Laser-Induced Alteration of Collagen Substructure Allows Microsurgical Tissue Welding

R. Schober, F. Ulrich, T. Sander, H. Dürselen, S. Hessel

Tissue welding is a potentially important biomedical application of laser technology. The structural alterations basic to this phenomenon were studied in experimental repair of lesions of the rat carotid artery and sciatic nerve. A modified neodymium-doped yttrium-aluminum-garnet laser operating at a wavelength of 1.319 micrometers was used in conjunction with conventional suture techniques. Histological and fine-structural analysis revealed a homogenizing change in collagen with interdigitation of altered individual fibrils that appeared to be the structural basis of the welding effect.

Tissue interactions with laser irradiation are based on either photochemical or thermal reactions. The thermal effects of photocoagulation and photovaporization have found many biomedical applications (*1*), including incision of tissue (*2*), hemostasis by coagulation of blood vessels (*3*), and selective photothermolysis (*4*). The use of the laser as a tissue-welding device was inaugurated with the treatment of retinal detachment (*5*). Apart from ophthalmological applications, however, it is still in the experimental stage. Pioneering work has been done with the repair of small blood vessels (*6*) and with the anastomosis of severed peripheral nerves (*7*). Jain and Gorish imputed fusion of collagen to the tissue-bonding laser effect (*8*), although the exact mechanism is still unclear.

We have investigated this problem by histological and fine-structural analysis of laser effects in microsurgically treated lesions of rat carotid arteries and sciatic nerves. A neodymium-doped yttrium-aluminum-garnet (Nd:YAG) laser was chosen because of the reported positive experience and because of its convenient handling through a flexible quartz fiber-optic delivery system. Disadvantages of the 1.06-μm wavelength commonly used (*9*) were overcome by use of a newly developed instrument operating at 1.319 μm. Although earlier reports about the biomedical utility of this wavelength were conflicting (*10*), comparative investigations have shown that its absorption in water is much stronger, entailing a more efficient transfer of energy (*11*).

An additional new development was a 200-μm focusing light conductor, which results in sufficient depth of field at a focal diameter of 200 μm with a 1:1 optical system. The maximum output power thus had to be restricted to 12.5 W, which, however, proved to be sufficient for the purposes of tissue welding (*12*).

In preliminary tests on carotid arteries of adult albino rats dissected in situ, a setting of 12 W and 0.1 second was found to be optimal for a single laser exposure. The result was a circumscribed coagulation point as seen in the operating microscope. No carbon deposits could be detected by histological analysis. The changes consisted of a localized swelling and homogenization of the adventitia as well as a coagulation necrosis of smooth muscle cells in the underlying media. The structural integrity of the latter was preserved since the elastic lamellae were essentially unaltered. Segments of the vessel wall adjacent to and opposite the beam impact appeared normal (Fig. 1A). Two to four such exposures were sufficient to seal vessel wall punctures made by a 26-gauge needle or small incisional lesions (Fig. 1B). Light- and electron-microscopic examination showed the sealing to be due to the homogenizing adventitial change bridging the lesion.

High-resolution analysis (Fig. 2) revealed that the collagen fibrils in this area had lost their periodicity, were greatly increased in caliber, and were split into fine fibrillary substructures with a roughly concentric arrangement on cross section. The altered collagen fibrils were still individually recognizable. However, they were not separated as they were in controls, but closely interdigitated with each other. This interdigitation seems to be the structural basis for the laser-induced welding effect.

The encouraging results with repair of smaller lesions led us to perform carotid artery anastomoses after the vessel was completely severed (*13*). Additional stay sutures that had to be used were left in place after application of six to ten laser welding points. Although almost all animals survived, histological examination after as long as 11 weeks showed a higher rate of thrombosis with ensuing recanalization and of aneurysm formation; these results are in accord with those acquired with other types of lasers. Such complications may be less likely to occur in anastomoses in which there are no pulsatile forces in action.

