a decrease in the area of capillary nonperfusion of >1.0 DA 1 month after the IVB (Patients 3, 13, 26, and 47, blue lines, Figure 2). However, only 1 eye showed an increase in the area of capillary nonperfusion of >1.0 DA 1 month after the IVB (Patient 23, red line, Figure 2).

Fundus photographs and FAs before and 1 month after IVB for 2 eyes are shown in Figures 3 and 4. The results from 1 eye of a 61-year-old man (Patient 47) in whom the area of capillary nonperfusion decreased from 12.49 DA to 9.52 DA 1 month after the IVB are shown in Figure 3. The best-corrected visual acuity before the IVB was 0.5 logMAR and improved to 0.7 logMAR 1 month after the IVB. In contrast, the results of 1 eye of a 62-year-old woman (Patient 23) in whom multiple soft exudates appeared in the fundus photograph and the area of capillary nonperfusion increased from 3.01 DA to 18.00 DA 1 month after the IVB are shown in Figure 4. The best-corrected visual acuity before the IVB was 0.8 logMAR and improved to 1.0 logMAR 1 month after the IVB.

Discussion

Our results demonstrated that the incidence of an increase in the area of capillary nonperfusion of >1.0 DA 1 month after the IVB therapy was very low in only 1 of 58 eyes (1.7%) in patients with macular edema secondary to BRVO. In addition, the difference in the area of nonperfusion before and 1 month after the IVB for 21 eyes that had nonperfusion before the
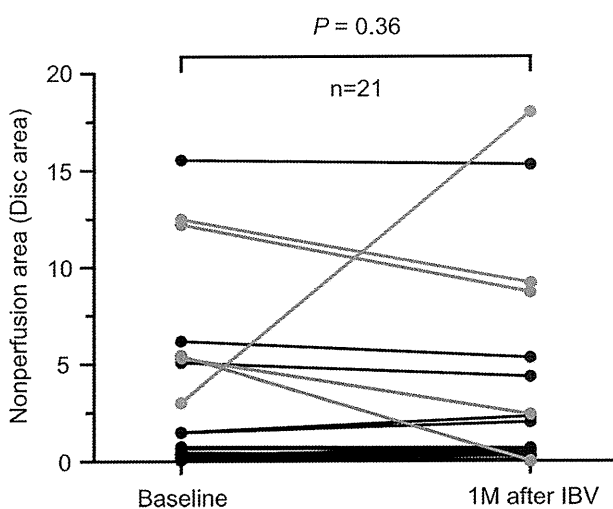

Fig. 2. Scatter plot for the area of capillary nonperfusion before and 1 month after IVB in patients with BRVO. Results from 21 patients who had capillary nonperfusion before the IVB are shown. There was no significant difference before and after the IVB in the area of nonperfusion (P = 0.36). Red line shows 1 eye whose nonperfusion area increased >1.0 DA after the IVB. Blue lines show 4 eyes whose nonperfusion area decreased >1.0 DA after the IVB.
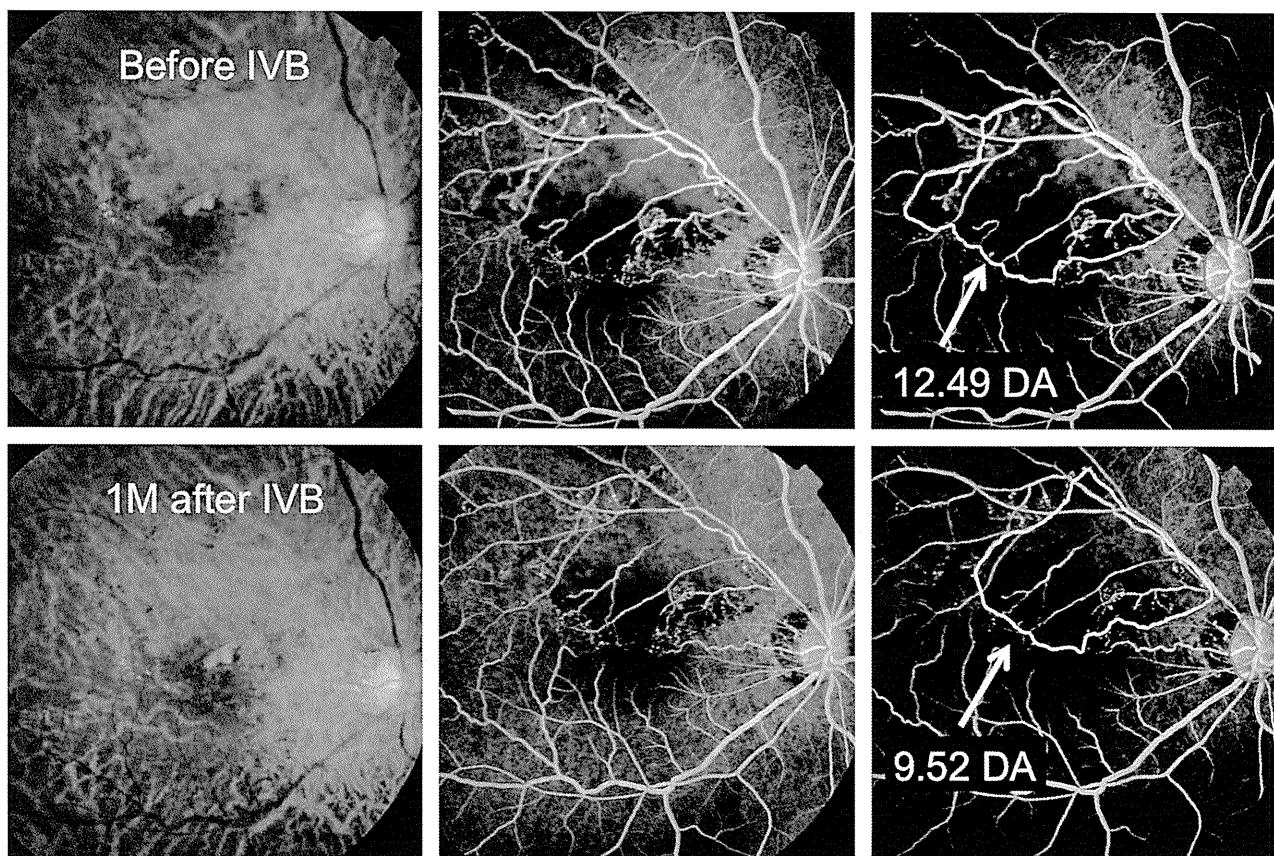
treatment was not statistically significant (P = 0.36). These results suggest that a single IVB injection does not worsen the retinal ischemia in eyes with BRVO.

Only 1 eye (Patient 23) showed a significant increase in the area of nonperfusion, namely, from 3.01 DA to 18.0 DA. This eye also developed multiple cotton wool spots (Figure 4), indicating a progression of retinal ischemia during the 1 month after the IVB therapy. However, it is uncertain whether these ischemic changes were truly related to the IVB. The patient was a 62-year-old woman with hypertension who had received the IVB very early after the onset of symptoms (2 weeks). It is known that the eyes with the nonischemic type of retinal vein occlusion often convert to ischemic type within 6 months after the onset.[42,43]

Three articles have been recently published that support our results.[29–31] Prager et al[29] followed 29 eyes with retinal vein occlusion after the IVB therapy, and while they did not measure the nonperfusion area quantitatively, they stated that there was no progression of the nonperfusion area in the FA images for all eyes. Kook et al[30] measured the degree of macular ischemia, that is, the diameter of the foveal avascular zone, before and after IVB therapy in 126 patients with chronic diabetic macular edema and reported that there was no significant change in the degree of macular ischemia. In addition, Neubauer et al[31] performed semiquantitative measurements of the nonperfused retina using an ultra wide-field scanning laser ophthalmoscope before and 1 month after IVB in 19 eyes with diabetic retinopathy. They found that the mean area of the peripheral ischemia decreased significantly 1 month after the IVB therapy. They suggested that IVB therapy may improve the peripheral retinal ischemia in the short term.

Contrary to these reports, other authors suggested that the IVB therapy may worsen retinal ischemia. Papadopoulou et al[32] reported that the diameter of the retinal arterioles significantly decreased after an intravitreal injection of ranibizumab (Lucentis, Novartis Pharma, Basel, Switzerland), a high-affinity, humanized, recombinant, antigen-binding fragment in patients with age-related macular degeneration. They suggested that intravitreal anti-VEGF agents may cause retinal circulatory disturbances. Although they reported a decrease in the vessel diameter after the injection of anti-VEGF agents, they did not show any progression of retinal ischemia associated with vasoconstriction by ranibizumab.

