·Basic Research·

Inhibitory effect of three biopolymer materials on scar formation following trabeculectomy

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Abstract

• AIM: To investigate the inhibitory effects of amniotic membrane, polylactic acid membrane and chitosan membrane on scar formation following trabeculectomy.

• METHODS: A total of 24 New Zealand white rabbits (48 eyes) were randomly divided into 4 groups: amniotic membrane group, polylactic acid membrane group, chitosan membrane group, and control group, with 6 rabbits (12 eyes) in each group. The left eyes underwent routine trabeculectomy, and the right eyes were considered as controls. Amniotic membrane, polylactic acid membrane and chitosan membrane were respectively installed under sclera flap in three groups, but any treatment was not applied in control group. Intraocular pressure, conjunctival filtering bleb, and anterior chamber inflammation responses were monitored at day 1, 3, 7, 14, 28 and 56 post-operatively. Eyeball tissue underwent histopathological examination at day 56 post-operatively.

• RESULTS: Fibrocytes and inflammatory cells were reduced in amniotic membrane, polylactic acid membrane and chitosan membrane groups compared to that in control group. At day 1 post-operatively, intraocular pressure was decreased in three membrane groups compared to that in control group. At day 14 post-operatively, the intraocular pressure was decreased significantly, while it of three membrane groups was significantly lower than that of preoperative ($P<0.01$). There were no significant differences among three membrane groups ($P>0.05$). Filtering bleb of four groups was clearly observed at day 7 post-operatively, but there was no significant difference in pair-wise comparison. At day 28 and 56 post-operatively, filtering bleb in control group was significantly narrowed compared to that in three membrane groups ($P<0.05$), but there was no significant difference in pair-wise comparison of three membrane groups.

• CONCLUSION: All amniotic membrane, polylactic acid membrane and chitosan membrane can effectively inhibit scar formation following trabeculectomy, the effect of amniotic membrane is the best.

• KEYWORDS: amniotic membrane; polylactic acid membrane; chitosan membrane; glaucoma; filtration surgery
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INTRODUCTION

Glaucoma filtering bleb scarring after surgery is the main reason for surgical failure. Scarring of the filtering bleb is associated with cellular proliferation, particularly fibroblast pro-liferation; Douglas and other eye surgery in the rabbit model of poly phospholipids by subconjunctival drug delivery system, the quantitative use of 5-fluorouracil and soaked in conventional topical 5-fluorouracil compared with a sponge, and reduced the antihypertensive effect of the considerable side effects of antimetabolites. At present, although inhibition of scar formation after glaucoma filtering surgery and administration of many drugs, but more is not ideal. Current clinical use more intraoperative antimetabolites combined with mitomycin to inhibit the proliferation of fibroblasts, to achieve the anti-adhesion effect. However, the application of anti-metabolism drugs, such as MMC increases the risk of wound leakage, ocular hypotony, hypotonous maculopathy, blebitis, and bleb-related endophthalmitis[1]. To maintain the filtering bleb without or with fewer complications caused by

conventional anti-fibrotic agents, an antifi brotic that is harmless to the eyes is desirable. Therefore, the search for efficiency, low toxicity anti-scar material is to improve the success rate of research. The development of polymer science, the research and application of polymer mateial, have provided a broader area for biomedical field, which amniotic membrane, polylactic acid membrane and chitosan membrane have become the representatives. In this study, we applied bio-medical experiments amniotic membrane, polylactic acid membrane and chitosan membrane to inhibit the scar formation in experimental filtration surgery, and hope to find an efficient and low toxicity method for inhibiting scar formation.

MATERIALS AND METHODS

Materials All experiments were carried out OD under a surgical microscope and general anesthesia. Rabbits weighing 2.5-3.5kg were anesthetized by intramuscular injection of ketamine hydrochloride (10mg/kg) and xylazine (20mg/kg). Topical anesthesia (4g/L oxybuprocaine drops) was applied to the eyes. All rabbits were treated in accordance with the ARVO Statement on the Use of the Animal Care and Use Committee of the China Medical University Animal Experiment Center. Twenty-four male rabbits underwent trabeculectomy and were randomly divided into 4 groups: amniotic membrane group, polylactic acid membrane group, chitosan membrane group and control group (6 rabbits/group, 12 eyes/group). A fornix-based conjunctival incision was made and a 4mm×4mm scleral fiap was created, a 1mm×3mm trabeculectomy was performed at the scleral spur, followed by iridectomy. A 5mm ×6mm biopolymer material was placed on the scleral fiap. The scleral fiap and conjunctival wound were sutured with 10-0 nylon. Dexamethasone 2mg was subconjunctival injected and eyes were packaged with eye pad.

