

Effects of the Pulsed Electron Avalanche Knife on Retinal Tissue

Daniel V. Palanker, PhD; Michael F. Marmor, MD; Andre Branco, MD; Philip Huie, MSc; Jason M. Miller, MSc; Steven R. Sanislo, MD; Alexander Vankov, PhD; Mark S. Blumenkranz, MD

Objectives: To evaluate the precision of retinal tissue dissection by the pulsed electron avalanche knife (PEAK) and to assess possible toxic effects from this device.

Methods: To demonstrate precision of cutting, bovine retina (in vitro) and rabbit retina (in vivo) were incised with the PEAK. Samples were examined by scanning electron microscopy and histologic examination (light microscopy). To evaluate possible toxic effects in rabbit eyes, 30 000 pulses were delivered into the vitreous 1 cm above the retina. Histologic examinations and electroretinography were performed at intervals up to 1 month after exposure.

Results: Cuts in postmortem bovine retina showed extremely sharp edges with no signs of thermal damage.

Full-thickness cuts in living attached rabbit retina were similarly sharp and were typically less than 100 μm wide. No signs of retinal toxic effects were detected by histologic examination or electroretinography.

Conclusions: The PEAK is capable of precise cutting through retinal tissue, and there are no demonstrable retinal toxic effects from its use. The precision and tractionless nature of PEAK cutting offers advantages over mechanical tools and laser-based instrumentation. We believe this new device will prove useful in a variety of vitreo-retinal surgical applications.

Arch Ophthalmol. 2002;120:636-640

From the Department of Ophthalmology, School of Medicine (Drs Palanker, Marmor, Branco, Sanislo, and Blumenkranz and Messrs Huie and Miller) and W. W. Hansen Experimental Physics Laboratory (Drs Palanker and Vankov), Stanford University, Stanford, Calif. Stanford University is the owner of a patent covering the use of the electric plasma-mediated cutter. Should the Stanford University receive royalties or other financial remuneration in the future related to the patent, Dr Palanker may receive a share in accordance with the Stanford University Institutional Patent Policy and Procedures, which include royalty-sharing provisions. Drs Palanker and Blumenkranz are consultants to Carl Zeiss, Inc, Jena, Germany.

RECENTLY, OUR group developed a new type of surgical instrument for precise microdissection of tissue in liquid media. This device, called the pulsed electron avalanche knife (PEAK), uses electrical pulses rather than laser light for the generation of plasma microstreamers in physiological medium or in tissue.¹ Nanosecond pulses of high voltage (2-7 kV) applied to an intraocular microelectrode produce plasma in a conductive fluid, which is confined to the immediate vicinity (several tens of micrometers) of the instrument's tip (**Figure 1**). High temperatures associated with this process cause explosive evaporation of the surrounding liquid that results in ablation or dissection of the adjacent tissue. Because of the short duration of the pulse, dissection takes place with no thermal damage to the surrounding tissue; the cuts are purely mechanical and can be very well controlled.

Lasers that generate plasma have been used within the eye for a number of years without evidence of toxic effects beyond the mechanical effects, and neither glass nor tungsten (which form the PEAK microelectrode) is known to have a toxic

effect on ocular tissue.² Nevertheless, free radicals produced by plasma in liquid,³ UV light emitted by plasma,⁴ and metal etched from the electrode tip could, in theory, have a toxic effect on tissue.

In this work, we demonstrate the precision of the PEAK in cutting of retinal tissue, both in vitro and in vivo, and evaluate whether there are any toxic effects from free radicals, UV light, or etched metal associated with application of the PEAK.

RESULTS

PRECISION OF CUTTING WITH THE PEAK

Cuts were made in bovine retina while the probe was held by hand and moved with a velocity of approximately 1 mm/s. Scanning electron microscopy (**Figure 2**) showed crisp cuts of about 50 μm in width. Some perforations from single PEAK pulses were seen at the edge of the cut. A histologic section of a similar cut (**Figure 3**) showed that the edges of the cut were remarkably abrupt and there were no signs of coagulation or cellular disruption beyond the edge.

