Transactions of the Materials Research Society of Japan 25 [3] 795-798 (2000)

Hydrogel Vitreous Substitute Containing Hyaluronic Acid

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Poly(vinyl alcohol) hydrogel (PVA hydrogel) cross-linked by gamma irradiation containing hyaluronic acid (HA) was assessed as a possible vitreous substitute. Two types of PVA hydrogel containing HA were prepared by a new refined method. Vitreous changes to PVA hydrogel were performed after vitrectomy and were followed clinically by ophthalmoscopy, tonometry, fundus photography and flare cell mater. Histopathological examination by light microscopy was performed 3 month after operation. PVA hydrogel containing HA exerted good biocompatibility with ciliary body, retina and original vitreous body. PVA hydrogel containing HA is a good candidate for vitreous substitute clinically.
Key Words: vitreous substitute, vitreous body, hydrogel, hyaluronic acid

1.INTRODUCTION

Recent advancement of vitrectomy has enabled us to treat incurable diabetic retinopathy, proliferative vitreoretinopathy and giant retinal breaks. For intraocular tamponade, several materials, including air, SF_6, C_3F_8, silicon oil and perfluorocarbon have been used during vitrectomy. But no ideal one has been found. We have sought for the ideal vitreous substitute that is optically clears, chemically stable and cause little inflammatory response years after surgery. Our previous investigations[1,2,3]suggest that the PVA hydrogel made of γ-irradiated polyvinyl alcohol solution, is a good candidate. Moreover, they reported hydrogels such as Poly(vinylpyrrolidone) (PVP), Poly(acrylamide), Poly (glyceryl-methacrylate) (PGMA), Poly(2-hydroxy-ethylacrylate) (PHEA) and Poly(methyl-2-acrylamido-2-methoxyacetate) for vitreous substitute. Still their biocompatibility is not so good as PVA hydrogel[4]. In vitro vitreous body is a complex gel of collagen fiber and HA. It is believed that 3-dimensional collagen fiber make gel network that contain HA. In this paper, PVA gel network is corrrespond to collagen fiber network. This biomimic structure of in vitro vitreous body improves water content and increase tamponade effect of gel network. The presenting paper reports biocompatibility of our improved PVA hydrogels containing hyaluronic acid that structure is similar to the original vitreous body[5].

2. EXPERIMENTAL

2.1 Materials and Sample preparation: PVA with polymerization grade 2000 (Kurare Poval120®) was prepared. PVA was washed in purified water, then was mixed with it, resulting in a concentration of 7%. The PVA was dissolved in an autoclave (120 C°, 50minutes). Then, dissolved HA (Touyou-zyouzou Co., M.W. 3000000) (conc. 1% and 0.2%) and PVA solution is mixed equal volume sufficiently. After centrifuged at 8000 rpm for one hour, the superficial layer was irradiated by γ-ray to make cross-linking (0.15 Mrad). The gel was diluted with same volume saline, and centrifuged at 12000 rpm for one hour. This procedure was repeated twice. The mixed gel was packed with nitrogen in sterile ampoules (Steri Ampule®10 cc Mita Rika Kogyo). They were sterilized in the autoclave (135C°, 20minutes) two days before operation. The final concentration of the gel was 3.5% in volume, which was the same concentration as that in the previous reports[1,2]. Improved points in the method were as follows.

1) Sterile, super-purified water was used to prepare the gel.
2) Clean appliances that is medical grade is used and sterile manipulation was performed in every synthetic process.
3) Repeated configuration of the gel was performed to remove unreacted monomers and contamination in the gel network.

This refined method has improved biocompatibility of the gel (see results). Figure 1 shows the three samples used in this experiment.

Table I. Sample Specification

No.	Sample Name	
1	3.5 % PVA gel	PVA (polymerization grade 200) Kurare Poval 120®
2	PVA/1% HA	
3	PVA/ 0.2% HA	3.5 % PVA gel + 0.2 % HA

2.2 Measurements of swelling ratio: As shown in Figure 2, a certain amount of gel is swelled to an equilibrium state. Then measured its weight ($W_{swollen}$). Next, the gel was dried up and dry weight was measured (W_{dried}). Swelling ratio is defined as follows. Swelling ratio = ($W_{swollen}$) / (W_{dried}). Added 1N hydrochloric acid to decompose HA, then swelling ratio is measured.

2.3 In vivo experiments: Twenty eyes of ten males colored rabbits (2.5-3.0kg) were used. Two port vitrectomy were performed under general anesthesia with intravenous injection of pentobarbital sodium (Nembutal®, Abbot company) (20mg/kg) in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. They were followed clinically by ophthalmoscopy, tonometry, fundus photography and flare cell mater. Further details of these in vivo experiments are reported in reference 1 and 2. At 3 months after operation, enucleation of the eye was performed. An overdose of Nembutal® was injected intravenously for the enucleation. Immediately after enucleation,the eyes were fixed in a Karnovski solution. After fixation, the eyes were bisected. A half was embedded in Epon, stained with uranyl acetate and lead citrate, and processed for electron microscopic evaluation (Data is not shown here). The other half was embedded in palaffin, stained with Hematoxilin & Eosin (H&E), PAS, and Azan and processed for light microscopic study.

