilia of carbowax and the feature that carbowax can reduce the immunogenicity of protein, hydrophilia and degradation of polyglycolic acid, drug permeability of polycaprolactone, and using single copolymer, people composed a series of polymer materials that are processed by chemical reshaping, such as poly glycolide-lactide copolymer, poly lactone-lactide copolymer, and poly glycolide-caprolactone copolymer.

Hybrid reshaping method

Hybrid reshaping is to hybrid materials of different properties together by various means and get a biological scaffold material with a series of properties. Chitosan is

the only obvious natural polysaccharide with alky among natural polysaccharide. It has an amino on the side. Chung *et al* [41] mixed Chitosan and polyvinyl alcohol by appropriate technology, and found amino was rich on the surface of membrane, inoculated fibroblasts had better cell affinity on the mixed membrane than pure ethanol membrane. The mixture of chitosan and polysiloxane can be used to make composite with separate structure, which is used as anti-coagulation biological material. The hybrid materials make up for the lack of a single material, and provide a method for making biocompatible materials.

Tissue-engineered KPros

Tissue-engineered KPros needs right seed cells and carrier support, and certain training techniques. Seed cells should be able to grow on the stent artificial organs which have the same or similar structure and function with cornea.

Seed cells

The seed cells selected for tissue engineering should have a high proliferation ability to maintain its physical functions and biological activity for a long time. In 1986, Schermer *et al* [42] confirmed that there are stem cells on corneal limbal epithelial. Stem cells have following features: low differentiation degree, long life-period, high proliferation ability, good self-renew capacity and stress proliferation capacity. Cultivating epithelial cells by corneal central tissues can pass to 2–3 generations, but cultivating corneal limbal cell can pass to 12 generations. Recent years, using this feature of corneal limbal stem cells to treat the diseases of the surface of the eyes has achieved good results. Therefore, corneal limbal stem cell is the best choice of tissue engineering.

Bearer stent

The construction of KPros using tissue engineering needs a suitable stent. It has good biocompatibility. The cells can grow normally and form normal structure which is similar to the organs in the body, and has corneal transparency, lucency and oxygen permeability.

(1) Amniotic membrane: Amniotic membrane is a transparent membrane without blood vessels. It is usually considered to have immunologic inertia, inhibit the growth of endothelial cells' proliferation, and promote the growth of corneal epithelial cells at the same time. It can also inhibit the inflammatory response, scar and the formation of blood vessels. Vitro expanding limbal stem cells by using amniocentesis as a carrier and forming corneal epithelial implants to treat alkali burns, has made a better clinical effect. Seoul-based KPros has applied amniotic membrane as a carrier.

(2) Collagen: The cell growth and metabolism environment provided by collagen stent is close to physiological state. Using tissue engineering collagen as matrix to train KPros epithelial cells, endothelial cells, and fibroblasts, people found that the corneal fibroblasts trained in the collagen increased more matrix synthesis as their own growth and corneal epithelial cells and endothelial cells can cover the collagen by electron microscope. They also found that the thickness of corneal epithelium is influenced by the soluble factor produced by endothelial cells, and the shape of epithelial base cells is influenced by corneal fibroblasts.

Recently, more recombinant human collagen is applied to avoid the potential immune response. The most applied is RCH I and RHC III. There was no significant difference between the collagen I and collagen III, both the refractive index was 1.35, its transparent cornea was very close with human. Also, recently the RHC III corneal replacements in the first phase clinical trials have been implemented in 10 patients with deep lamellar transplant in Sweden[44-45]. In 10 patients with epithelial regeneration, stromal cells were seeded in the plant. Another two young patients appeared epithelial nerve regeneration at postoperative 3 months.

(3) Fibrin: Fibrin is a common used protein material. Through the cross-linking, it can enhance the adhesion and migration of epithelial cells on the gel. Fibrin-gel implants can be completely degraded after implanting to the body, and eventually replaced by autologous collagen. By joining aprotinin, it can prevent its premature dissolution. Han *et al* [43] showed that the fibrin-based matrix supports the growth and differentiation of corneal epithelial cells.

(4) Acellular cornea stroma: Acellular cornea stroma comes from the acellular allogeneic or xenotransplantation. Because of removing lipid membranes, the immunogenicity of membrane-associated antigen and soluble protein greatly decreased. As a stent, the micro-environment is closest to their physiological state, conducive to cell adhesion, migration and proliferation, and promote tissue regeneration. Pig cornea stroma expresses that micro amount of paratose antigens α-antigen determinant site will not induce hyperacute rejection, but only cause minor cell rejection.