Methods Eight weeks later, after injected 30mL air into the vein of rabbits to death, we incised the skin along orbital margin, separated along orbital wall, retaining part of eyelids and intact conjunctiva, cut the eye muscles and optic nerve, removed the eyeball. After rinsed with saline, the eye tissues were fixed into 40g/L paraformaldehyde to product samples. The samples were made of the continuity of biopsy sections, stained by hematoxylin-eosin (HE). The situation of subconjunctival bleb and its around was observed at low magnification and the situation of scleral flap tissue neutrophils, fibroblasts and lymphocytes was observed at high magnification. Intraocular pressure (IOP) of both eyes was measured with a Tono-Pen (Medtronic, Jacksonville, FL, USA) before surgery and on days 1, 3, 5, and 7, and then each week until 4 weeks after surgery. Topical anesthesia (0.4% oxybupro-caine drops) was applied to the eyes before IOP measurements. Three measurements were taken from each eye and averaged. The rabbits were killed with an overdose of pen-tobarbital, and the eyes were enucleated for histologic evaluation 4 weeks after surgery. All enucleated eyes were fixed into 2% paraformaldehyde and 2.5% glutaraldehyde solution, dehydrated with serial alcohol solutions, and embedded in paraffin. Serial 4-μm-thick sections were stained by HE and examined under light microscopy. High degree of bleb divided into 0 to 4 points, the range of sizes from bleb 0 to 12 points, according to the size range of limbal division, the two together, such as less than 2 minutes non-functional bleb, more than 2 points for the functional filtering bleb. Postoperative tobradex eye drops were given 4 times per day, tobradex cream once a day, for one week. The conjunctival wound healing, conjunctival hyperemia, filtering bleb, intraocular pressure, corneal transparency, anterior chamber reaction and crystal transparency were observed daily.

Statistical Analysis Data are expressed as the mean ± standard deviation (SD), application of the United States by the first of the SPSS 12.0 statistical software for statistical analysis of each set of results. The four groups were compared by OneWay-ANOVA analysis, application of multiple comparison tests between groups (Tuky HSD; LDS method). An unpaired t test was used to compare the adhesive force between the biopolymer material and non-biopolymer material groups. The mean IOP curves between operative and nonoperative fellow eyes within the biopolymer material trabeculectomy and the non-biopolymer material trabeculectomy groups, and the mean postoperative IOP between the biopolymer material trabeculectomy and non- biopolymer material trabeculectomy groups. A paired t test was used to compare preoperative and postoperative IOP within the biopolymer material trabeculectomy and the non-biopolymer material trabeculectomy groups. $P<0.05$ was considered statistically significant.

RESULTS

Eyes Healing The mild corneal haze appeared after surgery in each group, disappeared at postoperative a week. There was no different between groups in pair-wise comparison. The harmful complications such as anterior chamber shallow or disappear were occurred in each postoperative group. There was no difference between groups in binary comparison. There were 6 eyes of 24 operative ones occurred different degrees of anterior chamber hemorrhage. However, the hemorrhage was absorbed within 3 days after surgery. There was no difference between groups in binary comparison. Comparing the four groups, filtering bleb on 1 week of postoperative had not showed the difference ($P>0.05$). However, filtering bleb in control group was

Table 1 Post-operative evaluation of filtering bleb (mean±SD, score)

Groups	1d	3d	7d	14d	28d	56d
Control	3.5±0.43	4.6±1.0	4.1±0.4	3.4±0.4	1.9±0.3	1.5±0.6
Amniotic membrane	2.9±1.7[a]	5.7±0.9[a]	5.0±0.6[a]	4.5±0.5[a]	3.2±0.5[a]	3.1±0.4[a]
Polylactic acid membrane	2.5±1.0	4.8±0.4	5.2±0.5	3.7±0.5	2.27±0.4	2.1±0.4
Chitosan membrane	3.2±0.8	4.9±0.9	5.2±0.4	3.7±0.4	2.3±0.5	2.1±0.4

