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In vivo cannulation of retinal vessels*

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Abstract. In vivo cannulation of retinal blood vessels has not been reported but would be useful in exploring local vascular parameters and new treatment modalities. We developed a microsurgical technique for cannulating rabbit and cat retinal vessels, using glass micropipettes with curved shanks made from standard capillary tubing. The micropipette was held by a micromanipulator, which was fitted with a fiberoptic sleeve for intraocular illumination and for micropipette tip protection during insertion through the sclera. Direct aspiration from and injection into retinal blood vessels were possible.

Introduction

Frontiers in microsurgical techniques continue to expand. Microvascular surgeons can reanastomose blood vessels with outer diameters of 500 μm (Urbaniak 1985) and renal physiologists can cannulate 15 μm renal cortical vessels (Andreucci 1978). In enucleated eyes, the free end of transected retinal vessels can be cannulated (Ashton 1950), Similar techniques are now used in studying retinal vessel permeability (Murta et al. 1986). However, cannulation of retinal vessels in vivo has not been reported. The purpose of our study was to develop a method for cannulating retinal vessels in living animals. This would allow exploration of new treatment modalities for retinal vein occlusion and other retinal vascular diseases and enable determination of local vascular parameters such as pH, oxygen tensions, perfusion pressures, and metabolites (Andreucci 1978; Wiederhielm et al. 1964; Johannsson et al. 1977).

Materials and methods

Micropipette preparation

Nonfilamented Pyrex glass tubing, approximately 20 cm long, 1.6 mm outer diameter, and 0.91 mm inner diameter (Drummond Scientific, Broomall, Pa.), was pulled near one end to an outer diameter of approximately 80 μm on a micropipette puller (Simpson, Elgin, Ill.). The tapered shank was broken into two with sharp forceps, the shorter end discarded, and the remaining microsyringe mounted vertically in a microforge (Stoelting, Chicago, Ill.). The tapered end was shaped into a hook and lowered through the heating element loop to the level where the pipette diameter began increasing rapidly (about 120 μm outer diameter). A 0.5 g weight was attached to the hook and the shank was pulled by gravity to an outer diameter of 20–70 μm for a length of at least 500 μm. Additional heat was applied as the pipette was rotated to near horizontal, thus curving the tapered shank. The hook was manually broken off, leaving the completed micropipette with an outer diameter of 20 to 40 μm at the tip, enlarging to 100–150 μm at the curve, then to the original 1.6 mm outer diameter behind the curve (Fig. 1). If the tip had not broken to a bevel, the greater curvature was beveled on an electrically powered, rotating grindstone. An alternate method that allowed beveling of the lesser curvature (thus allowing the bevel to be "up", i.e., away from the vessel during micropuncture) was to bevel the tip before bending the shank. However, the heat required for bending would fire-polish the tip, making it less sharp (Fig. 2).

Microcannulation

Micropipettes were filled with a filtered (0.22 μm filter unit, Millipore, Bedford, Mass.) heparin solution (20 units/ml in

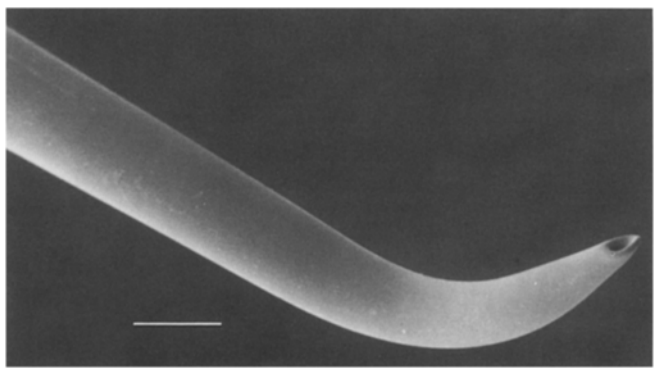

Fig. 1. Scanning electron micrograph of a micropipette: outer diameter is 25 μm at the tip, 40 μm at 100 μm behind the tip, and 60 μm at 200 μm behind the tip. This shank is curved approximately 30°. (Bar is equal to 200 μm)

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normal saline) by aspirating through the tip with suction from a 10-cc syringe connected to the micropipette by a 50 cm length of 1.6 mm inner diameter silastic tubing. The micropipette was then mounted in a Zeiss electronic (Spitznas 1983), or a mechanical (Wolbarsht et al. 1976) micromanipulator that had been fitted with a fiberoptic light pipe or a plain metal sleeve, respectively (Figs. 3 and 4). Once mounted, the micropipette was reattached to the silastic tubing and 10-cc syringe for aspirations and injections. However, in the case of the electronic micromanipulator, the silastic tubing was not attached directly to the micropipette, but to a metal tube that was embedded in a plastic adaptor into which the micropipette was inserted and sealed by a standard "0" ring/screw system (Fig. 5).