Severed sciatic nerves of adult albino rats were operated on under similar conditions and examined immediately and at intervals of 1 to 4 weeks, by using both paraffin and plastic embedding techniques. In two cases an autologous nerve transplant from the opposite limb was interposed between the stumps and welded on both sides. As in the carotid arteries, the principal acute laser effect was collagen homogenization and tumefaction of the supporting structures, predominantly of the epineurium. Despite the enormous expansion, individual layers of flat perineurial cells were still preserved (Fig. 3A).

Reactive changes were minimal, and foreign body granulomas were observed exclusively around the silk sutures. In this respect, the 1.319-μm Nd:YAG laser seems to have distinct advantages over other lasers that induce carbonaceous deposits under comparable experimental conditions (*7*). Such deposits can potentially give rise to

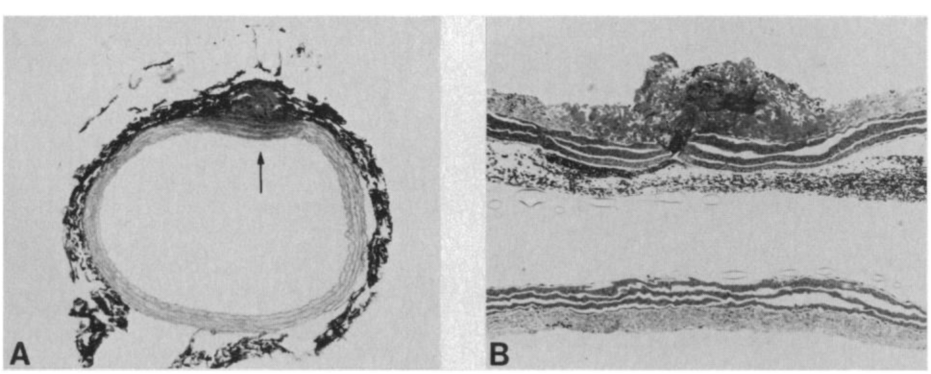

Fig. 1. Acute laser effect with localized adventitial change in rat carotid artery. (A) Cross section of control exposed to a single impulse (arrow), Masson-stained paraffin section, ×40. (B) Longitudinal section of sealed stab lesion, toluidine blue–stained plastic section, ×50. Note the lack of alterations in the opposite side of the wall.

R. Schober, Department of Neuropathology, University of Düsseldorf, 4000 Düsseldorf 1, West Germany.
F. Ulrich, T. Sander, H. Dürselen, Department of Neurosurgery, University of Düsseldorf, 4000 Düsseldorf 1, West Germany.
S. Hessel, Messerschmitt-Bölkow-Blohm Medizintechnik, 8000 Munich 80, West Germany.

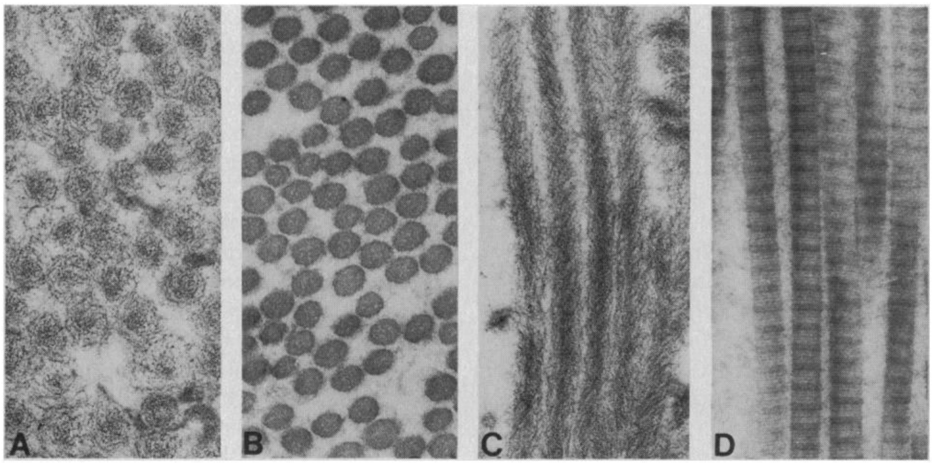

Fig. 2. Ultrastructure of laser-induced adventitial collagen alteration in cross (A) and longitudinal (C) section. The welding effect becomes apparent by comparison with nonirradiated areas at the same magnification (B and D), ×32,000.

scarring detrimental to nerve repair and even to adverse immunologic reactions (*14*).