[a]P<0.05 *vs* polylactic acid membrane and chitosan membrane

Table 2 Intraocular pressure pre- and post-operation (mean±SD, mmHg)

Groups	Preoperative	1d	3d	7d	14d	28d	56d
Control	2.31±0.09	3.11±0.18	1.38±0.22	1.55±0.27[d]	2.40±0.40[d]	2.92±0.47	2.94±0.52
Amniotic membrane	2.23±0.06	3.03±0.22	0.96±0.31[a,d]	1.11±0.28[a,d]	1.65±0.40[b,c]	1.78±0.53[b,c]	1.55±0.28[b,c]
Polylactic acid membrane	2.23±0.06	2.96±0.34	1.09±0.28[a,d]	1.97±0.42[a,c]	1.65±0.47[a,c]	1.79±0.42[b,c]	1.50±0.55[b,c]
Chitosan membrane	2.23±0.06	2.97±0.19	1.06±0.32[a,d]	1.53±0.43[a,c]	1.94±0.42[a,c]	2.08±0.44[b,c]	1.97±0.31[b,c]

[a]P<0.05, [b]P<0.01 *vs* control group; [c]P< 0.05, [d]P<0.01 *vs* pre-operation

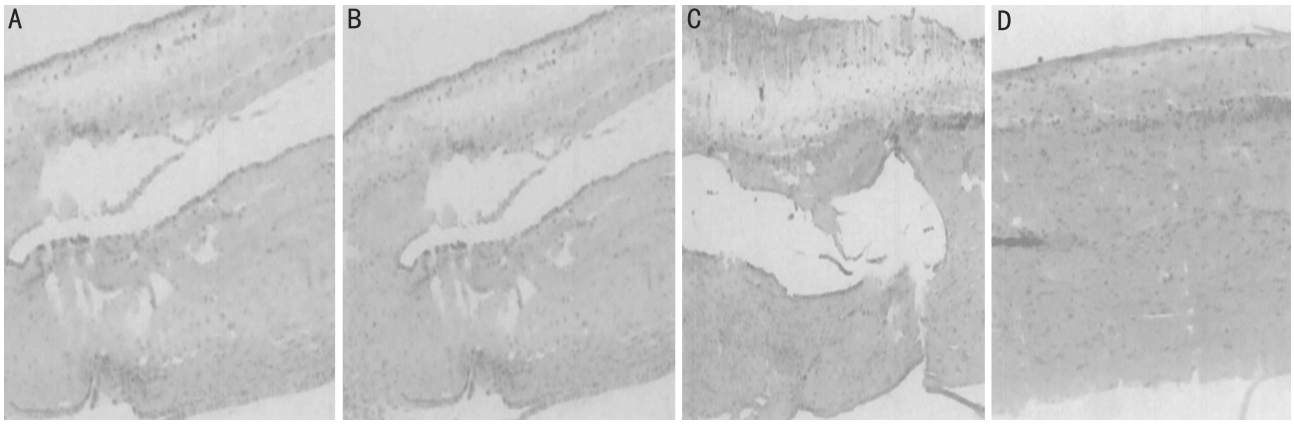

Figure 1 Corneal histopathological changes (HE×40) A: Control; B: Amniotic membrane; C: Polylactic acid; D: Chitosan

progressive narrowing and narrowed obviously. After two weeks, there were significant differences among control group and experimental groups (P<0.05). Four weeks of postoperative, the control group's filtering bleb changed into nonfunctional, and eight weeks of postoperative, experimental groups still had functional filter. At each observation time points after operation, there was significant difference among experimental groups (P<0.05), and there was no difference between polylactic acid membrane and chitosan membrane group. (P>0.05, Table 1).

Intraocular pressure The average preoperative intraocular pressure was 2.50kPa. After a week, the four groups' IOP decreased significantly compared with the preoperative ones, with a significantly worse different (P<0.01). After two weeks, markedly elevated IOP appeared in control group and the bright show higher than that in experimental groups (Table 2).