Ameri et al[33] evaluated the effects of IVB in a rabbit retinal neovascularization model produced by an intravitreal injection of VEGF. They observed the development of severe capillary nonperfusion in 4 of 5 rabbits in which the bevacizumab was injected 1 week

Fig. 3. Fundus photographs and FAs before and 1 month after the IVB in a patient with BRVO (Patient 47, 61-year-old man). The results of the measure of capillary nonperfusion are shown in the right column. The area of nonperfusion decreased from 12.49 DA to 9.52 DA after the IVB.

after the VEGF injection. Based on these results, they suggested that a sudden drop in the VEGF concentration may be responsible for the closure of the normal capillaries. The incidence of severe progression of capillary nonperfusion after the IVB was higher in their animals (80%) than that of our study (1.7%). This difference may be because of the difference in the species and/or degree of retinal ischemic condition before the IVB. When compared with the human BRVO retina, the retina of rabbit neovascularization model shows more severe ischemic changes in which the neovascular membrane develops within 1 week.

There have been recent case reports that showed rapid ischemic changes,[34,35] multiple retinal hemorrhages,[36] and retinal artery occlusion[37] after the IVB therapy. Kim et al[34] reported a case of nonischemic central retinal vein occlusion, which transformed to severe ischemic central retinal vein occlusion 3 weeks after IVB. However, it is questionable whether these rapid ischemic changes are related to the IVB because such changes can occur even during the natural course of retinal vein occlusion. Hayreh et al[42] reported that the incidence of conversion

of nonischemic to ischemic central retinal vein occlusion within 6 months was 13.2% in patients aged ≥65 years.

There are four limitations in this study. The first limitation was the lack of control subjects. Without the control subjects, we cannot conclude whether the incidence of severe ischemic changes seen after IVB (1.7%) was really because of the blockage of VEGF or spontaneous changes during the natural course of BRVO. The second limitation was the difficulty in identifying the exact area of capillary nonperfusion for some patients with BRVO. We did not set any observational period before the IVB therapy and started the IVB at a relatively early stage, a mean period from the onset to the injection of 11.1 weeks. At this early stage of BRVO, the extensive blockage because of the retinal hemorrhage often makes the exact measurement of the nonperfused retinal area difficult. The third limitation was that we could not measure the area of peripheral nonperfused retina exactly using our standard fundus camera system. The recently developed ultra wide-field scanning laser ophthalmoscopy combined with a digital area measurement program may be useful in measuring the area
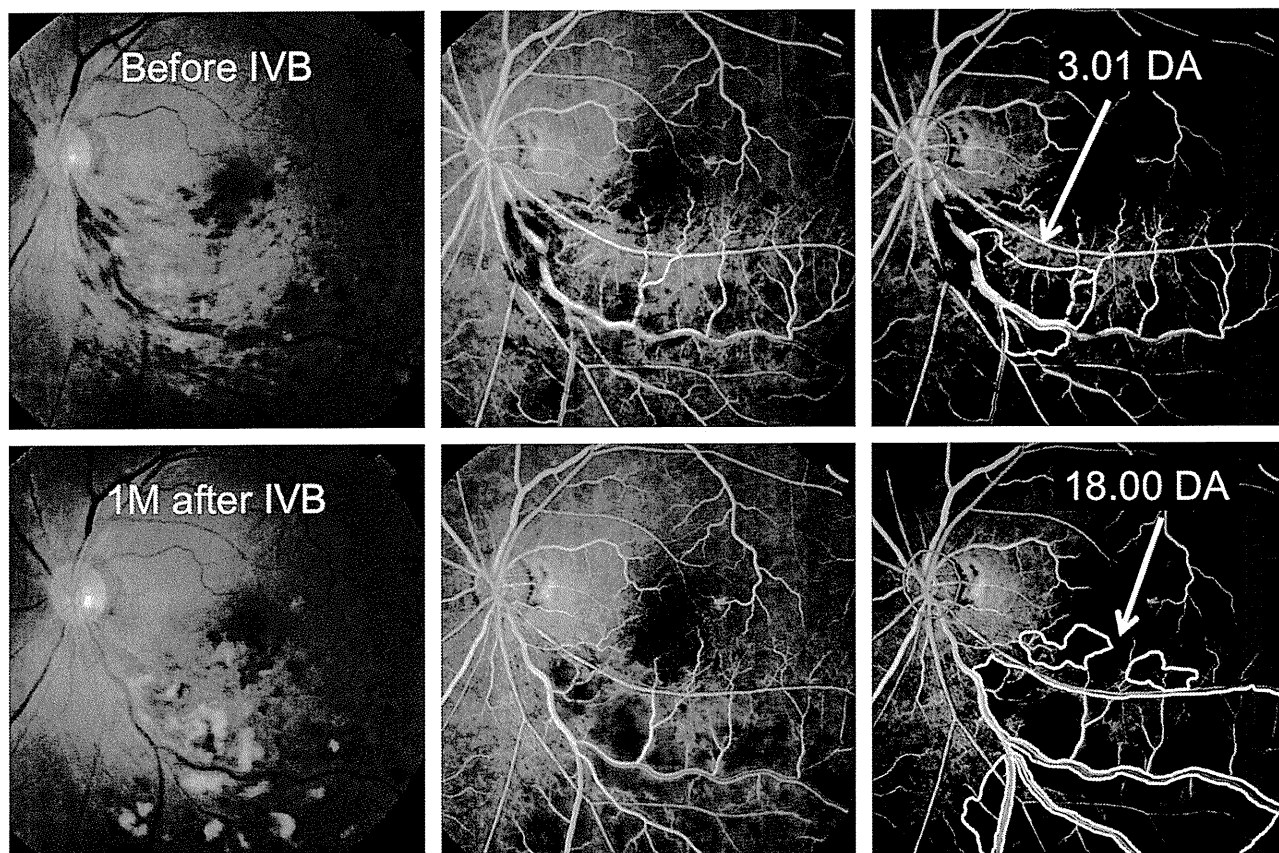
Fig. 4. Fundus photographs and FAs before and 1 month after the IVB in a patient with BRVO (Patient 23, 62-year-old woman). The results of the measure of capillary nonperfusion are shown in the right column. The area of nonperfusion increased from 3.01 DA to 18.00 DA after the IVB, and multiple cotton wool spots developed in the fundus photograph.

of all nonperfused retinas explicitly. The fourth limitation was the small sample size. While the number of eyes treated in this study may be sufficient to rule out a large risk, a much larger sample size would be necessary to rule out a smaller risk or an infrequent complication.

In conclusion, our results demonstrated that the incidence of a significant increase (>1.0 DA) in the area of capillary nonperfusion within 1 month after the IVB was very low (1.7%) in our 58 eyes with BRVO. Furthermore, randomized controlled trials and longer follow-up are needed to clarify whether the IVB therapy exacerbates the retinal ischemia.

Key words: branch retinal vein occlusion, bevacizumab, vascular endothelial growth factor, capillary nonperfusion, ischemia.

Acknowledgment

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眼 科

臨時増刊号：眼科最新手術

顕微鏡による新しい観察システム

門之園一明

毒性も軽減できる可能性があるなどの利点が多い[8]。しかし，大量の硝子体出血を認める症例にシャンデリア照明を用いる場合，まれに照明ファイバーの先端に凝血塊が付着し，それに伴う吸熱反応が原因でファイバー先端に熱溶解を生じることがある[9]。このような懸念がある症例では手術開始時に照明ファイバー先端部周囲の硝子体を先に切除してしまうと良い。また，シャンデリア照明ファイバーは空気灌流下でもその先端が高熱となることが知られており，液・空気置換後は光量を下げることや必要以上に長時間にわたって空気灌流下で使用しないなどの対処が必要である。

III 今後の展望

キセノンや水銀蒸気灯のような低出力かつ高輝度の照明を実現できる光源を用いた照明装置は，今後の眼内照明ファイバーのサイズやバリエーションの開発のニーズに応えるべく，従来のハロゲンライト光源にとって代わり，硝子体手術照明の主役になることに疑う余地はない。実際のところ，次世代の硝子体手術装置として最近発売されたAlcon社のConstellation®やBausch & Lomb社のStellaris PC®のいずれもキセノン光源や水銀蒸気灯が内蔵の照明光源として標準装備されている。

これらの新しい光源装置，眼内照明と次世代の硝子体手術装置を組み合わせることによって，今後の小切開硝子体手術のシステム開発はさらに低侵襲な方向に向かって発展することは間違いないであろう。

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8 顕微鏡による新しい観察システム

はじめに

硝子体手術においてクリアな観察系は最も重要な手術の成功のカギである。顕微鏡手術においては，非接触広角観察システムが近年の硝子体手術の術中観察系において注目される顕微鏡による手術観察システムである。広角観察システムには，非接触と接触があり，非接触は BIOM，OFF-ISS，Resight の3種類の機種が主に本邦でよく使用されている（表）。また，それぞれの機種には独自の広角観察画像を得る工夫が施されている。本稿では，主に最近話題となっている非接触広角観察システム，Resight（Lumera 700，Zeiss）についてその概略を説明する。