Axonal outgrowth at the site of anastomosis was vigorous, and myelination of regenerated axons in the distal stump progressively increased during the examined intervals. The capacity for neurite regeneration is apparently not limited by laser effects, a result in accord with experiments on single cells in tissue culture (*15*). The direction of sprouting, however, was not confined to the distal stump undergoing Wallerian degeneration. We observed various degrees of neuroma formation, predominantly in the broadly widened sheath structures where miniature fascicles replaced the altered collagen. Surprisingly, and probably because of the framework of residual or regenerated perineurial cell layers, the fascicles seemed to be oriented longitudinally and not in the irregular and tortuous fashion characteristic of neuromas (Fig. 3B). Comparable changes were recorded in experimental cialit-conserved homologous transplants and were termed "neuromatous neurotization," implying that the extrafascicular guidance of outgrowing fibers might aid in the restoration of proper connections and thus improve the chance for functional recovery (*16*).

The feasibility of laser-induced tissue welding is apparent from these two experimental investigations. In regard to anastomoses, further technical modifications and long-term evaluation by morphological methods are required before the techniques can be safely applied clinically. The 1.319-μm Nd:YAG laser seems particularly useful as an auxiliary instrument in microsurgery of peripheral nerves where the ideal repair would use no stitches (*17*). Its ability to weld by altering intrinsic supportive tissue structures without inducing a foreign reaction may render it advantageous for other microsurgical applications as well.

Note added in proof: Independently conducted vessel welding studies with other lasers (*18*) have reported light microscopic collagen changes comparable both to our results and to those obtained with electrocoaptive anastomoses (*19*). Their specificity therefore remains to be determined by comparative ultrastructural investigations.

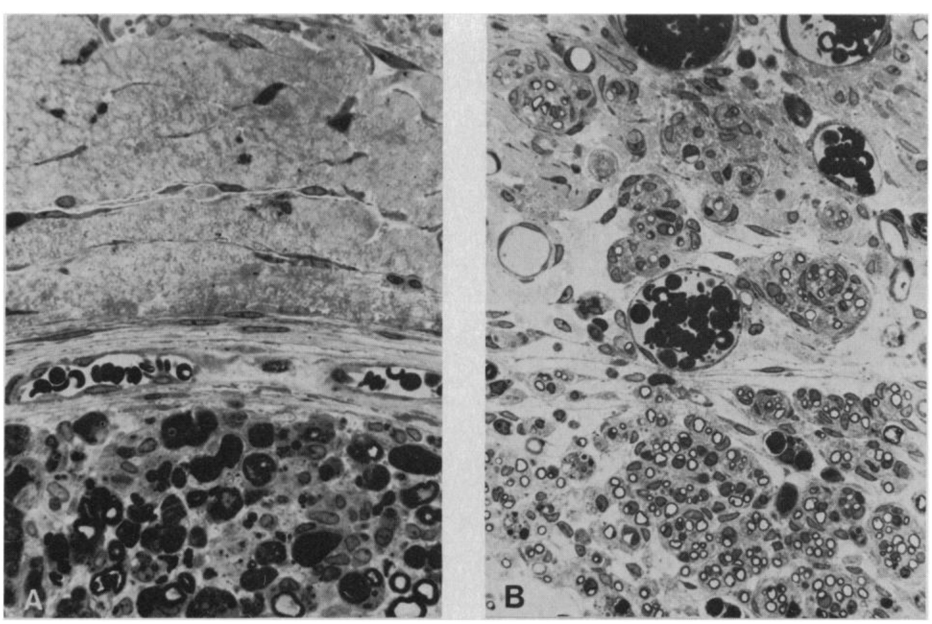

Fig. 3. Laser-assisted peripheral nerve anastomoses after 1 week (A) and after 3 weeks (B) in toluidine blue–stained 1-μm plastic sections, ×360. Wallerian degeneration with ensuing vigorous regeneration of the endoneurium can be seen in the lower half; concentric collagen alteration with ensuing vascularization and neuromatous neurotization of the supportive nerve structures appears in the upper half.