Corneal pathological changes In control group, lymphocytes, fibroblasts, multinucleated cells were more common, filtration Road healing occlusion, conjunctiva and sclera between the union and the emergence had more inflammatory cells (Figure 1A). In amniotic membrane group, visible fibroblast proliferation and collagen fibers were less than those in the first two experimental groups. Lymphocytes 1 to 2/H, two scleral flap separation and filtration Road gap could be seen, organized under the loose conjunctiva (Figure 1B). In polylactic acid group and the chitosan group, a little fiber cell proliferation could be seen. A small amount of collagen fibers, few lymphocytes, two scleral flap separation and filtration Road gap was visible, organized under the loose conjunctiva (Figures 1C, 1D).

DISCUSSION

Polylactide and its derivatives in recent years have been a big progress in the field of medical materials, because its inside body hydrolysis defatted generating lactic acid monomer, and in lactate dehydrogenase under the action of oxidation as pyruvate, as energy material attend inside the Krebs cycle, final degradation products for water and carbon dioxide carbon, the lung and kidney, skin eduction body. The body that does not contain accumulation peptides, carp, and immunogenicity, have good biocompatibility. Machine body eyewinker rejection tiny, on the surrounding neighboring tissues and systemic no obvious poison reaction and stimulation[2]. Synthetic polymer biodegradable materials together for the first time with glycolic acid biological catabolic seam listed, for the basis of medicine research investigate and clinical research provides a broad prospect[3]. Apel *et al* [4] with polycarboxylate lactose acid as a carrier which join ring cell element diameter of 5mm, made a thick 0.15mm membranous implant copolymer, in areas not seen

localized tissue inflammation, organization necrosis and fiber hyperplasia etc pathological changes, implants once can maintain effective drugs concentration 60d. Yasukawa *et al* [5] made with adriamycin polylactic acid microspheres, resection immediately after the rabbit of conjunctival filtering bleb parts infuse, found the experimental intraocular pressure obviously lower than those of the control group, and filtration time than the control group was significantly longer, and no posterior cornea and other section of complications. Berger *et al* [6] containing 5-fluorouracil and dapsone membranous implant monkey eyes subconjunctival, proof postoperative 3 months maintain intraocular pressure lower level, and does not affect wound healing. Biological amniotic membrane is by freeze-drying process and ^{60}Co sterilization technology system prepared of biological material, has the histology, immunology and physiological characteristics, manner body the thickened basement membrane, the cortex, basement membrane layer, the stromal layer, fiber mother layer of cells, sponge layer. It contains a variety of collagen fiber, protein, and layer adhesion secretion multiple factor etc. Current creatures amniotic membrane transplantation has been applied to glaucoma filtration in the operation, the trabecular resection combined amniotic membrane transplantation with possible reasons for the successful application the following factors: the clinical application of confirmed, amniotic membrane transplantation matrix can adjust fibroblast growth factor expression transformation of level, also can inhibit the epithelium sources of stimulating muscles fibroblasts differentiation of signal thus plays restrain fiber Dimension of role [7]. Aimed at young patients' fiber proliferation obvious characteristic, should with amniotic membrane can restrain the fibre hyperplasia, maintain the functional filtering bleb the formation; 2 to 1 amniotic interleukin alpha and beta 1 before factors such as inflammation express have inhibition [8] and at the same time can secrete a variety of factors, directly or in between pick in the role of eye, reduced the sclera after transplantation, and conjunctival flap of inflammatory reverse should be; implanted in sclera amniotic membrane formed continuity of collagen thin pad slice, therefore it is probably inhibit sclera mechanical barrier under fibrosis [9].

Chitosan, also called for chitosan, a derivative of chitin off acetylation, its exist widely in insects, animals and fungi carapace outline in their cell is nature yield after cellulose natural polysaccharides in the body, it is biodegradable synthesis and biodegradability, excellent biocompatibility, non-toxic, degradation processes produce low molecular sugar, *in vivo* without accumulation, almost no immunogenicity. Due to its structure, and have already in hydroxyl lively amino, easy to chemical change sex, can be introduced multi-functional group and widening the application fields [10]. LeGrand [11] and Sun *et al* [12] research found that chitosan and its derivatives have hemostatic, acetanilide, inhibit microbes growth to help wound healing role, and its to mesangial fine sphrulocyte, fibroblasts, *etc* to adjust action may be the direct action mechanism.

Therefore, the three kinds of biological materials can all effectively inhibit the scar formation after glaucoma filtration operation, and at present biological amniotic membrane transplantation has been widely used in clinical, and has achieved satisfactory curative effect.

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