I 広角観察システム手術顕微鏡

非接触レンズにより術者は，広角の眼底画像を得ることができるが，その画像は反転している。このため，広角観察系を硝子体手術中に用いるためには画像を正立像にする必要があり，手術顕微鏡に取り付けられた画像反転装置（SDI と呼ばれる）により，術者は正立像として術野を認識して手術を遂行することが可能である。

一般に，レンズを角膜面上に近付けることにより得られる眼底画像はより広角になり，その焦点は角膜面上に近くなる。一方，レンズを遠ざけることにより得られる眼底画像は狭く，その焦点は網膜面上に近くなる。すなわち，接触レンズの移動のみでは，的確な広角画像を得ることはできない（図1）。このため，従来は術中の広角画像の焦点合わせの習得が非常に難しく，広角観察系は，

表

製造元	Carl Zeiss Meditec		OCULUS		TOPCON	
名称	Resight		BIOM 4e		OFFISS	
レンズディオプター(D)	60	128	60	120	40	120
レンズ自体の径	17 mm		19 mm		25 mm	21 mm
レンズ枠を含めた径	20 mm		20 mm		28 mm	23.5 mm
取付可能な顕微鏡	Zeiss 社製の Lumera 700，Lumera T，VISU 210，VISU 200 など		ほぼすべての顕微鏡(Zeiss，Leica，Topcon)		トプコン社 OMS-800，850 のみ	
像の見え方	倒像		倒像		倒像	
追加のインバーター	不要(電動インバーター鏡筒)		必要(SDI)		必要(SDI)	
追加のフットスイッチ	不要		必要(Zeiss との組み合わせの場合)		不要	

門之園一明
Kazuaki KADONOSONO 横浜市立大学附属市民総合医療センター眼科
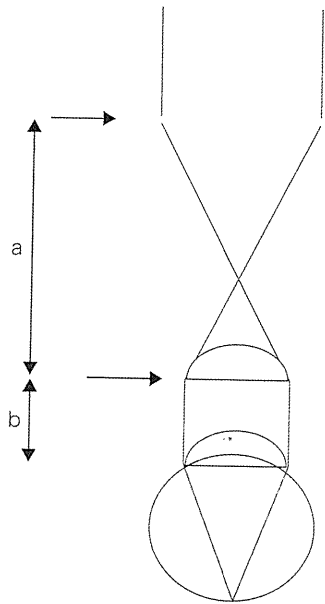
図1：広角観察システムの光学的特徴

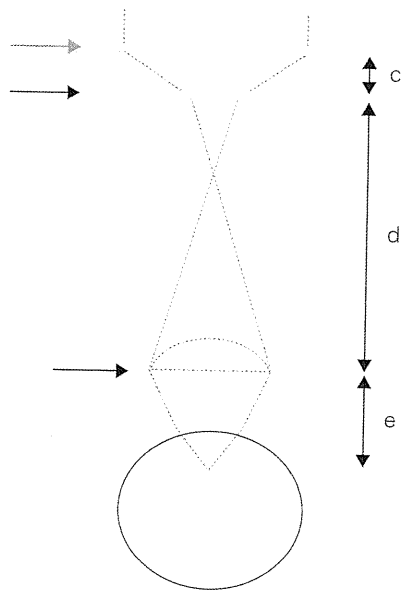顕微鏡の接眼レンズ（上の矢印）と非接触レンズ（下の矢印）との距離（a）および非接触レンズと角膜面との距離（b）が眼底に対する焦点合わせに関連する。主に BIOM で用いられている。

図2：内部焦点合わせを用いた広角観察システムの光学的特徴

焦点は顕微鏡鏡筒内の内部レンズ（赤矢印），および顕微鏡の接眼レンズ（上の矢印）と非接触レンズ（下の矢印）との距離（c）および非接触レンズと角膜面との距離（d）により決定される。焦点合わせは，内部レンズの上下移動により簡便に行われる。Resight 手術顕微鏡において用いられる。角膜面上と非接触レンズとの距離（e）は一定である。

接触レンズに比較して劣っていると考えられてきた。結果的にわが国では，硝子体手術においては，より簡便である接触レンズが広く使用され，歴史的に欧米と異なり広角観察システムは十分な普及をみることはなかった。ところが，近年使用が可能となった Resight（Lumera 700）は，reduction lens を顕微鏡鏡筒内に内蔵することで，焦点合わせを広角合わせと独立して行うことを可能とした（図2）。このため，術者は，適切な画角で，適切な対象物を手術中に簡便に確認することができるようになった（図3）。

　眼科手術にとって，観察対象物の奥行きは眼球の大きさを考慮すると，2～3 cm 程度と非常に小さい。このため，眼科手術顕微鏡では，従来より焦点合わせ（focus）は対物レンズの上下移動により行われてきた。一方，脳神経外科など手術対象物の奥行きが広く，手術中の出血などによるレンズへの損傷が生じる可能性のある臓器を扱う分野では，焦点合わせは顕微鏡の上下移動ではなく，内部レンズの上下移動による焦点合わせシステム
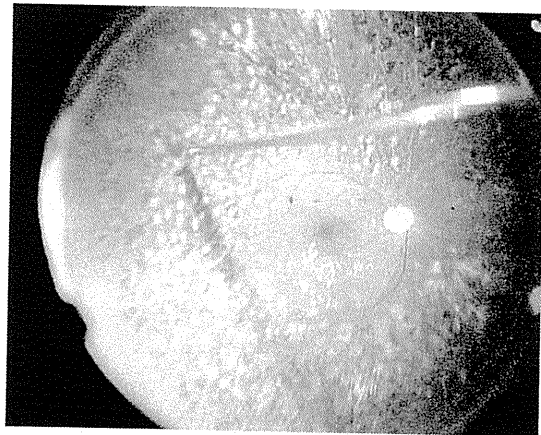

図3：広角観察システムによる眼底画像

ほぼ周辺部網膜まで網膜は広く観察される。また，光凝固を施行している網膜の画像に焦点は良く合っている。

により行われていた。すなわち，顕微鏡本体と対象物との距離は変わらない。Resight は，従来より他科手術顕微鏡で使用されていたこの内部焦点システムを眼科顕微鏡に初めて応用した。このアイデアは，結果的に広角でかつ的確な焦点合わせ（focus）機能を可能とした。

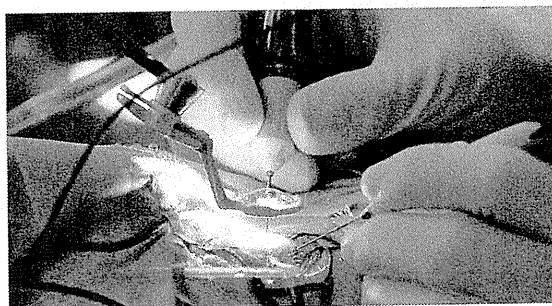問題点：非接触レンズ広角観察システムでは，前置レンズの曇りおよび器具との干渉が問題になる（図4）。また，レンズ固定の支持バーのずれにより術野のずれが生じることがある。

Ⅱ 広角観察システムの応用例

症例1

　67歳の男性で7年前に白内障手術を受けた。数日前に突然の視力障害をきたし来院した。眼底検査で，眼内レンズの眼底への落下が観察され眼内レンズの摘出術を受けた。図5は，手術中の眼内レンズが液体パーフルオロカーボン（PFC）上に浮かび注入に伴い眼内レンズが角膜側に移動してきている手術画像である。ここで注目すべきは，周辺部の網膜の状態を確認しつつ，落下した眼内レンズを観察し続けることがこの広角顕微鏡システムでは可能なことである。眼内レンズは，PFC上で氷の上のようにつるつると移動し術野の死角に入る可能性がある。このため，眼内レンズをPFCで摘出する場合は，周辺部網膜を確認することが重要となる。広角観察手術顕微鏡は，このような症例には有用である。その後，眼内レンズは首尾よく摘出され術後視力は矯正1.0まで回復した。