Rabbits were anesthetized intramuscularly with 30 mg/kg ketamine hydrochloride and 6 mg/kg of xylazine, with maintenance doses of 10 mg/kg ketamine hydrochloride and 1.2 mg/kg xylazine given as needed to prevent excessive movement. Cats were anesthetized intramuscularly with 20 mg/kg ketamine hydrochloride and 0.5 mg/kg acepromazine maleate. Pupils were dilated with one drop of 10% phenylephrine and 1% cyclogel instilled in the conjunctival cul-de-sac. The anesthetized animal was placed on an operating table and fixed with tape (rabbits) or a stereotactic device (cats) so that the eye was easily accessible by the micromanipulator. The animal's head was positioned so that the imaginary axis connecting the animal's optic nerve and the surgeon's eye was oriented vertically. This enabled the entire retina in the microscope field to be focused simultaneously in one plane; it also allowed a 2 cm × 2 cm glass, microscope coverslip to be floated over the cornea on a cushion of healon (methyl cellulose). The coverslip functioned as a contact lens in reducing light reflections and neutralizing intraocular optics, but had the advantage over a hand-held lens of preventing interference from slight movements or changes in intraocular pressure, and obviating the need for an assistant.

The medial canthus (rabbits) or lateral canthus (cats) was crushed with a large hemostat and then incised to improve access to the sclera. The nictitiating membrane was excised or pulled out of the microscope field with a coarse suture. The conjunctiva was cut 360° near the limbus and retracted. A sclerostomy was made 5 mm posterior to the limbus in the superonasal quadrant (rabbits) or superotemporal quadrant (cats) with an 18-gauge trochar and cannula. The trochar was removed, leaving the cannula in the sclera. The cannula was connected to an intraocular infusion line and checked to ensure it was in the vitreous cavity. In cats, because of their relatively thick sclera, sclerostomies were first established with an 18-gauge needle or a no. 11 blade (Becton Dickenson, Rutherford, NJ). In rabbits, because of their relatively thin sclera, sutures (6-0 vicryl) were sometimes required to hold the cannula in place. A second cannula was similarly placed in the inferonasal quadrant (rabbits) or the inferotemporal quadrant (cats). Air was ejected from the micropipette tip to prevent introduction of intraocular bubbles. The protective sleeve, attached to the micromanipulator and loaded with the micropipette, was inserted through the second cannula and manually (mechanical micromanipulator) or electrically (electronic micromanipulator) advanced toward the optic nerve (about a 45° angle) under direct visual control. Near the retina, the micropipette tip was advanced out of the sleeve toward the selected vessel, observed at low-power magnification; the micropuncture was then performed at higher magnification (× 29). The tip of the micropipette was rotated to point peripherally, with the shank running parallel to the vessel, then advanced until it touched the vessel near the disc. This often caused some constriction with slight peripheral venous distention. The tip was then moved peripherally, parallel to the vessel, and simultaneously advanced slightly until the vessel was punctured. Micropuncture was confirmed by observing spontaneous filling of the micropipette tip with blood. Subsequently, aspirations and injections were made manually using the syringe/silastic tube system. Cannulation was confirmed by blood aspirations without or before perivascular bleeding and by fluid injections displacing intravascular blood. Results were recorded on videotape and by still motion photography. Postoperatively, micropipettes were connected to wall suction for 30 min and then stored on a clay strip for reuse.