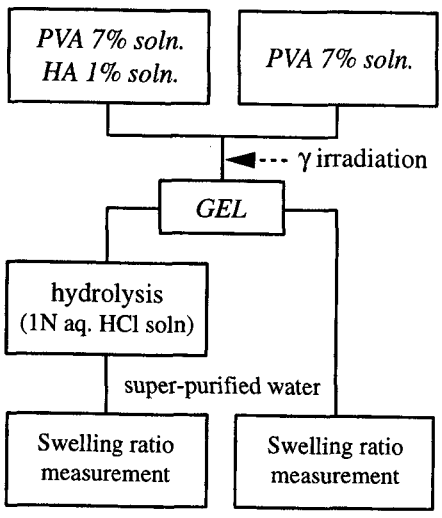

Figure 2. Sample preparation and experimental procedure.

3. RESULTS AND DISCUSSION

3.1 Swelling ratio: Figure 3 shows the swelling ratio of each sample gels. Swelling ratio of PVAgel containing HA increased compared to PVAgel only. For example, at 0.9Mrad irradiation, swelling ratio of PVAgel is about 42. But swelling ratio of PVAgel containing HA increased about 300. After hydrolysis. swelling ratio of PVA gel containing HA approaches to PVAgel only. From those hydrolysis data, these hypotheses are estimated. HA is enclosed in the PVAgel network. And HA is not destroyed by gamma rays. Probably, HA is destroyed in the vitreous cavity in a short time. But, PVAgel networks remain.

Table II. Swelling Experiment

Irradiation dose (Mrad)		0.90	2.25	3.75	6.00
Gels	No.				
PVA + 1% HA	1	321	56.5	32.4	18.2
	2	277	59.5	37.9	23.6
	3		60.2	31.6	18.2
Hydrolysis	1	56			
	2	50			
PVA	1	43	12.5	9.9	7.0
	2	42	12.7	9.6	7.0

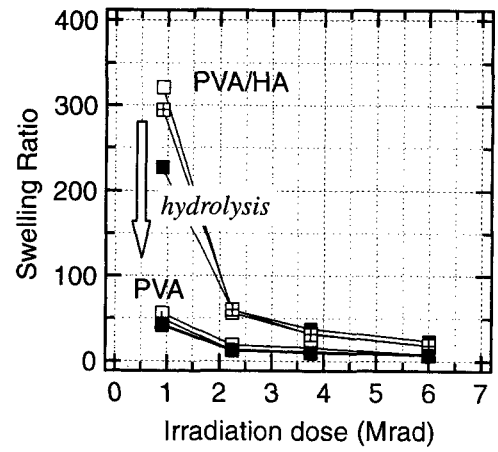

Figure 3. Irradiation dose dependence of swelling ratio of PVA hydrogel containing HA and hydrolyzed gel.

3.2 Intraocular Pressure (IOL): A pneumatic tonometer (Alcon) was used. The central value of a measurement of 5 seconds was recorded. Intraocular Pressure(IOP) mesured by manometer is 9-21mmHg in normal eye. When inflammation occur in the eye or the substance injected into the eye expand, IOP goes up. Therefore IOP is a good parameter that represent the course after the operation. Figure 4 indicate IOL changes after operation. In PVA/1%HA at 14 days after operation, IOP rise can be regarded as significant(t-test, 5%) . But there are no significant differences in 21days

after operation.

3.3 Flare cell mater: After dilation of the pupil with Midriatic agent, A laser-flare cell meter (FC5000,Kowa) was used to evaluate the anterior chamber inflammation. The photon of the multiplier was recorded. The average of a five measurements was recorded. Flare cell mater is a rapid non-destructive method to measure the concentration of protein in the anterior chamber. The principle of this method is based on the laser light scattering. This is used widely clinically. And photon counting of PMT reveals the damage of blood retinal barrier of the eye. So this numerical value is one of the indication of biocompatibility to the retina when something is injected into the vitreous cavity. Results is shown in figure 5. In PVA/1%HA's case, damage of blood-aqueous barrier is relatively large. In PVA/0.2%HA's case, damage of blood-aqueous barrier is smaller than that of PVA gel only. Because PVA/1%HAgel has a poor handing during the operation due to its non-homogeneity, damage during operation may be relatively large.