図4：問題点
非接触128°レンズは角膜上約1.0～1.5mm程度の前方ある。このため，しばしばレンズの曇りおよび硝子体器具が操作の際にレンズに当たる。

症例2

　53歳の女性，術前視力は手動弁であった。網膜剥離手術後の再剥離の症例であった。眼底は，視神経乳頭が閉じている漏斗状の増殖硝子体網膜症（PVR）であり，ナプキンリングと呼ばれる網膜下増殖の存在も確認された。本症例に対して硝子体手術が行われ，増殖膜が除去された。図6は，広角観察手術顕微鏡を用いた術中画像である。膜剥離のための硝子体鑷子の先端は網膜面上の増殖膜にあり，かつそれと同時に周辺部網膜は十分に確認可能である。このようなPVR症例では，増殖膜の処理に気を取られしばしば周辺部の異変に気づくことが遅れることがある。広くかつ良好な術野を得ることで，PVR手術に伴う網膜
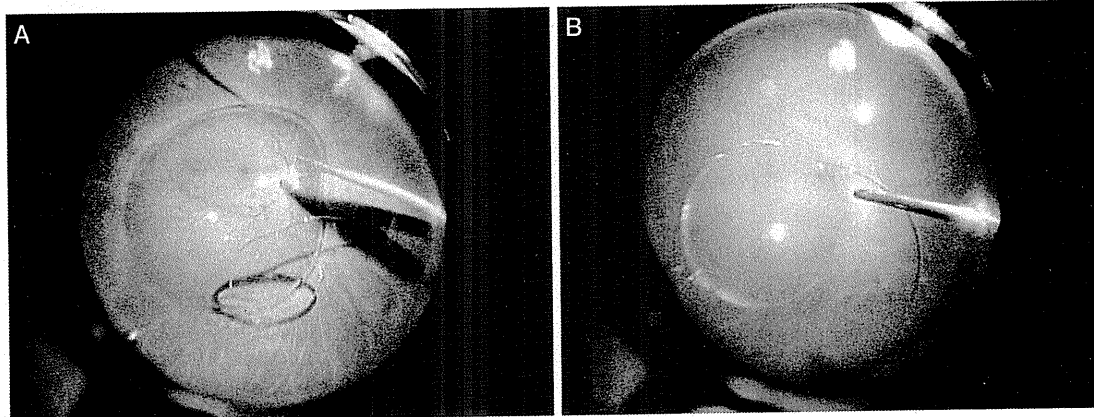

図5：落下眼内レンズの眼底画像
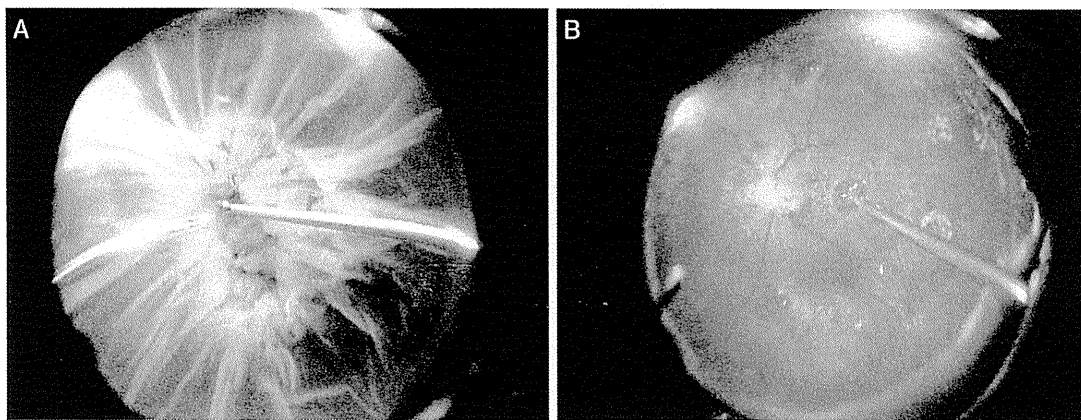パーフルオロカーボンを注入して眼内レンズを浮揚させている。ほぼ周辺部網膜まで観察される（A）。また，眼内レンズはよく確認されかつほぼ赤道部網膜まで観察される（B）。

図6：増殖硝子体網膜症の硝子体手術術野画像

全剥離した周辺部網膜が確認される。また，鑷子にて増殖膜の切除がよく観察される（A）。その後，網膜はよく復位されパーフルオロカーボンの粒子をフルートニードルにて受動吸引しているのがよく観察され，同時に眼底にシリコーンオイルがほぼ充填されつつあるのがよくわかる（B）。
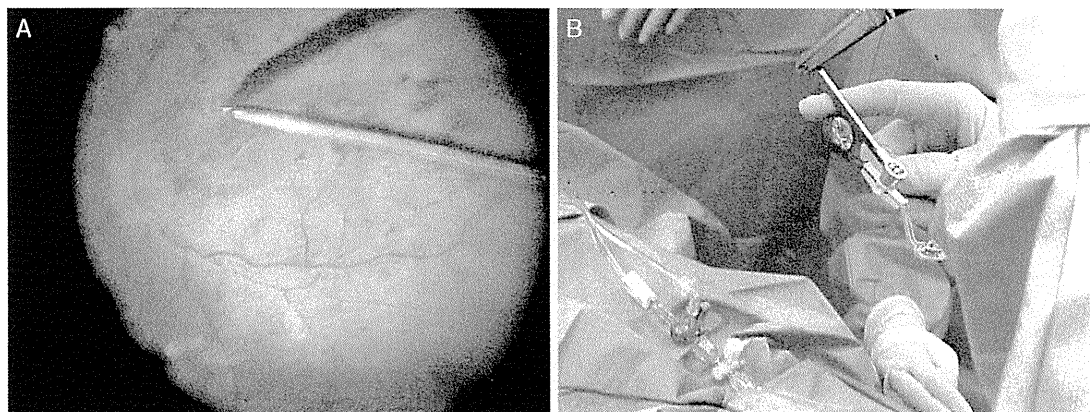

図7：黄斑円孔の内境界膜剥離

広角観察システムを用いているが後極部網膜をよく観察できる。内境界膜は非接触レンズでもよく確認できる（A）。手術中に簡便に Resight 手術顕微鏡の 60°非接触レンズを使用することが可能である（B）。

裂孔や出血などの合併症は軽減することができる。

また，PFC は，多くの場合最終的に術中にシリコーンオイルへ置換される。PFC-シリコーンオイル置換において重要なことは，PFC の粒子を見失わないことと，周辺部網膜にシリコーンオイルの注入過程にて異変がないか否かを確認することである。さらに，視神経乳頭の色調の変化を確認することも重要である。このため，手術中に広角かつ的確な画像を得ることは安全なシリコーンオイル置換術にとって重要であり，広角観察システムの利点が生かされる症例である。

症例3

56 歳の男性，黄斑円孔の患者である。黄斑円孔に対する硝子体手術が行われた。図7A は内境界膜剥離を行っている術中写真である。通常この繊細な手技は，接触レンズ下に行われていたが，Resight 手術顕微鏡では，非接触レンズのまま，内境界膜を剥離することが可能である。60°レンズを使用することで（図7B），染色された内境界膜を剥離することが可能となる。従来，広角観察システムの欠点として黄斑部網膜の処理を行うことができない点が挙げられていたが，Reisght 手術顕微鏡では，広角観察システムと同時に黄斑部
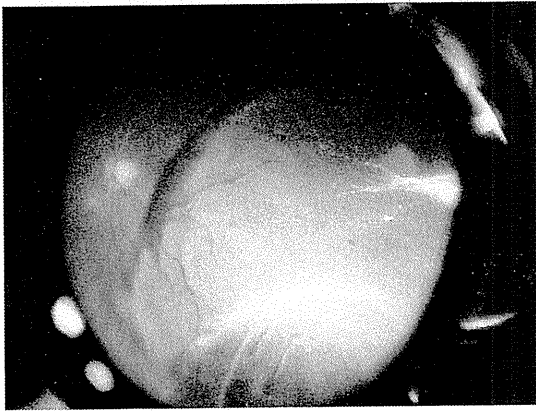
図 8：強膜内陥術
広角観察システムにより，萎縮性円孔に対する網膜冷凍凝固がより的確に簡便にできる。

治療を行うことができる。

4 症例4

　28歳の男性。萎縮性円孔を原因とする裂孔原

性網膜剝離に対する強膜内陥術を施行された（図8）。Resight 手術顕微鏡により，術中のバックリング操作を顕微鏡下で行うことが可能である。

まとめ

　近年，硝子体手術において広角観察手術顕微鏡は急速に普及しつつある。さまざまな種類の広角観察手術顕微鏡が市販されている。なかでも，内部焦点をもつ手術顕微鏡は，広角かつクリアな術野という相反する事象を具現化した。今後，本邦でも欧米のように，硝子体手術の観察系は広角へとシフトしてゆくと予想される。それは，硝子体手術の治療成績をさらに進歩させるであろう。

*　　　　　　　　　　　　　*

An Experimental Study of Retinal Endovascular Surgery with a Microfabricated Needle

Kazuaki Kadonosono,[1] *Akira Arakawa,*[1] *Shin Yamane,*[1] *Eiichi Uchio,*[2] *and Yasuo Yanagi,*[3]

PURPOSE. To study the feasibility of performing retinal endovascular surgery with a microfabricated needle-based cannulation system at the level of the retinal microvasculature.

METHODS. A total of 40 retinal vein vessels, and 40 porcine eyes were used, and the eyecups were prepared under an operating microscope. Twenty retinal veins each were pierced with a microfabricated needle having an outer diameter of 50 μm and with a micropipette having an outer diameter of 50 μm, respectively, and each vessel that was successfully pierced was injected with a solution. The piercing success rates and injection success rates were calculated, and a histologic examination of the site was performed in each eye.