Results

We repeatedly cannulated retinal vessels in rabbits and cats using the curved glass micropipette. Micropuncture was

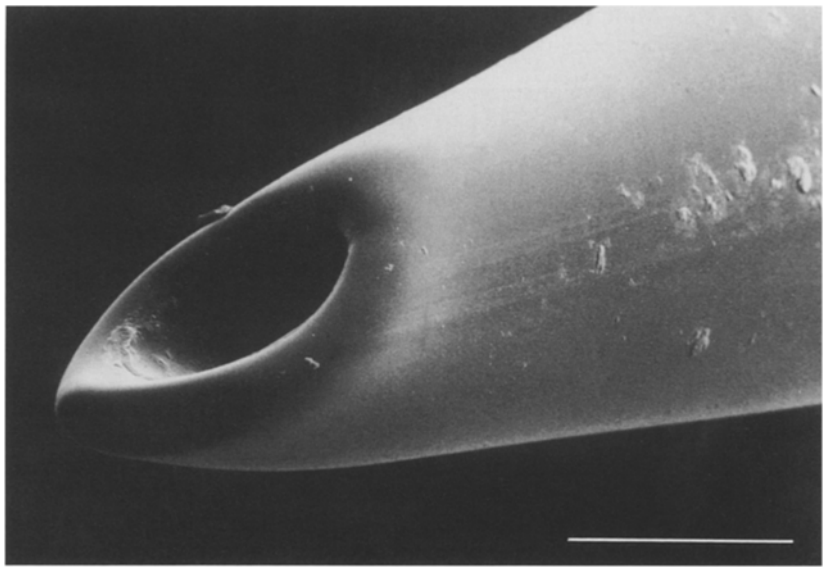

Fig. 2. Higher magnification of the same micropipette depicted in Fig. 1. The tip is beveled approximately 45° on the lesser curvature side, with some rounding of the tip due to fire-polishing from bending a prebeveled microtool. (Bar is equal to 50 μm)

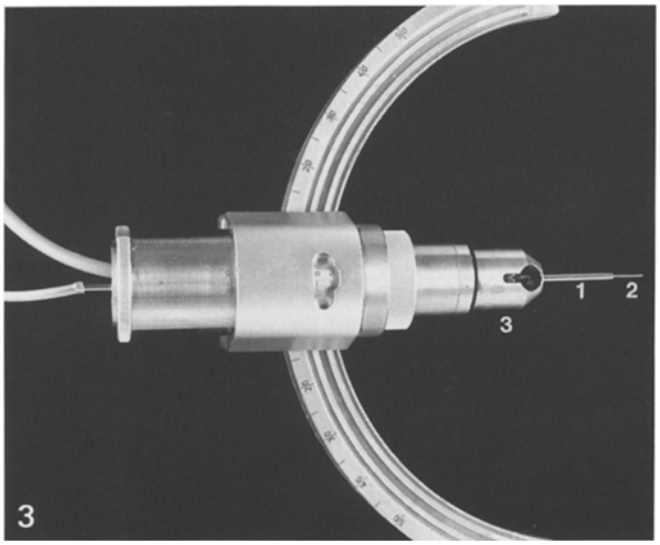

Fig. 3. Photograph of the electronic micromanipulator arm ready for surgery. Exiting the back of the micromanipulator sleeve is the fiberoptic cable (superiorly) and the metal tube (inferiorly) to which the silastic tube is attached. Exiting the front is the fiberoptic sleeve (*1*) and an unsharpened micropipette (*2*). Metal joint guard (*3*) stabilizes the fiberoptic sleeve