MATERIALS AND METHODS

Ten bovine eyes were obtained from a local slaughterhouse 3 hours after death and packed on ice during delivery. Retinal dissection with the PEAK was performed through the vitreous after removal of cornea, lens, and iris with the use of 100-nanosecond pulses at 50 μJ with a repetition rate of 20 Hz. This energy level is near the minimum required to produce a full-thickness retinal cut.

Fifteen albino rabbits weighing 3.0 to 3.5 kg were used in accordance with the Association for Research in Vision and Ophthalmology Statement Regarding the Use of Animals in Ophthalmic and Vision Research. Before retinal cutting, a partial vitrectomy was performed via a 1-mm sclerotomy made 3 mm behind the limbus. The PEAK probe (shown diagrammatically in Figure 1) was inserted through the same sclerotomy, and 2 linear cuts of about 4 mm in length were made parallel to the medullary ray with the use of 100-nanosecond pulses of 17 μJ with a repetition rate of 10 Hz. This energy level was just sufficient to cut the full thickness of the retina without producing hemorrhage. The eyes were enucleated immediately thereafter and fixed in glutaraldehyde-paraformaldehyde fixative, postfixed in osmium tetroxide, and embedded in epoxy resin (Epon 812; Electron Microscopy Sciences, Fort Washington, Pa). One-micrometer sections were cut on an ultramicrotome and stained with toluidine blue for light microscopy.

For scanning electron microscopy, retina was fixed in glutaraldehyde-paraformaldehyde fixative (1.25%/1% in 0.2M sodium cacodylate buffer) at room temperature for 30 minutes. The tissue was then fixed for an additional 18 hours at 4°C, rinsed in buffer, and postfixed in osmium tetroxide. The tissue was washed with distilled water, dehy-

drated in a series of ethanols, transferred to absolute acetone, and critical point dried. The dried tissues were then mounted on stubs and plasma coated with gold.

To test for possible toxic effects from the PEAK probe, we applied the discharges into the vitreous of 10 rabbit eyes. To maximize concentration of the substances released during the procedure, no infusion and no irrigation were attached to the eye. Electroretinography (ERG) was performed on 5 rabbits before, and at intervals after, the procedure. For ERG recordings, the pupils were dilated and the eyes dark adapted for 20 minutes. A jet contact lens electrode was positioned on the cornea with a reference wire placed under the eyelid and a ground attached to the ear. The stimulus flashes at setting 1 were applied with a flash lamp (PS22; Grass Instruments Co, West Warwick, RI). The signal was amplified with a preamplifier (SR530; Stanford Research Systems Inc, Mountain View, Calif) and recorded on an oscilloscope (TDS 620B; Tektronix Inc, Irvine, Calif). In 5 other rabbits, we applied the same PEAK treatment and enucleated the eyes 4 and 14 days after surgery for histologic evaluation with light microscopy.

Light emission from the PEAK was measured with a large-area silicon photoreceiver (model 2031; New Focus Inc, San Jose, Calif).

To compare the rates of metal erosion from the PEAK with that of conventional diathermy, we applied the Wet-Field Hemostatic Eraser (Mentor Ophthalmic Inc, a division of Goldman and Shurtleff Inc, Randolph, Mass) powered by a Wet-Field Coagulator (Mentor Ophthalmic Inc) at settings typical for retinal applications (25/70 on the instrument's scale).

In all the surgical applications of the PEAK in this study, pulses of 100-nanosecond duration and negative polarity were applied to the central wire of the PEAK probe, while the coaxial needle served as a return electrode (see Figure 1).