(5) Chitin and chitosan: Chitin is the linear polysaccharide composed by N-acetyl-D glucose amine. Chitosan is the deacetylation derivatives of chitin. As a biodegradable material, Chitin is possible for the production of KPros.

Nerve regeneration

Reports show that hybrid neuroblastoma cell has the ability to differentiate into neurons, bumps, functional control target cells. Nerve growth factor-contained, dimethyl sulfoxide or dexamethasone serum free medium can be used to cultivate neuroblastoma cells. The experimental results indicate the medium combined neuroblastoma cell contributes to nerve regeneration *in vitro* [46-47].

Tissue-engineered KPros

A study has transferred E6/E7 double gender recombinant retroviral gene to human corneal epithelial cells and

endothelial cells[45], and fibroblasts by using immortalized cell technology. Before cultivating active corneal tissues, the converted cell lines were subject to morphological, biochemical and electrophysiological examinations, which is similar to human cornea. Cell phenotype is identified by detecting the expression of biochemical protein. People use collagen as stent, corneal fibroblast was incubated in collagen stent, epithelial cells were cultured at the bottom of the stent, while endothelial cells on the surface of the collagen stent. After the epithelial cells formed a layer of smooth cells, they were exposed to the air and divided into multilayer structure. To construct the active corneal tissues, we can also cultivate epithelial cells at the bottom of the stent, and form the equivalent of cornea, which is similar to human cornea in shape, structure, and tissue functions. It can be used after two weeks' cultivating. The constructed KPros is very similar to human cornea in shape, transparency and histology.

Germain *et al* used corneal epithelial cells and fibroblast to cultivate. The carrier is produced by the mixture of bovine collagen, human collagen liquor medium and human corneal fibroblast. Four days later, human corneal limbal epithelial cells were inoculated to the three-dimensional gel surface containing human corneal fibroblasts and adhered for two hours, then added medium and cultivated. Three days later, there were corneal fibroblasts alive in the collagen, and the epithelial cells equally covered the collagen matrix surface full of fibroblasts. Masson trichrome staining showed the epithelia had 4–5 layers, and the cells on the basal layer were cubic. The thickness of reconstructed cornea was close to normal cornea. Basal membrane component could be detected between epithelial layer and three- dimension collagen.

CONCLUSION

In recent years, the KPros research is mainly focused on the use of porous polymer materials as a central-surrounding stent material, and improving the biological combining ability of the materials[51-55]. Over the past ten years, the hot spots of KPros the long-term stability of porous, needs further discussion. Growing evidence shows that the epithelialization of KPros material surface can not only reduce the heterogeneity stimulation, more importantly, the continuous stable corneal epithelium can prevent the destruction of collagenase, inhibit the secretion of interleukin-1, prevent corneal solution, and guarantee good optical surfaces and lower the resistance of bacterial infection. In addition, active tissue-engineered KPros is under positive research stage in experiment because of its high production requirements and complex technology. We can expect that in the next two decades, people could copy the KPros similar with donor cornea outside the body. In many cases, the key for the reconstruction of cornea is to provide some adhesion and growth factors *in vivo*. Tissue engineering cornea stroma either through nutrition preparation for living cells for transplantation, at the same time, the sterile nature and no cytotoxicity contribute to the cell growth, and form an extension of the nerve adjacent to the tissue. The function of the cornea is also improved. Transparent tissue-engineered KPros is applied in not only surgery, it also has a inner pore and cell adhesion factor, allowing the growth of the stromal cells, epithelial proliferation and functional nerve plexus growth. Recently, constructing a substitute that is close to human corneal structure and physiological function, may alleviate the current shortage situation of global cornea donors, thus brings a hope for the blindness caused by corneal disease. Although tissue-engineered artificial cornea has not been widely applied to clinical practice, much effort is needed to further test the function of cornea reconstructed. We believe in the near future, tissue-engineered artificial cornea will be successfully and widely used in clinical corneal transplantation, and bring the light back to the patients.