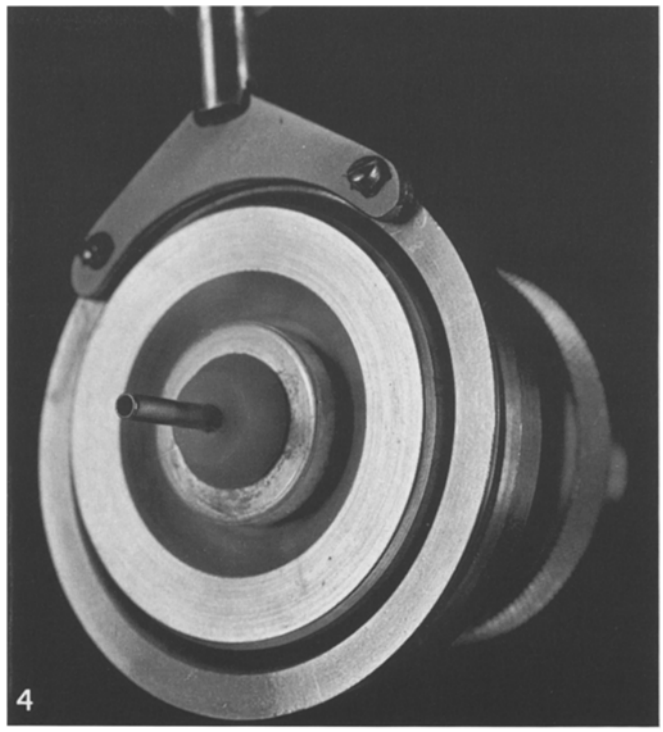

Fig. 4. Photograph of the mechanical micromanipulator mounted with metal sleeve

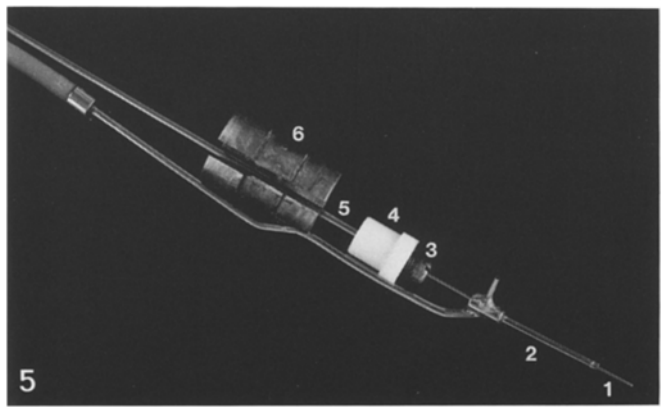

Fig. 5. Exploded picture of the electronic micromanipulator arm. The different pieces, from the tip back, are: (*1*) micropipette (unbeveled); (*2*) fiberoptic tube – metal stem is for an infusion line in the event that a single sclerostomy is desired; (*3*) metal tightening screw; (*4*) plastic adaptor ("0" ring inside); (*5*) metal tube; (*6*) metal adaptor. The syringe/silastic tubing is attached to the metal tube which, through the plastic adaptor, forms an air tight seal with the micropipette

demonstrated by the tip spontaneously filling with blood. Cannulation was verified by fluid injections filling the intravascular space and displacing intravascular blood and by blood aspirations without perivascular bleeding.

Successful micropipettes tips were curved between 50° and 95° with curves beginning up to 320 μm behind the tip, and ending between 400 and 2000 μm behind the tip. Less curved tips simply pushed the vessel aside or punctured front and back vessel walls without achieving cannulation. Micropipette outer diameters ranged from 20–40 μm at the tip, 25–50 μm at 100 μm behind the tip, and 30–70 μm at 200 μm behind the tip. Larger tips would not puncture the vessels while smaller tips restricted injection and aspiration. Tip bevels ranged 15°–70°, but beveled surfaces of successful micropipettes were often not smooth by microscopic inspection.

Both the electronic and the mechanical micromanipulators effectively fixed the micropipette, dampened hand movements, allowed the tip to be rotated around the axis of pipette advancement (z-axis), and moved in all directions perpendicular to that axis (x/y-plane). Compared with the mechanical micromanipulator, the electronic micromanipulator was more cumbersome and could not be adapted to our cat stereotactic device. Furthermore, the electronic micromanipulator would occasionally jam, requiring manual force to restart movement in the z-axis (the axis of micropipette advancement), and inertia of the gears sometimes allowed slight overshooting of an intended movement. Otherwise the electronic micromanipulator was preferred for several reasons: (1) it was more stable; (2) it lacked the large fixed ring of the mechanical micromanipulator that blocked the globe and interfered with the operating microscope; (3) its range of motion arc was greater in the axes perpendicular to the axis of advancement (200° in the x-axis and 100° in the y-axis compared with 30° for the mechanical micromanipulator); (4) it revolved around the scleral insertion point, thus preventing globe movement during manipulations.

Other findings included: Pyrex was less fragile and performed better than borosilicate or other softer glasses. Pyrex micropipette tips would withstand harsh intraocular movements and sometimes even being dropped on the floor without breaking. Extensive cleaning, drying, and dust-free, humidified storage of micropipettes was not necessary, as

in other micropuncture experiments (Andreucci 1978). Cat retinal vessels were easier to cannulate than rabbit retinal vessels. This was at least partly because even heavily sedated rabbits frequently masticated and these movements severely disrupted cannulation efforts. Our two major unresolved problems were bleeding from the micropuncture site, which sometimes obscured much of the fundus, and optical difficulties in obtaining good intraoperative photographs. Our best documentation of cannulation is a videotape showing fluid repeatedly being injected into the vessel with retrograde displacement of intravascular blood.