3.4 Histological findings: 3 month after the operation, there in no abnormal changein ciliary body (Figure 6) and retina (Figure 7). Also, surface between PVA gel network and original vitreous body do not arise no significant findings (Figure 8). At 3 months after surgery, an enucleation of the eye was performed. An overdose of anesthetic agent was injected intravenously for the enucleation. Immediately after enucleation,, the eyes were fixed in a Karnovski solution. After fixation,the eyes were bisected. The half was embedded in paraffin, stained with Hematoxilin & eosin(H&E), PAS,and Azan, and processed for light microscopic study. Because, at that time, ocular tissue become stable condition. If some undesirable reaction will arise, it will be at ciliary body, retina or/and surface between PVAgel network and original vitreous gel. Therefore, we examine those tissues.

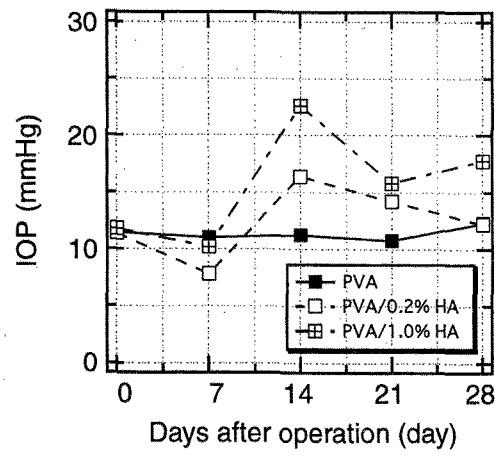

Figure 4. Chnges of IOP after operation.

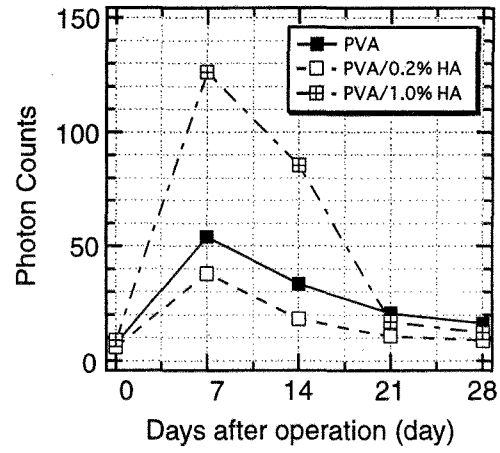

Figure 5. Flare cell mater photon counts after operation.

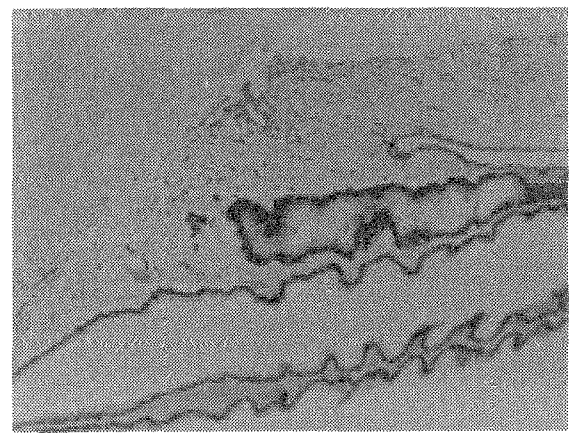

Figure 6. Ciliary body. H.E .stain x200

Figure 7. Retina. H.E .stain x200

4. CONCLUSION

Flare cell mater and histologial findings tells that biocompatibility of the PVA/0.2%HAgel in the vitreous cavity is better than that of PVA gel. Also,

PVA/0.2%HAgel has a good handing during the operation. Considered in vivo structure of vitreous body, it is made up of collagen and HA[5]. In our experiments, PVAgel networks correspond to collagen. This biomimic structure is useful for retain water and keep tamponade effect. This compound gel is a good candidate for vitreous substitute in clinically.

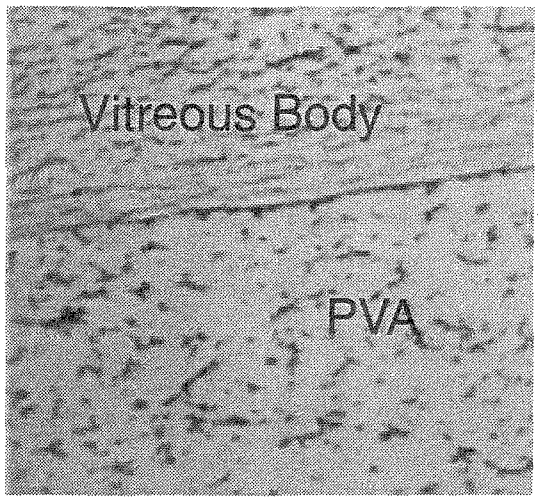

Figure 8. PVA gel and vitreous body. Orange G and Aniline blue double staining (for PVA staining) x400.

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(Received December 17, 1999; Accepted March 31, 2000)