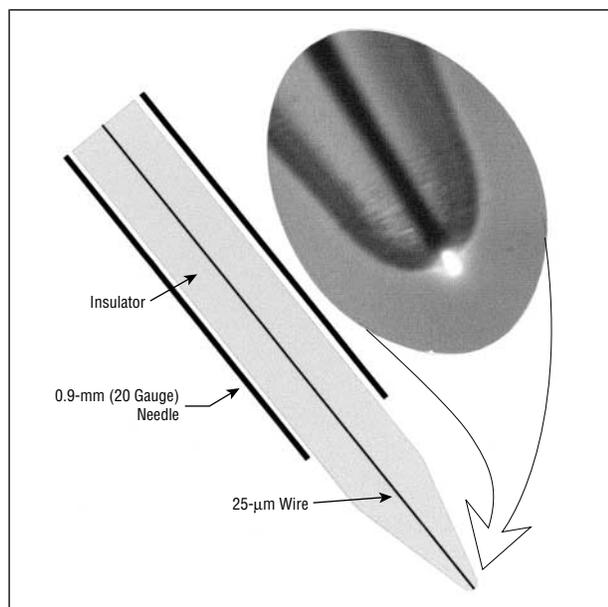


Figure 1. Diagram of the intraocular pulsed electron avalanche knife probe. Inset is a photograph of the plasma formed in saline in front of the probe.

In living rabbit eyes, a single PEAK pulse produced a small crater from which tissue was removed to about half retinal thickness (**Figure 4**). By applying pulses at a rep-

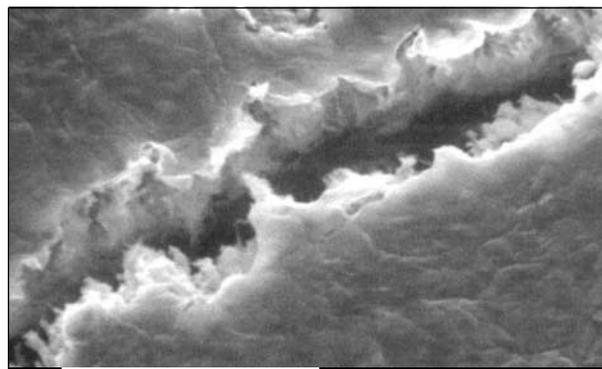


Figure 2. Scanning electron micrograph of the cut in bovine retina produced by pulses of 50 μJ at 20-Hz repetition rate. Bar (black as well as white lines) indicates 0.1 mm (original magnification $\times 503$).

etition rate of 10 Hz and scanning across the retina at a rate of 0.5 to 1.0 mm/s, we made full-thickness cuts through retina without choroidal damage. There was no bleeding from cuts through avascular rabbit retina, but the major retinal vessels bled if cut, since the device does not coagulate tissue. Typical cuts, as shown in **Figure 5**, were remarkably clean and sharp. The width of the dissected zone was generally less than 100 μm , and neither retinal detachment nor signs of thermal damage were observed near the cut. If multiple pulses were delivered to one location, there

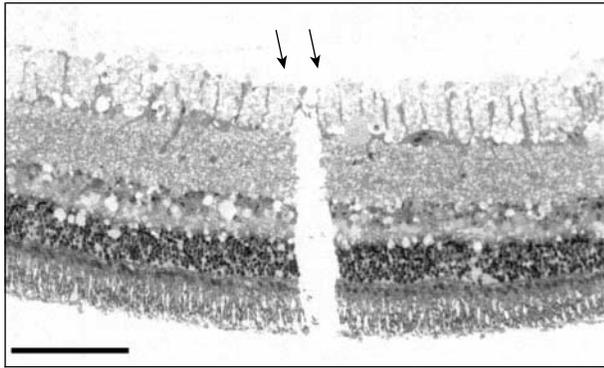


Figure 3. A full-depth cut in bovine retina resulting from application of 50- μ J pulses at 20 Hz and 1 mm/s scanning rate. The walls of the cut are remarkably sharp, and no signs of coagulation or other thermal damage are observed at the edges (arrows). Bar indicates 0.1 mm.