REFERENCES

[1] Yang ZL, Liu L. Modern cornea transplantation. Beijing: People's Military Medical Publishing House. 1998;167-170.
[2] Zhu ZZ, Zhou DF, Li MQ. Keratonosus. Beijing: People's Military Medical Publishing House. 1986;84-97.
[3] León CR, Barraquer JI Jr, Barraquer JI Sr. Coralline hydroxyapatite keratoprosthesis in rabbits. J Refract Surg. 1997;13(1):74-77.
[4] Perry JD. Hydroxyapatite implants. Ophthalmology. 2003; 110(7):1281.
[5] Yin XT, Sun XG, Jin XY, et al. The experimental study on hydroxyapatite for supporting material of keratoprosthesis. Yanke Yanjiu. 2001;19(3):193-194.
[6] Liang D, Chen JQ, Li YP, et al. Tissue interaction with bioglass ceramic implanted in the rabbit cornea. Yanke Xuebao. 2001;17(4):198-201.
[7] Linnola RJ, Happonen RP, Andersson OH. Titanium and bioactive glass-ceramic coated titanium as materials for keratoprosthesis. Exp Eye Res. 1996;63(4):471.
[8] Stone W Jr, Herbert E. Experimental study of plastic material as replacement for the cornea; a preliminary report. Am J Ophthalmol. 1953;36:168-173.
[9] Knowles WF. Effect of intralamellar plastic membranes on corneal physiology. Am J Ophthalmol. 1961;51:1146-1156.
[10] Lamberts DW, Grandon SC. A new alloplastic material for ophthalmic surgery. Ophthalmic Surg. 1978;9(6):35-42.
[11] White JH, Gona O. "Proplast" for keratoprosthesis. Ophthalmic Surg. 1988;19(5):331-333.
[12] Trinkaus-Randall V, Capecchi J, Newton A, et al. Development of a biopolymeric keratoprosthetic material. Evaluation in vitro and in vivo. Invest Ophthalmol Vis Sci. 1988;29(3):393-400.
[13] Legeais JM, Rossi C, Renard G, et al. A new fluorocarbon for keratoprosthesis. Cornea. 1992;11(6):538-545.
[14] Chirila TV, Constable IJ, Crawford GJ, et al. Poly(2-hydroxyethyl methacrylate) sponges as implant materials: in vivo and in vitro evaluation of cellular invasion. Biomaterials. 1993;14(1):26-38.
[15] Chirila TV, Thompson-Wallis DE, Crawford GJ, et al. Production of neocollagen by cells invading hydrogel sponges implanted in the rabbit cornea. Graefes Arch Clin Exp Ophthalmol. 1996;234(3):193-198.