RESULTS. Piercing and injection with the microneedle were successful in all 20 eyes (100%). Histologic examination showed that the retinal vasculature was well preserved in all eyes in which piercing had been performed with the microneedle. Piercing with the micropipette, on the other hand, was successful in only 8 eyes (40%), and injection with the micropipette was successful in only 5 eyes (25%). The tip of the micropipette broke in 12 vessels during piercing and in 3 vessels during injection.

CONCLUSIONS. The feasibility of performing microvascular piercing and intravascular injection of retinal veins with a microneedle was demonstrated in porcine eyes. It may be possible to administer solutions into retinal vessels more effectively with a microfabricated needle, and that may contribute to improving retinal endovascular surgery in human eyes. (*Invest Ophthalmol Vis Sci.* 2011;52:5790–5793) DOI:10.1167/iovs.11-7327

Because retinal vascular occlusions may be initiated by endovascular pathophysiologic mechanisms,[1,2] endovascular surgery has been considered as a potential treatment. However, despite several earlier intensive experimental and clinical studies, this surgical approach has never become completely established.[3-11] Retinal vein cannulation is one of the surgical procedures that are performed on the retinal vasculature.[12,13] It involves puncture, injection, and cannulation, and has been performed in studies on both animal and human eyes. However, several problems related to the surgical technique remain

in retinal endovascular surgery, and as a result it is still challenging and has never been evaluated as useful clinically.[14-19]

Glass micropipettes have been produced with very fine tips and diameters that enabled them to be used for such applications as pressure injection, ion sensing, and microvascular puncture.[20] Glass micropipettes are so sharp that they easily pierce the retinal microvasculature, and thus have been considered the most suitable surgical tools for retinal endovascular surgery.[3-13] However, micropipettes have the disadvantage of being so frangible and delicate that it is difficult to maneuver them during cannulation procedures on retinal vessels.

In recent years, microneedles have been fabricated by leveraging tools from the microelectronics industry, and they have been assessed as devices to facilitate administration delivery.[21-23] Because fabricated microneedles may be sharp and rigid enough to serve as tools for microvascular surgery, we compared the performance of microneedles and conventional micropipettes as a means of cannulating and injection of retinal veins in porcine eyes.

MATERIALS AND METHODS

Porcine Eyes

More than 40 porcine eyes were prepared for use in this study. The eyes were delivered fresh, and they were used within 24 hours of enucleation. All maneuvers were performed on the eyecup in room air. The anterior segment was excised by circumferential incision at the level of the pars plana. The vitreous was removed by the en bloc method, which exerts minimal traction on the retinal surface. The vitreous base was gently massaged with a dry cotton-tipped applicator until it separated from the vitreous base, making it possible to remove and roll the vitreous out of the eye en bloc with the applicator. The residual fluid on the retinal surface was aspirated with a blunt 30-gauge cannula. All procedures were approved by the institutional animal care and use committee of Yokohama City University Medical Center and complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Preparation of the Microneedles

An application-independent optimal design for the microneedle was prepared. The microfabricated needles were manufactured to be angled and jointed with an angled 23-gauge steel pipe (Medical Planning Laboratory Ltd., Tochigi, Japan) (Figs. 1 and 2). In general, needles having a smaller diameter made of stainless steel are sharper and can withstand higher pressure without fracturing or buckling. On the other hand, the flow rate through microneedles declines as their diameter decreases, and the pressure increases. With this in mind, the outer micrometer size domain of the microneedle was designed to be 40 to 50 μm, because the diameter of first-order retinal veins in human eyes is approximately 100 μm.[24]

Also, because needles with a steep angled cutting plane at the tip, whose angle from the axis of the needle shaft is greater than 60°, are sharper and capable of piercing the microvasculature more smoothly, the cutting plane of the needle was designed to have a 30° angle, i.e.,
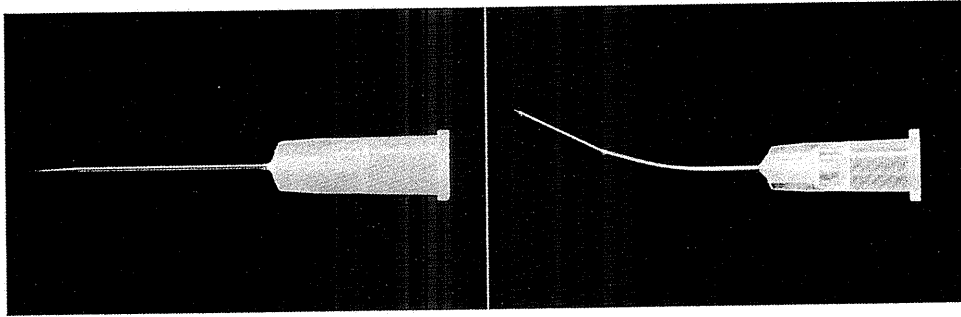
FIGURE 1. Photograph of a microfabricated needle and a micropipette. *Left:* The micropipette. Glass tubes 26 mm in length having an outer diameter of 50 μm and a 30° angle tip were designed to be attached to a 23-gauge cannula. *Right:* The microfabricated needle. The microneedle was manufactured with a curved shaft attached to a 23-gauge holder. The outer micrometer size domain of the microneedle was designed to be 50 μm, and the length of the front of the needle was designed to be 1.5 mm.

60° angle from the axis of the needle shaft (Fig. 3). Shorter needles of the same diameter and material can withstand higher pressures without fracturing or buckling. Lower microneedle height allows cannulation of smaller needle diameters without inducing buckling. A smaller tip diameter results in a much higher ratio of fracture force to insertion force into the microvasculature. Accordingly, the front of the microneedle was designed to be 1.5 mm long and to be connected to a 23-gauge needle. A photograph of the microneedle is shown in Figures 1 and 2.

Preparation of the Micropipettes

Micropipettes were prepared and manufactured from unsharpened standard glass pipettes (Primetech Laboratory Ltd, Ibaragi, Japan). Glass tubes 26 mm in length having an outer diameter of 50 μm with a 30° angle tip were designed to be attached to a 23-gauge cannula (Figs. 1 and 2).

Piercing and Injection

Retinal veins were punctured at a site near the optic nerve head where the diameter of the vein was maximal and the vein was tightly tethered to the optic nerve head. The vein was injected with balanced saline solution (BSS) at high pressure of approximately 50 mm Hg created with a viscous-fluid control machine (Accurus, Alcon, TX), and retrograde blood flow was considered evidence of successful injection of the solution. The flow rate and the inside diameter in the micropipette were approximately 0.077 mL per second and 30 μm, respectively, while those of the microneedle were approximately 0.083 mL per second and 35 μm, respectively. The duration of the injection was three minutes. These procedures were performed manually and the instruments were held with the hands, and there was no significant movement or distortion of the vessels during the procedures. The

success rate of piercing and injection with each instrument was evaluated in all procedures, and a histologic examination was performed on all eyes. After successful piercing and injection of the retinal vein, the specimen was preserved and photographed, and the site of the piercing was identified under a microscope. The specimen was then embedded in paraffin, and every section up to a distance of 500 μm from the site of the piercing was mounted. Serial sections were examined to determine the integrity of the retinal vasculature after the piercing.

RESULTS

Piercing and injection were performed with micropipettes and microneedles on a retinal veins in 20 porcine eyes each, and a total of 40 retinal veins in 40 porcine eyes were used (Table 1).

All attempts to pierce the retinal vein with a microneedle were successful. The resistance of the retinal vein to piercing was low, and no microneedles broke as a result of unintentional tremors or movements of the microneedles. After the vein had been pierced, a BSS injection was performed (Fig. 4). Blood flow was clearly observed in all 20 eyes. The success rates of both piercing and injection with the microneedle procedure were 100%.