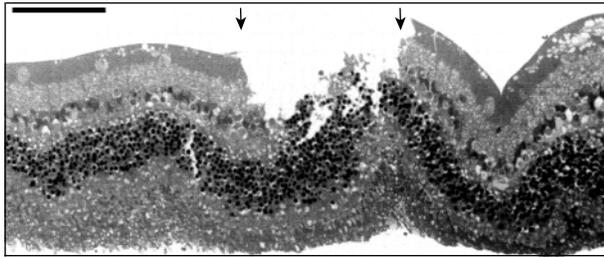


Figure 4. Center of the crater produced in the rabbit retina by a single shot of 17 μ J. The retina was detached from the retinal pigment epithelium and choroid during the sample preparation. Arrows show the edges of the cut; bar indicates 0.1 mm; photograph was taken with $\times 20$ objective.

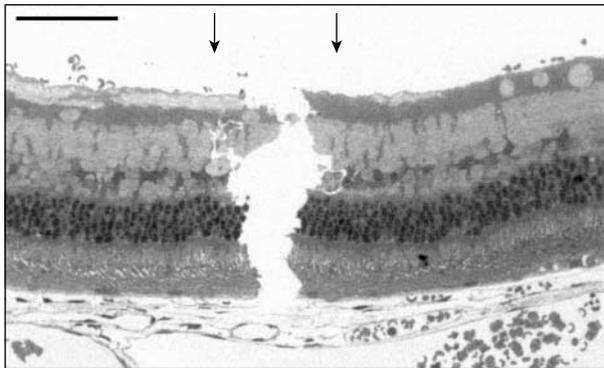


Figure 5. Histologic section of the full-thickness cut in rabbit retina produced at discharge energy of 17 μ J per pulse, repetition rate of 10 Hz, and scanning rate of about 1 mm/s. Arrows show the edges of the cut; bar indicates 0.1 mm; photograph was taken with $\times 20$ objective.

was deeper penetration into the choroid, resulting in hemorrhage (**Figure 6**). Although PEAK effects at these settings were readily apparent histologically, they were nearly invisible to a surgeon using an operating microscope; the surgeon saw only very faint whitening of the retina along the line of scanning with the probe. In contrast, the cuts in detached retina and epiretinal membranes were readily observable, since these tissues are more opaque, and the gap was easily seen as they split apart during dissection.

TOXIC EFFECTS FROM PEAK APPLICATIONS

To measure the erosion rate of the tungsten wire in a PEAK probe, we took micrographs of the electrode after pro-

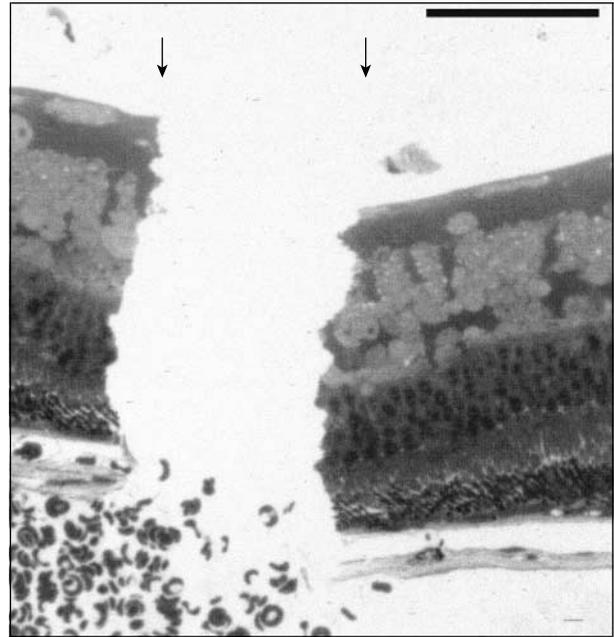


Figure 6. Histologic section of the same cut at the site of choroidal bleeding. Note the sharp edges of the cut (arrows) and lack of retinal detachment and of thermal damage at the edges. Bar indicates 0.1 mm; photograph was taken with $\times 20$ objective.