[16] Hicks CR, Fitton JH, Chirila TV, et al. Keratoprostheses: advancing toward a true artificial cornea. Surv Ophthalmol. 1997;42(2):175-189.
[17] Strampelli B. Osteo-odontokeratoprosthesis. Ann Ottalmol Clin Ocul. 1963;89:1039-1044.
[18] Choyce DP. The Choyce 2-piece perforating kerato-prosthesis seventy cases, 1967-1973. Trans Ophthalmol Soc U K. 1973;93:333-342.
[19] Ricci R, Recorella I, Ciardi A, et al. Strampelli ostro-odonto-keratoprosthesis. Clinical and histological long term features of three prostheses. Br J Ophthalmol. 1992;76(4):232-234.
[20] Khan B, Dudenhoefer EJ, Dohlman CH. Keratoprosthesis: an update. Curr Opin Ophthalmol. 2001;12(4):282-287.
[21] Dohlman CH, Schneider HA, Doane MG. Prosthokeratoplasty. Am J Ophthalmol. 1974;77:694-700.
[22] Dohlman CH, Doane MG. Some factors influencing outcome after keratoprosthesis surgery. Cornea. 1994;13: 214-218.
[23] Dohlman CH, Netland PA, Fung WC, et al. Keratoprosthesis update. Forth World Congress on the Cornea Abstracts, The Castroviejo Society. 1996.
[24] Cardona H. Keratoprosthesis; acrylic optical cylinder with supporting intralamellar plate. Am J Ophthalmol. 1962;54: 284-294.
[25] Doane MG, Dohlman CH, Bearse G. Fabrication of a keratoprosthesis. Cornea. 1996;15:179-84.
[26] Aquavella JV, Rao GN, Brown AC, et al. Keratoprosthesis. Ophthalmology. 1982;89:655.
[27] Huang YF, Wang IQ, Wang FX. Clinical application of keratoprosthesis implantation. Shengwu Yixue Gongcheng Zazhi. 2002;19:112-116.
[28] Мороэ ЗИ, Борэеиок СА, Ковшуи ЕВ, et al. Кератпротезу 20 лет. Новое в офталъмологии. 1998;2:23.
[29] Hicks CR, Chirila TV, Clayton AB, et al. Clinical results of implantation of the Chirila keratoprosthesis in rabbits. Br J Ophthalmol. 1998;82(1):18-25.
[30] Crawford GJ, Hicks CR, Lou X, et al. The Chirila keratoprosthesis: phase I human clinical trial. Ophthamology. 2002;109:883-889.
[31] Hicks CR, Chirila TV, Werner L, et al. Deposits in artificial corneas: risk factors and prevention. Clin Experiment Ophthalmol. 2004;32(2):185-191.
[32] Lee JH, Wee WR, Chung ES, et al. Development of a newly designed double-fixed Seoul-type keratoprosthesis. Arch Ophthalmol. 2000;118(12):1673-1678.
[33] Von Fischern T, Langefeld S, Yuan L, et al. The“Aachen” keratoprosthesis: a new approach towards successful keratoprosthesis-surgery. Int J Artif Organs. 1999;22(1): 52-57.
[34] Sipehia R. The enhanced attachment and growth of endothelial cells on anhydrous ammonia gaseous phasma modified surfaces of polystyrene and poly (tetrafluoroethylene). Biomater Artif Cells Artif Organs. 1990; 18(3):437-446.
[35] Latkany R, Tsuk A, Shen MS, et al. Plasma surface modification of artificial corneas for optimal epithelialization. J Biomed Mater Res. 1997;36(1):29-37.
[36] Wu XY, Tsuk A, Leibowitz HM, et al. In vivo comparison of three different porous materials intended for use in a keratoprosthesis. Br J Ophthalmol. 1998;82(5):569-576.
[37] Pettit DK, Hoffman AS, Horbett TA. Correlation between corneal epithelial cell outgrowth and monoclonal antibody binding to the cell binding domain of adsorbed fibronectin. J Biomed Mater Res. 1994;28(6):685-691.
[38] Kobayashi H, Ikada Y. Corneal cell adhesion and proliferation on hydrogel sheets bound with cell-adhesive protein. Curr Eye Res. 1991;10(10):899-908.
[39] Lee SD, Hsiue GH, Chang PC, et al. Plasma-induced grafted polymerization of acrylic acid and subsequent grafting of collagen onto polymer film as biomaterials. Biomaterials. 1996;17(16):1599-1608.
[40] Aucoin L, Griffith CM, Pleizier G, et al. Interactions of corneal epithelial cells and surfaces modified with cell adhesion peptide combinations. J Biomater Sci Polym Ed. 2002;13(4):447-462.
[41] Chuang WY, Young TH, Yao CH, et al. Properyies of the poly(vinyl alcohol) chitosen blend and its effect on the culture of fibroblast in vitro. Biomaterials. 1999;20(16): 1479-1487.
[42] Schermer A, Galvin S, Sun TT. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial cell. J Cell Biol. 1986;103(1):49-62.
[43] Han B, Schwab IR, Madsen TK, et al. A Fibrin-based bioengineered ocular surface with human corneal epithelial stem cells. Cornea. 2002;21:505-510.
[44] Koizumi N, Inatomi T, Quantock AJ, et al. Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. Cornea. 2000;19(1):65-71.
[45] Merrett K, Fagerholm P, McLaughlin CR, et al. Tissue-engineered recombinant human collagen-based corneal substitutes for Implantation: performance of type i versus type iii collagen. J Ophthalmol Vis Sci. 2008;49(9): 3887-3894.
[46] Fagerholm P, Lagali N, Carlsson DJ, et al. Corneal regeneration following implantation of a biomimetic tissue-engineered substitute. J Clin Transl Sci. 2009;2(2): 162-164.
[47] Hackett JM, Ferguson C, Ferguson CR, et al. Optimal neural differentiation and extension of hybrid neuroblastoma cells (NDC) for nerve-target evaluations using a multifactorial approach. Toxicol In Vitro. 2010; 24(2):567-577.
[48] Ma X, Shi H, Huang YF, et al. Biocompatibility of hydroxyapatite surface modified titanium skirt for keratoprosthesis: scanning electron microscopy observation. Zhongguo Zuzhi Gongcheng Yanjiu yu Linchuang Kangfu. 2013;17(51):8875-8880.
[49] Tan DT, Tay AB, Theng JT, et al. Kemtoprosthesis surgery for end-stage corneal blindness in asian eyes. Ophthalmology. 2008;115(3):503-510.
[50] Kim MK, Lee SM, Lee JL, et al. Long-term outcome in ocular intractable surface disease with Seoul-type keratoprosthesis. Cornea. 2007;26:546-551.
[51] Zellander A, Gemeinhart R, Djalilian A, et al. Designing a gas foamed scaffold for keratoprosthesis. Mater Sci Eng C Mater Biol Appl. 2013;33(6):3396-3403.