On the other hand, piercing the retinal vein near the optic nerve with 8 micropipettes was attempted, and then BSS was injected after the piercing procedure in all 8 eyes in which piercing was attempted (Fig. 4). The other 12 micropipettes broke near their tip during the piercing procedure. Thus, the piercing procedure and the injection procedure were both successful with only 5 micropipettes. No blood flow at all was seen or it stopped during the injection in 3 eyes because the
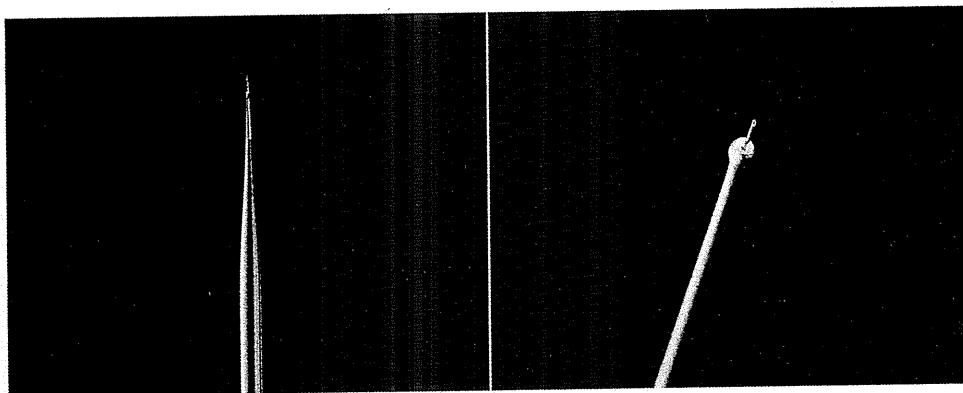

FIGURE 2. Magnified photograph of the tip of the micropipette (*left*) and the microfabricated needle (*right*).
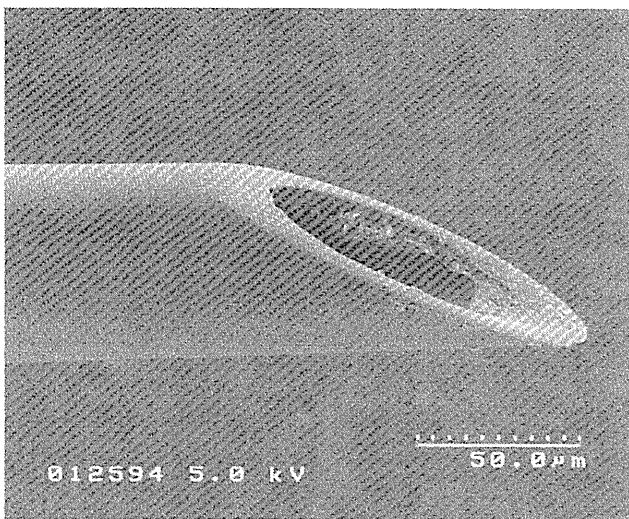
FIGURE 3. The electron microscope of the microneedle shows that the cutting plane of the needle is designed to have a 30° angle. Bar, 50 μm.

TABLE 1. Summary of Results

	Number of Successful Procedures/ Total Procedures	
Procedure	Micropipette	Microneedle
Retinal vein piercing	8/20 (40%)	20/20
Retinal vein injection	5/20 (25%)	20/20

micropipette was damaged by the delicate movement of the instrument during the injection procedure. The piercing success rate was 40% (8/20 eyes), and the injection success rate was 25% (5/20 eyes).

The specimen was then embedded in paraffin, with the cut edge marked for keratome sectioning. For a distance of 1000 to 1500 μm spanning the vessel piercing site, every fifth section was mounted. Histologic examination of all eyes in which the microneedle procedure was successful demonstrated that the vascular penetration site and the other retinal vascular wall was clear but the site of the piercing was not damaged (Fig. 5). On the other hand, in all eyes pierced with a micropipette, the wall was severely damaged, the laceration was not clearly identified, and vitreous hemorrhages were seen (Fig. 6).

DISCUSSION

In this study, the microvessels were completely punctured with microneedles and the solution was successfully injected. On the other hand, it was difficult to pierce the vessels with the micropipettes, which easily broke, and the maneuvers were very complicated. The results showed that the microneedles that had been fabricated were more feasible instruments for microvascular surgery than the micropipettes.

The microfabricated needles used in this study have several advantages over micropipettes, and those advantages may have contributed to the more favorable results. The greatest advantage of the microneedles is their rigidity, because they are made of stainless steel. Even if there is some movement or distortion during the piercing or the injection procedure, they hardly ever break. Moreover, the tip of the microneedle can be easily seen during the piercing procedure, whereas the tip of micropipettes is hard to see, because it is transparent and glistens. Furthermore, the microneedles are fabricated to be attached to the angled handle-shaft shown in Figure 1, which enables a smooth approach to retinal veins.

Micropipettes are used to physically interact with microscopic structures, such as during microinjection and patch clamping procedures.[20] Most micropipettes are made of borosilicate, aluminosilicate, or quartz, and many types and sizes of glass tubing are available. Because of the above characteristics, they have also been used for retinal endovascular surgery for a decade,[3-13] but they have several disadvantages, including fragility and poor visibility.[11-13] In recent years microneedles having diameters as small as 5 μm have been fabricated in the electronics industry[21-23] and they appeared to be quite suitable for use in microvascular surgery. In our experimental studies, all retinal vessels pierced with microneedles were smoothly pierced, and a solution was injected into the microvessel. Also, microneedles are easy to maneuver during eye surgery because of their rigidity.

The results of the histologic examination in this study showed that the cutting plane of the retinal vessel walls pierced with the micropipettes was rougher and more severely damaged than the walls pierced by the microneedle procedure and that the operation with the microneedle caused less damage to the retinal veins. Although it was possible to successfully pierce and inject vessels with 25% of the micropipettes used in this study, the structure of the retinal vessels at the sites where they were successfully pierced with the micropipettes was severely damaged.
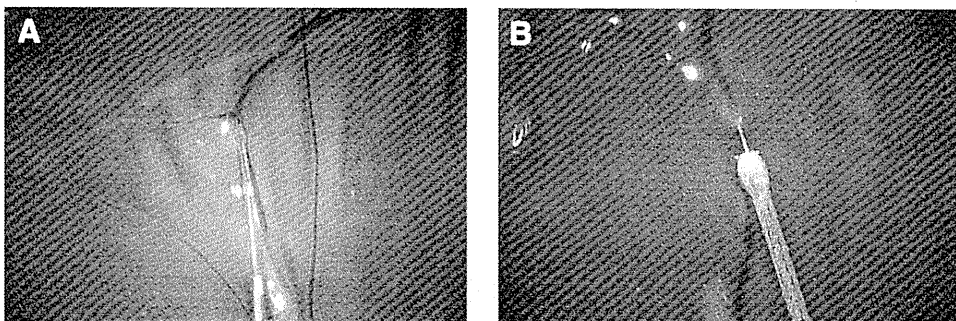

FIGURE 4. Retinal vein cannulation procedure in a porcine eye with a micropipette (A) and a microfabricated needle (B). A major retinal vein branch close to the optic nerve head was pierced with the micropipette or the microneedle. Retrograde blood flow was seen in both successful procedures (A and B).
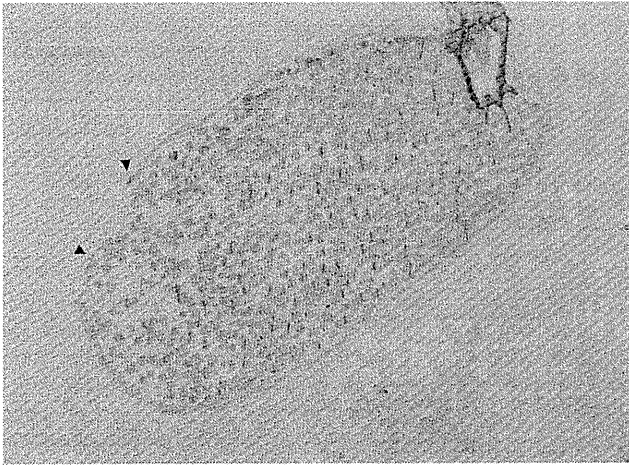
Figure 5. Photomicrograph of a cross-section of a retinal vein pierced with a microneedle in a porcine eye. The retinal vasculature is completely patent, and the laceration of the retinal vein wall is very clean (*arrowheads*). No preretinal hemorrhages are present.

There was a limitation in this study. The micropipettes used in this study are shown in Figures 1 and 2 and, unfortunately, were not the same as the micropipettes that Weiss and Bynoe used in their study.[5] If we had used the same micropipettes and compared the feasibility of the piercing procedure with the two instruments, the results would have been more reliable. Because the micropipettes used in this study were manufactured with a 30° beveled tip, they may be comparable to those used by other investigators.

The findings in this study may contribute to the development of the endovascular approach to the retinal vein. However, because fibrinolysis by injection of such drugs as the tissue plasminogen activator (t-PA) can be effective against central vein occlusion only in eyes with recently formed clots, the effectiveness of the tissue plasminogen activator injection of retinal veins is still unclear. Therefore, further investigation will be needed to ascertain the effectiveness of retinal endovascular surgery for eyes with retinal vein occlusion.

In conclusion, the feasibility of performing microvascular punctures and intravascular injections of retinal veins was demonstrated in porcine eyes. Solutions can be administered into the retinal vessels more effectively with a microfabricated needle, and that may contribute to improving the success of retinal endovascular surgery in human eyes.
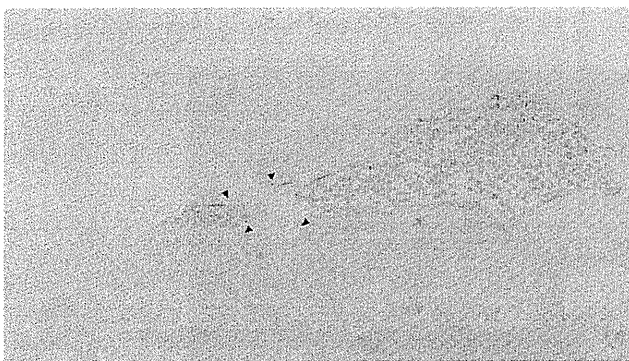

Figure 6. Photomicrograph of a cross-section of a retinal vein pierced with a micropipette in a porcine eye. The retinal vasculature is severely damaged, and the vessel is penetrated by the microneedle (*arrowheads*).