gressively larger numbers of discharges (**Figure 7**). At pulse energy of 150 μ J, the erosion rate was on the order of 0.065 μm^3 per pulse. During “maximal” surgery, which we estimate might involve 10 minutes of cutting time and 3×10^4 pulses, the total eroded volume would be on the order of $2 \times 10^3 \mu\text{m}^3$ (equivalent to 0.04 μg or 0.2 nmol).

For comparison, we measured the amount of metal released by a standard wet-field diathermy probe in typical operational conditions. As can be seen in **Figure 8**, about $3 \times 10^5 \mu\text{m}^3$ of iron was removed during 100 seconds of application. The total duration of coagulation treatment in typical vitreoretinal surgery is less than 20 seconds (with pulses of 1-3 seconds in duration), which would correspond to $6 \times 10^4 \mu\text{m}^3$ (0.5 μg , 8 nmol) of iron released into vitreous during a coagulation-intensive operation.

To directly evaluate possible toxic effects of the PEAK probe to retina, we applied 3×10^4 pulses of 180 μ J in 5 rabbit eyes. The energy was delivered into vitreous toward the posterior pole about 1 cm from the retina, and the eyes were not perfused to maximize possible toxic effects. Preoperatively and at 7, 11, and 30 days after the operation, ERG was performed. The results from all 5 animals were essentially identical and showed no influence of the procedure on the ERG (**Figure 9**).

Five additional animals were exposed to the same procedure and were killed 4 and 14 days after the treatment. Light microscopy of histologic sections showed no retinal changes in the eye exposed to the PEAK compared with the control eye.

COMMENT

The PEAK makes cuts through bovine and rabbit retina that are remarkably sharp, with no evidence of cellular

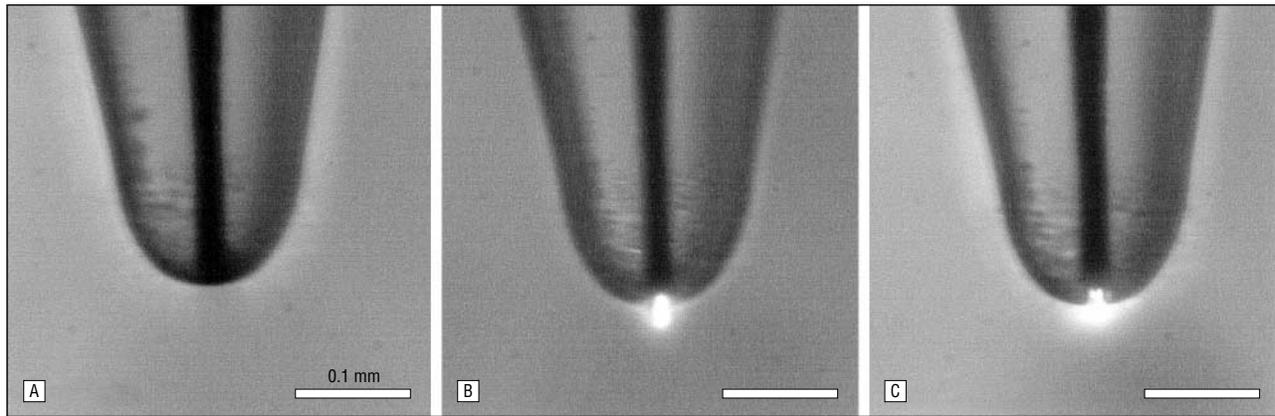


Figure 7. Pulsed electron avalanche knife probe photographed on inverted microscope via $\times 20$ objective during the treatment. A, Probe before the irradiation. B, Erosion after 50 000 pulses at 150 μJ per pulse. Bright plasma streamer is seen in front of the tip. C, Erosion after 106 000 pulses. Plasma originates at the surface of metal deeper inside the probe; 13 μm of 25- μm -thick tungsten wire was etched.