[52] Du LQ, Chen HM, Yan Y, et al. In vivo biological stability of chemically pretreated silicone gel inserts intended for use in keratoprostheses. Chin Med J (Engl). 2012;125(23): 4239-4244.
[53] Laattala K, Huhtinen R, Puska M, et al. Bioactive composite for keratoprosthesis skirt. J Mech Behav Biomed Mater. 2011; 4(8):1700-1708.
[54] Santos L, Ferraz MP, Shirosaki Y, et al. Degradation studies and biological behavior on an artificial cornea material. Invest Ophthalmol Vis Sci. 2011;52(7):4274-4281.
[55] Kim MK, Lee JL, Wee WR, et al. Comparative experiments for in vivo fibroplasia and biological stability of four porous polymers intended for use in the Seoul-type keratoprosthesis. Br J Ophthalmol. 2002;86(7):809-814.

组织工程化人工角膜的临床应用：距离还有多远？

陈佳佳，王文战，朱　豫(郑州大学第一附属医院眼科，河南省郑州市　450052)

陈佳佳，女，1982 年生，河南省新乡市人，汉族，2008 年南京大学毕业，硕士，主治医师。
通讯作者：陈佳佳，郑州大学第一附属医院眼科，河南省郑州市　450052

文章亮点：

1 此问题的已知信息：以往研究介绍了多种人工角膜材料的设计及应用利弊。

2 文章增加的新信息：人工角膜的研制在近年来有了较大的进步，非组织工程化人工角膜在材料选择，处理和设计方式上有所创新，医生可在临床中逐步改良人工角膜材料性质，增加材料与组织相容性。具有活性的组织工程化人工角膜的出现为人工角膜的研制开辟了一条新途径。文章结果为选择最佳的人工角膜提供多种思路。

3 临床应用的意义：重建角膜的关键是给细胞提供一些细胞黏附和生长的体内因子。组织工程化角膜基质既可以通过营养活细胞为移植做准备，同时其无菌无细胞毒性的性质也使细胞的生长，毗邻组织神经的延伸成为可能。最近构建接近人角膜组织结构和生理功能的角膜替代物之出现，将可能缓解全球角膜供体短缺的现状，从而给因角膜病而致盲的患者带来了重见光明的希望。

关键词：
组织构建；组织工程；人工角膜；角膜移植；生物相容性
主题词：
人工角膜；角膜移植；生物相容性；组织工程

摘要

背景：人工角膜是取代混浊角膜组织而用异质成形材料制成的一种特殊屈光装置，通过手术植入患眼，以取得一定视力。

目的：介绍近年来人工角膜研究进展。

方法：由第一作者用计算机检索万方医学网和 Medline database 数据库，检索词分别为“人工角膜、角膜移植、生物相容性、组织工程”和“keratoprosthesis, corneal transplantation, biocompatibility, tissue engineering”，对人工角膜移植的动物实验、临床进展、植入方式及生物相容性、并发症等方面进行探讨。

结果与结论：共检索到 204 篇文章，按纳入和排除标准对文献进行筛选，共纳入 50 篇文章。人工角膜的研制在近年来有了较大的进步，非组织工程化人工角膜在材料选择，处理和设计方式上有所创新，同时具有活性的组织工程化人工角膜的出现为人工角膜的研制开辟了一条新途径。

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利益冲突：文章及内容不涉及相关利益冲突。

伦理要求：没有与相关伦理道德冲突的内容。

学术术语：人工角膜-是用医用高分子材料制成的类似人体角膜的产品，人工角膜一般包括光学镜柱和周边支架两部分。光学镜柱是用光学特性优良、物理化学性质稳定的透明材料制成，用以替代病变后阻碍眼球光学通路的浑浊角膜；周边支架相当于连接光学镜柱和周边组织的桥梁，故而要求具有良好的组织相容性。

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