REFERENCES AND NOTES

1. F. Hillenkamp, R. Pratesi, C. A. Sacchi, Eds., *Lasers in Biology and Medicine* (Plenum, New York, 1980).
2. R. R. Hall, A. D. Beach, E. Baker, P. C. A. Morison, *Nature (London)* **232**, 131 (1971); M. S. Litwin, S. Fine, E. Klein, B. S. Fine, *Arch. Surg.* **98**, 219 (1969).
3. F. Leheta and W. Gorisch, in *Laser Surgery I*, I. Kaplan, Ed. (Jerusalem Academic Press, Jerusalem, 1976).
4. R. R. Anderson and J. A. Parrish, *Science* **220**, 524 (1983).
5. F. A. L'Esperance, Jr., *Trans. Am. Acad. Ophthalmol. Otolaryngol.* **75**, 609 (1971).
6. W. Z. Yahr and K. S. Strully, *J. Assoc. Adv. Med. Instr.* **1**, 28 (1966); K. K. Jain, *Microsurgery* **1**, 436 (1980).
7. D. W. Fisher, J. L. Beggs, D. L. Kenshalo, Jr., A. G. Shetter, *Neurosurgery (Baltimore)* **17**, 300 (1985).
8. K. K. Jain and W. Gorisch, *Surgery* **85**, 684 (1979).
9. T. A. Fuller, *Lasers Surg. Med.* **1**, 5 (1980); M. L. Saunders *et al.*, *Surg. Neurol.* **14**, 1 (1980).
10. A. Vasko, *Infra-Red Radiation* (Iliffe, London, 1968), p. 308; J. E. Harry, *Industrial Lasers and Their Applications* (McGraw-Hill, London, 1974), p. 171; L. F. Stokes, D. C. Auth, D. Tanaka, J. L. Gray, C. Gulacsik, *IEEE Transact. Biomed. Eng.* **BME-28**, 297 (1981).
11. F. Frank, O. J. Beck, S. Hessel, E. Keiditsch, *Lasers Surg. Med.*, in press.
12. F. Ulrich *et al.*, *ibid.*
13. F. Ulrich and W. J. Bock, *Adv. Neurosurg.*, in press.
14. B. Helpap, *Res. Exp. Med.* **183**, 215 (1983).
15. E. Rieske and G. W. Kreutzberg, *Brain Res.* **148**, 478 (1978); G. W. Gross, J. H. Lucas, M. L. Higgins, *J. Neurosci.* **3**, 1979 (1983).
16. J. M. Schröder and K. E. Seiffert, *Virchows Arch. B* **5**, 219 (1970); W. Krücke, in *Handbuch der Neurochirurgie*, H. Olivecrona, W. Tönnis, W. Krenkel, Eds. (Springer-Verlag, Berlin, 1964), vol. 7, part 3.
17. M. E. Jabaley, *J. Hand Surg.* **9-B**, 14 (1984).
18. O. H. Frazier, G. A. Painvin, J. R. Morris, S. Thomsen, C. R. Neblett, *Surgery* **97**, 585 (1985); M. R. Quigley *et al.*, *Lasers Surg. Med.* **5**, 357 (1985); S. Pribil and S. E. Powers, *J. Neurosurg.* **63**, 771 (1985).
19. B. Sigel and M. R. Dunn, *Surg. Gynecol. Obstetr.* **121**, 823 (1965).

20. Dedicated to Prof. Dr. W. Krücke, Frankfurt, on occasion of his forthcoming 75th birthday. Supported by the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen, Federal Republic of Germany.

2 December 1985; accepted 18 February 1986