An Experimental Study of Retinal Endovascular Surgery with a Microfabricated Needle

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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. 5–13 However, micropipettes have the disadvantage of being so fragile 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 angular 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.,
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
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 \( \mu \text{m} \) 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.

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<th>Procedure</th>
<th>Micropipette</th>
<th>Microneedle</th>
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<tr>
<td>Retinal vein piercing</td>
<td>8/20 (40%)</td>
<td>20/20</td>
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<tr>
<td>Retinal vein injection</td>
<td>5/20 (25%)</td>
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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 \( \mu \text{m} \) have been fabricated in the electronics industry21–23 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.
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.

References