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Review

HYALOCYTES: ESSENTIAL CELLS OF VITREOUS CAVITY IN VITREORETINAL PATHOPHYSIOLOGY?

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Purpose: To review the present understanding of hyalocytes.

Methods: A review of recent studies that investigated the roles of hyalocytes in the pathophysiology of vitreous cavity.

Results: Studies on immunocytochemistry and chimeric mice with green fluorescent protein transgenic mice show that hyalocytes belong to the monocyte/macrophage lineage and derive from bone marrow. The effects of hyalocytes on vitreous cavity environment can be divided into three categories: synthesis of extracellular matrix, regulation of the vitreous cavity immunology, and modulation of inflammation. In noninflamed eyes, vitreous cavity is an immune-privileged site that is maintained by a system called vitreous cavity–associated immune deviation, in which hyalocytes play the role of antigen-presenting cells. However, cultured hyalocytes proliferate in response to inflammatory molecules and secrete vascular endothelial growth factor and urokinase-type plasminogen activator. A collagen gel embedded with hyalocytes contracts over time, which is enhanced by transforming growth factor-β but is inhibited by Rho kinase inhibitor. These results suggest that hyalocytes can be an exacerbating factor in inflamed eyes. Clinically, hyalocytes are frequently found in the surgically removed specimens of epiretinal membrane or proliferative vitreoretinopathy.

Conclusion: Elucidating the properties of hyalocytes is important to understand the biology of vitreous cavity and to develop novel treatments for vitreoretinal diseases.

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Recently, a number of novel therapies have been emerging in clinical ophthalmology, especially for vitreoretinal diseases. They include a new type of

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pars plana vitrectomy, novel drugs such as ranibizumab and pegaptanib, and gene-mediated therapy. Of note is that these therapies often use the vitreous cavity as a therapeutic place or platform; thus, a more detailed knowledge of the environment of the vitreous cavity is required.

The vitreous cavity is composed mainly of collagen fibers, hyaluronan, and some cells.[1] It has been reported that there are groups of cells in the cortical or peripheral vitreous.[1–5] These cells, currently called hyalocytes, have a lobulated nucleus, cytoplasmic projections, and moderate numbers of mitochondria. Hyalocytes are located at an average distance of 50 mm from the inner surface of the retina and are concentrated anteriorly at the vitreous base and posteriorly in the vicinity of optic disk. According to previous publications,[6–8] hyalocytes were regarded as resting cells, and hyalocytes have been studied less

extensively in comparison with other intraocular cells, such as retinal pigment epithelial cells. However, recent studies[9–12] have shown that hyalocytes play a significant role in maintaining the vitreous body as a transparent and avascular system actively rather than passively. Hyalocytes have been found to be present in various aspects of pathophysiology, which involve epiretinal membrane (ERM) formation, diabetic macular edema, proliferative vitreoretinopathy, and others.[10,12–14] Therefore, we would like to review our present knowledge of hyalocytes to better understand vitreoretinal pathophysiology and to develop new treatments.

Origin, Morphology, and Turnover of Hyalocytes

Hyalocytes are variously described by light microscopy as being spindle-shaped, rounded, or even star-shaped cells. Their nuclei are lobulated, and the cytoplasm is characterized by the presence of many secretory granules and a well-developed Golgi apparatus[4,15] (Figure 1, A–D). They are mainly distributed close to the retina at the vitreous base and in the posterior hyaloid.[15] Morphologic studies demonstrate that hyalocytes belong to the monocyte/macrophage lineage.[4,7,17–19] However, Hogan et al[15] reported that hyalocytes differ from macrophages because of a paucity of lysosomes. Immunocytochemical analysis shows that hyalocytes express a monocyte/macrophage cell marker but not CD68, glial fibrillary acidic protein, cellular retinaldehyde-binding protein, and cytokeratin.[9,20–23] These results indicate that hyalocytes are derived from a monocyte/macrophage lineage but not from glial cells or retinal pigment epithelial cells. Furthermore, a study on rat hyalocytes revealed a positive reaction for ED2 but not for ED1, confirming that hyalocytes have characteristics of tissue macrophages.[16]

Recently, enhanced green fluorescent protein transgenic mice have been generated; the tissues of enhanced green fluorescent protein transgenic mice are green under excitation light. Using enhanced green fluorescent protein transgenic mice, cell movements can be tracked in an in vivo model. We created chimeric mice by transplanting bone marrow from enhanced green fluorescent protein transgenic mice into irradiated wild mice.[10,16] The results show that hyalocytes were green fluorescent protein negative directly after bone marrow transplantation in chimeric mice; however, the number of green fluorescent protein–positive hyalocytes increased over time. More than 60% of hyalocytes were replaced by green fluorescent protein–positive cells within 4 months, and 90% of hyalocytes were green fluorescent protein

positive within 7 months after bone marrow transplantation (Figure 2). The levels of residual macrophages might not have been maintained by their proliferation but by being produced in bone marrow under a physiologic condition with a turnover time of several months.[16] However, van Meurs et al[24] showed the half-life of vitreous macrophage was 4.8 days by allowing vitreous macrophages to phagocytose [141]Cerium (γ-emitter)-labeled microspheres. It is difficult to conclude that these groups studied the same type of vitreous cells; however, there might be several different cell lineages within the so-called hyalocytes.

Conversely, Gloor[25] described that hyalocytes would be in an independent tissue layer, in which the cells are replaced by reproduction because the hyalocytes showed increased mitotic activity after photocoagulation. Haddad and André observed that ^{3}H-thymidine was detected in the hyalocytes of the cortical vitreous after ^{3}H-thymidine injection and concluded that hyalocytes renew themselves inside the eye.[26] It is not clear whether hyalocytes are composed of cells of different origins or those of the same origin at different developmental stages. Although more detailed studies are necessary to answer these questions, it is safe to say that most hyalocytes originate from bone marrow, at least under physiologic conditions.

Functions of Hyalocytes

The functional properties of hyalocytes can be divided into the following three categories: synthesis of extracellular matrix (ECM), modulator of immune reaction, and modulator of inflammation.

Synthesis of Extracellular Matrix

Because hyalocytes appear in the vitreous at an early embryonic stage, it is reasonable to assume that hyalocytes produce vitreous collagen. It has been reported that chick vitreous collagen is synthesized by the neural retina at early embryonic stages, whereas the major contribution derives from cells within the vitreous body later in the development.[27] Also, hyalocytes are reported to be responsible for the production of hyaluronic acid in calf and primate.[28,29] Recently, it has been further confirmed that production of hyaluronan is modulated by cytokines, such as transforming growth factor (TGF)-β or platelet-derived growth factor-BB using cultured hyalocytes.[30] It was also shown that cultured porcine hyalocytes produce glycosaminoglycans and ECM, which is modulated by basic fibroblast growth factor and

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Fig. 1. Hyalocytes in animals. **A.** Scanning electron microscopic photograph of rat hyalocytes. Hyalocytes are present in collagen fibers. Bar 1 mm. **B.** Transmission electron microscopic photograph of rat hyalocytes. Hyalocytes are located close to the ciliary epithelium (arrow). Bar 1 mm. **C.** Phase-contrast microscopic photograph of bovine hyalocytes. Numerous hyalocytes are scattered in the vitreous (original magnification, ×10). **D.** Transmission electron microscopic photograph of bovine hyalocytes. Bar 1 mm. Reproduced with permission from Qiao et al,[16] Noda et al,[11] and Sakamoto.[10]
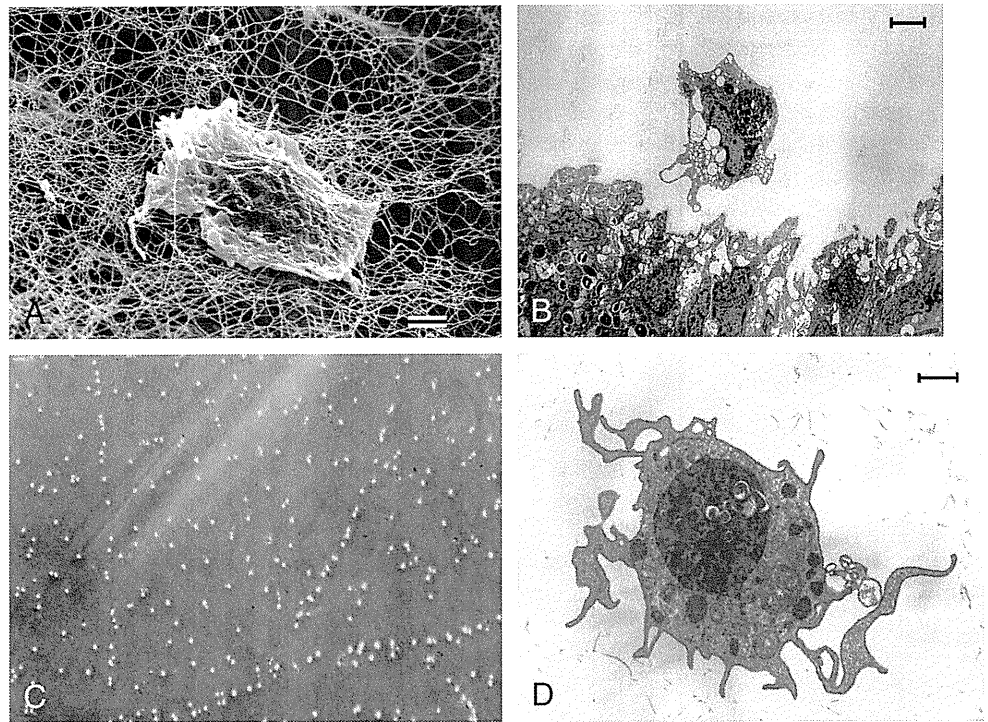
TGF-β1.[31] Because glycosaminoglycans are the stimulators of contraction of ECM with cells, the formation of a membrane with cells and ECM might be an important first step in the progression of ERM or proliferative vitreoretinopathy.[10,32] It is likely that hyalocytes play a certain role in this pathology by producing ECM in addition to other cells.[10,33–38]