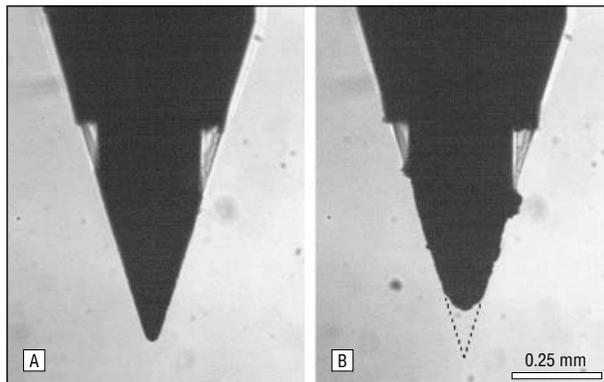


Figure 8. Diathermy probe after 20 seconds (A) and 100 seconds (B) of treatment in isotonic sodium chloride solution. Photograph was taken with $\times 10$ objective; dashed lines schematically indicate the initial shape of the tip.

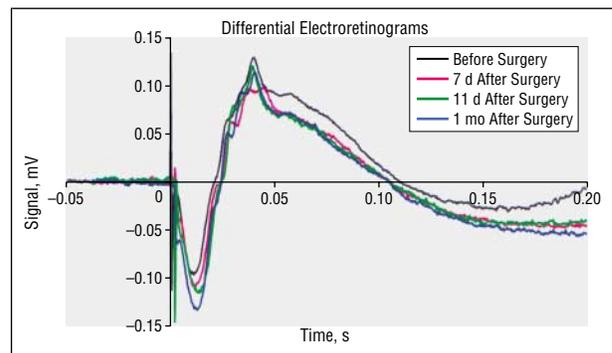


Figure 9. Electroretinographic measurements before and after (7, 11, and 30 days) treatment of the rabbit eye with 30 000 pulses of 180 μJ . The results from the other 4 animals were essentially identical.

damage at distances beyond the first layer of cells adjacent to the edge. Such cutting resembles cracking rather than ablation of tissue, since very little material seems to be ejected from the application site. The cuts are so clean, in fact, that in attached retina they are nearly invisible to a surgeon using an operating microscope. For this reason it will be important to perform further studies to define the proper and safe pulse energies for different applications on the retina. Visibility of cuts is less of a problem with detached retina and epiretinal membranes, since the cuts are readily observable in these more opaque tissues, and because these tissues tend to spread apart during dissection (leaving a gap that is easily observed on a dark background). Longer-term studies are needed to assess whether there is any injury to cells at the margins of the PEAK cuts that was not visible in tissue enucleated immediately after surgery. Our present experiments, which were designed to show only the precision of cutting, did not look beyond the immediate effects of the surgery.

Since histologic examination of the cut retina shows no signs of coagulation effects, the faint whitening of the retinal surface that we observed during cutting of the attached retina may be a result of axoplasmic flow abnormalities from damage to nerve fiber layer in the cut area. It should be emphasized that, since energy deposition is

localized at the end of the probe, the instrument cuts most efficiently if it is held close to the tissue (within 100 μm). Cutting width and depth are highly reproducible with a fixed position of the probe. However, reproducibility of cutting *in vivo*, especially on attached retina, will be influenced by hand control and the fact that natural tremor of a human hand itself is on the order of 100 μm (or more). Pushing the probe into attached retina would eliminate this problem, but it must be avoided to prevent mechanical damage. On the other hand, on detached retina and epiretinal membranes, higher energy can be applied and the probe can safely touch the tissue to ensure a close contact. As a result, clinical cutting of detached retina and epiretinal membranes is more reproducible than cutting of attached retina.