Discussion

Although the eye is unique in providing direct visual access to nerves and blood vessels, manipulation of these structures in vivo is limited by the globe's small size, the retina's delicateness, and the need to work indirectly through small incisions while viewing through the subject's pupil and media. We have demonstrated the feasibility of cannulating retinal blood vessels in living animals. This permits the study of local vascular parameters, such as metabolites, pressures, flow rates, volumes, and fluid compositions, all of which are measured with micropipettes in renal vessels (Andreucci 1978). Such measurements in retinal vessels may have basic and clinical value. For example, abnormal blood flow, perfusion pressure (Archer et al. 1974), and blood rheology (Peduzzi et al. 1986) have been associated with retinal vein occlusion and can now be studied directly. New treatment modalities for retinal vascular diseases can also be explored. For example, retinal vein occlusion, a serious and relatively common retinal vascular disorder (Magargal et al. 1981; Murakami et al. 1983; BVO Study Group 1984, 1986), has no adequate treatments (Newell 1982). Because the underlying vascular defect may be thrombotic (Green 1981), local injections of fibrinolytic agents may achieve thrombolysis without the systemic side effects observed with systemic injections of these agents (Kohner et al. 1976; Aldrich et al. 1985).

Several problems were adequately overcome with our technique: the protective sleeve prevents micropipette tip breakage during insertion through the sclera; the curved and beveled micropipette tip facilitates micropuncture; the joint guard prevents breakage at the mobile plastic adaptor/light pipe junction (Fig. 5); the cannula reduces the risk of retinal detachment with repeated micropipette insertions into the globe; the micromanipulator guides the micropipette with greater precision than can be achieved manually, a virtual necessity when working with object sizes near the resolving power of the human eye. Presumably, the curved tip functioned by enabling the tip to be advanced along the vessel lumen rather than be directed through the back vessel wall and by allowing retinal tissue to provide traction during micropuncture. Traction is otherwise minimal since the vessel is not directly accessible.

Other problems were inadequately overcome with our technique: although our method of micropipette tip preparation was adequate, it was tedious and time consuming. Increased efficiency could be achieved with more sophisticated beveling techniques (Vurek et al. 1967; Baldwin 1980) and by taking advantage of similar micropipette technologies that have progressed to where tip diameters of even 0.02 μm can be made (Brown and Flaming 1975). We found cannulation easier in the cat retina and attribute this to better sedation that prevented reflex mastication, better blood vessel stability, presumably due to their intraretinal location, and availability of a stereotactic device. Unfortunately, we could not use the electronic micromanipulator with the cat stereotactic device. Other micromanipulators have been designed specifically for use in animal eyes and may improve results (Wormington and Jaeger 1983). The problem of puncture site bleeding may improve with better beveling techniques (Baldwin 1980) that would permit the micropipette tip to cut a precise aperture that effectively seals itself (Brown and Flaming 1975), by injecting oil before withdrawing the tip from the vessel (Andreucci 1978), or by tamponading the vessel by increasing intraocular pressure (Hilton 1986). The problem of obtaining good intraoperative photographs may be addressed by using a beam splitter that directs more of the light beam to the camera or by inserting an endoscope camera through a third cannula port.

Retinal vessel cannulation in humans may be easier than in cats and rabbits. While there are similarities between their vascular architectures (Mutlu and Leopold 1964; Hyvarinen 1967; de Juan 1986), human retinal veins are larger: on the order of 200 μm outer diameter near the disc (Wise et al. 1971) compared with approximately 60 μm in rabbits (Hyvarinen 1967). They also lack smooth muscle in their media and should not spasm to touch. Additionally, human vessels are intraretinal like the cat, rather than preretinal like the rabbit, which should provide better vessel fixation and traction, facilitating micropuncture as we found with the cat. Furthermore, retinovascular surgery in humans is facilitated by better anesthetic protocols and by combination stereotactic/micromanipulator devices designed specifically for use in humans such as the Zeiss electronic micromanipulator. Finally, because metal tubing can be made with outer diameters on the order of 80 μm (Uniform Tubes, Collegeville, Pa.), it and presumably other materials could be used to cannulate retinal vessels in humans, while they still would be too large for laboratory animal experimentation.

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