Modulator of Intraocular Immune System: Vitreous Cavity–Associated Immune Deviation

The eye is an immune-privileged site that is styled to keep the visual pathway clear while at the same time to provide defenses against invading organisms.[39] Above all, the anterior chamber–associated immune deviation is a unique system to keep the eye immune privileged. Anterior chamber–associated immune deviation can be induced by antigen injection for peripheral tolerance to that antigen.[40] It is demonstrated that anterior chamber–associated immune deviation is induced by bone marrow–derived antigen-presenting cells, which are positive for F4/80, a marker of a wide range of mature tissue macrophages, localized in the iris and ciliary body in the eye and carrying an antigen-specific signal to the spleen.[39,41]

We investigated the mechanisms by which ocular inflammation associated with the vitreous cavity is reduced by injecting either ovalbumin or allogeneic splenocytes into the vitreous cavities of mice and

assessed the effects of this on delayed-type hypersensitivity responses. After antigen inoculation into the vitreous cavity, antigen-specific delayed-type hypersensitivity responses were significantly impaired, and we named this phenomenon the vitreous cavity–associated immune deviation.[42] Vitreous cavity–associated immune deviation could also be induced by inoculating antigen-pulsed macrophages into the
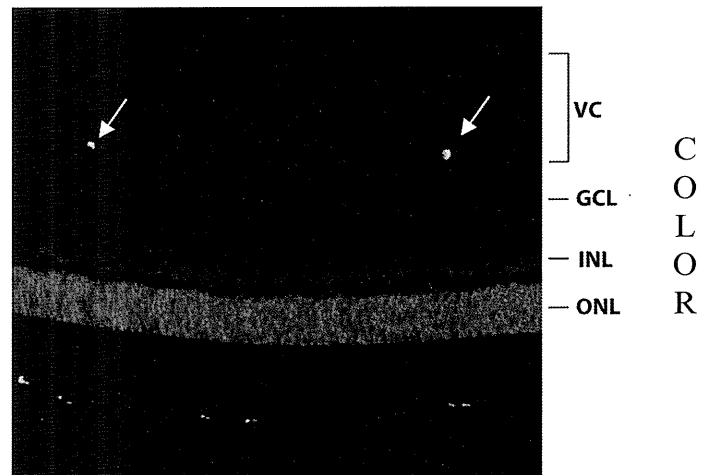

Fig. 2. Fluorescent microscopic photograph of GFP chimeric mouse. The hyalocytes (arrows) are GFP positive, indicating their bone marrow origin. VC, vitreous cortex; GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; GFP, green fluorescent protein (original magnification, ×40).

vitreous cavity. However, vitreous cavity–associated immune deviation did not develop either in mice with inflamed eyes, whether as a result of experimental autoimmune uveitis or coadministration of interleukin-6 in the vitreous cavity, or in knockout mice deficient in natural killer T cells.[42] In this system, we found that hyalocytes are the only cells present in the vitreous cavities. Interestingly, hyalocytes express F4/80, suggesting that hyalocytes are candidate antigen-presenting cells responsible for mediating vitreous cavity–associated immune deviation (Figure 3).[42] These findings suggest that hyalocytes would play a pivotal role in inhibiting intraocular inflammation in noninflamed eyes.

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Modulator of Intraocular Inflammation

Almost three decades ago, human hyalocytes were reported to have characteristics of macrophages, such as phagocytic activity with surface receptors for IgG and complement components.[20] Macrophages are major cells in the inflammation of most tissues, so it is natural to assume that hyalocytes play a major role in intravitreous inflammation.

Cultured bovine hyalocytes proliferate in response to platelet-derived growth factor and secrete urokinase-type plasminogen activator (uPA).[11] Because uPA has a strong fibrinolytic activity by converting proenzyme plasminogen into serine protease plasmin, it might be beneficial to keep the vitreous cavity clear by removing fibrin and fibrin-related materials. At the same time, uPA is a multifunctional protein that also affects growth factor bioavailability; for example, uPA is a potent inducer of angiogenesis and tissue

remodeling, so the role of uPA in ocular pathology would be complicated.[43] Similarly, cultured hyalocytes secrete VEGF, which is upregulated by hypoxia-inducible factor-1 or tumor necrosis factor-α.[44] Hyalocytes might be one of the cellular sources of intravitreous VEGF, which is a well-proven angiogenic and vascular permeability factor in diabetic retinopathy and exudative age-related macular degeneration.

Recently, the role of hyalocytes in ocular pathology has been investigated from a different viewpoint. Contraction of the preretinal cortical vitreous is one of the most critical steps in various intraocular diseases. Three-dimensional collagen gel preparations have been used to assess the mechanism of membrane contraction in vitro, and this system is suitable for evaluating the cortical hyaloid contraction.[45] Using this system, the contractile property of hyalocytes was studied (Figure 4). As a result, a collagen gel embedded with hyalocytes contracts significantly in response to various stimulants, such as TGF-β2, and this effect is mediated through Rho and Rho kinase (ROCK)–dependent pathways.[46] This in vitro cell-mediated collagen gel contraction is more potent with hyalocytes than with retinal glial cells or retinal pigment epithelial cells.[10] Therefore, the presence of hyalocytes might be a potent exacerbating factor of preretinal membrane contraction in proliferative vitreoretinopathy after retinal detachment.

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Clinical Implication

In histologic studies, several types of cell were found in preretinal membrane or posterior hyaloids, and macrophage-like cells were found frequently.[9,12,47–52] It is certain that some of them are hyalocytes (Figure 5). Kohno et al[33] found that cells located at the contractile epicenter of ERMs are mostly hyalocytes, not glial cells. Because hyalocytes have a strong contractile property, it may be assumed that hyalocytes play a critical role not only in the pathology of ERM but also in tractional retinal detachment.[32] Gandorfer et al[14] studied specimens of flat mount internal limiting membrane and found that macular hole formation is caused by the insertion of the cortical vitreous into the foveal internal limiting membrane and that cellular proliferation including hyalocytes is involved in vitreofoveal traction, resulting in a foveal tear.

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To treat these pathologic conditions, removing ERM and posterior hyaloid is the preferred and logical approach at present because these membranes contain a number of cells including hyalocytes. The
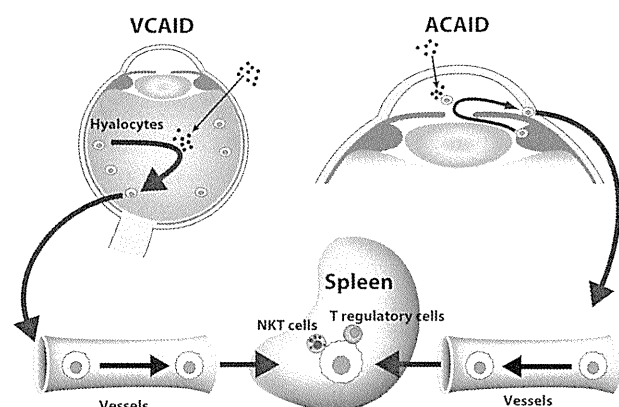

Fig. 3. Schema of vitreous cavity–associated immune deviation (VCAID) and anterior chamber–associated immune deviation (ACAID). Antigens inoculated into the vitreous cavity are captured by resident macrophage hyalocytes and carried via the bloodstream to the spleen. Both VCAID and ACAID require eye-derived antigen-presenting cells and CD1d-restricted natural killer T cells to induce antigen-specific regulatory T cells.