Advantage of the PEAK as compared with scissors or blades is mainly in the tractionless nature of its cutting. Erbium:YAG^{3,6} and argon fluoride excimer lasers^{7,8} have also been applied for tractionless vitreoretinal cutting, but these devices did not gain widespread acceptance in practice mostly because of the high cost, large size, and somewhat rigid fibers. The PEAK is a much smaller and less expensive device, with a light handpiece and very flexible cable. In addition, PEAK probes can be bent at any angle without losing cutting efficiency.

The availability of a precise microsurgical tool capable of producing microscopic cuts of controlled width

and depth without traction should facilitate many aspects of vitreoretinal surgery. The PEAK will be useful in the performance of the circumferential and radial retinotomies and in the dissection of complex epiretinal and fibrovascular membranes. Its value will be particularly evident in eyes where excessive traction or pressure could cause collateral damage, such as those with atrophic underlying retina susceptible to tractional tears, and with membranes adjacent to blood vessels or overlying detached retina. In addition, this tool may be applied to posterior and anterior capsulotomy and other ocular surgical procedures.

The generation of plasma in liquids could, in theory, be toxic to tissue on the basis of the formation of free radicals, the generation of UV light, and the erosion of a metal electrode. To calculate the maximal exposure that is likely to occur, typical applications of PEAK require discharge energies between 50 and 150 μJ , at a pulse repetition rate of about 30 Hz, and the total number of pulses does not exceed 5000. An exceedingly large exposure, which we shall use for the sake of discussion, would be 10 minutes of total application time at 100 μJ and 50 Hz (3×10^4 pulses). With this exposure, the total deposited energy is 3 J, and the average cumulative energy density in the posterior pole of a human eye would be about 0.75 J/mL.

Free radicals ($\cdot\text{H}$ and $\cdot\text{OH}$) can be produced directly by plasma-mediated discharge, but their lifetime in water is only about 70 nanoseconds, during which they can only diffuse about 20 nm³. Thus, free radicals will have no effect beyond the zone of mechanical damage (which may vary between a few and a few hundred micrometers). The recombination of 2 OH radicals can produce hydrogen peroxide (H_2O_2), which is a strong and long-living oxidizer. However, it has been shown³ that the threshold energy density to produce detectable H_2O_2 toxicity is about 500 J/mL, which is 3 orders of magnitude greater than would occur with the PEAK.

Our measurements of light emission by plasma indicate that UV toxicity is also unlikely to occur during PEAK surgery, since the total dose of photic irradiation during a PEAK-intensive operation (involving 3×10^4 pulses) would be 6 orders of magnitude lower than the established danger levels for retinal exposure to UV light (5 J/cm^2).⁹ One possible source of PEAK toxicity involves deposition of eroded metal from the microelectrode. We compared the amount of metal etched from the PEAK probe in typical operational conditions with that released by a standard wet-field diathermy probe. Diathermy electrodes are made of steel and erode during electrosurgery, producing iron ions in various oxidation states. Iron metal and iron compounds have important toxic actions on the eye. These have been studied for more than a century and have been the subject of an extensive body of literature.^{10,11} According to our measurements, 0.5 μg (or 8 nmol) of steel is eroded during 20 seconds of application of diathermy at typical vitreoretinal settings, which is about 25% of the threshold amount of iron shown to be toxic if injected into the vitreous.¹¹ However, eyes are perfused during retinal surgery, which should reduce the toxic load by a considerable margin (a factor of 2 to 10 on the basis of typical perfusion rates and duration). To our knowledge, no observations of retinal (or other) toxic effects from dia-

thermy probes have been reported so far, even though some operations may have involved even longer exposures.

The central electrode in the PEAK probe is made of tungsten. No toxic effect from tungsten metal has been found in experiments with intravitreal implants in rabbit eyes.^{10,12} During typical surgery involving 3×10^4 pulses, 0.04 μg (or 0.2 nmol) of tungsten will be eroded. This amount is only 2.5% of the number of moles of iron left in the eye after application of diathermy for 20 seconds. Since 8 nmol of rather toxic metallic iron released by diathermy does not seem to have any adverse effects on ocular tissues during surgery, the much smaller amount (0.2 nmol) of nontoxic tungsten is probably safe. These estimations have also been supported by the lack of adverse effects of PEAK treatments on the ERG and on retinal histologic findings in our experiments.

We conclude that the PEAK is capable of precise cutting through retinal tissue, and that there are no demonstrable retinal toxic effects from the use of this device. The precision and tractionless nature of PEAK cutting offers potential advantages over mechanical tools, as well as over laser-based cutting instrumentation. We believe this new device could be useful in a variety of vitreoretinal surgical applications.

Submitted for publication May 31, 2001; final revision received November 7, 2001; accepted December 21, 2001.

This study was supported by grant 1 R01 EY12888-01A1 from the National Eye Institute, Bethesda, Md, and by Carl Zeiss Inc, Jena, Germany.

We thank Rupa Dalal for her help with sample preparations.

Corresponding author and reprints: Daniel V. Palanker, PhD, W. W. Hansen Experimental Physics Laboratory, Stanford University, Stanford, CA 94305-4085 (e-mail: palanker@stanford.edu).

REFERENCES

1. Palanker DV, Miller JM, Marmor MF, Sanislo SR, Huie P, Blumenkranz MS. Pulsed electron avalanche knife (PEAK) for intraocular surgery. *Invest Ophthalmol Vis Sci.* 2001;42:2673-2678.
2. Grant MW, Schuman JS. Tungsten metal. In: *Toxicology of the Eye*. Springfield, Ill: Charles C Thomas Publisher; 1993:1480.
3. Sato M, Ohgiyama T, Clements JS. Formation of chemical species and their effects on microorganisms using a pulsed high-voltage discharge in water. *IEEE Trans Ind Appl.* 1996;32:106-112.
4. Sun B, Sato M, Clements JS. Optical study of active species produced by a pulsed streamer corona discharge in water. *J Electrostatics.* 1997;39:189-202.
5. Brazitikos PD, D'Amico DJ, Bernal MT, Walsh AW. Erbium:YAG laser surgery of the vitreous and retina. *Ophthalmology.* 1995;102:278-290.
6. D'Amico DJ, Blumenkranz MS, Lavin MJ, et al. Multicenter clinical experience using an erbium:YAG laser for vitreoretinal surgery. *Ophthalmology.* 1996;103:1575-1585.
7. Palanker D, Hemo I, Turovets I, Zauberman H, Fish G, Lewis A. Vitreoretinal ablation with the 193-nm excimer laser in fluid media. *Invest Ophthalmol Vis Sci.* 1994;35:3835-3840.
8. Hemo I, Palanker D, Turovets I, Lewis A, Zauberman H. Vitreoretinal surgery assisted by the 193-nm excimer laser. *Invest Ophthalmol Vis Sci.* 1997;38:1825-1829.
9. Ham WT Jr, Mueller HA, Ruffolo JJ Jr, Guerry D III, Guerry RK. Action spectrum for retinal injury from near-ultraviolet radiation in the aphakic monkey. *Am J Ophthalmol.* 1982;93:299-306.
10. Grant MW, Schuman JS. Iron metal and iron compounds. In: *Toxicology of the Eye*. Springfield, Ill: Charles C Thomas Publisher; 1993:845-859.
11. Gardner HB. Deferoxamine: effects on intravitreal iron. *Exp Eye Res.* 1976;23:333-339.
12. Laurant L, Wergeland FL Jr. Ocular toxicity of newer industrial metals. *Mil Med.* 1970;135:1171-1174.