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FINAL TECHNICAL REPORT
CONTRACT (N00014-87-C -0146)
OFFICE OF NAVAL RESEARCH
VANDERBILT UNIVERSITY FREE-ELECTRON LASER CENTER
FOR BIOMEDICAL AND MATERIALS RESEARCH

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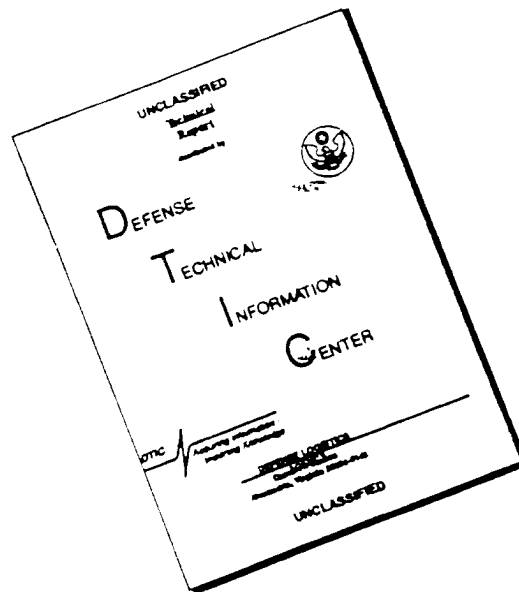
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Bibliography and Meeting Abstracts

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FORWARD

During the period 2/1/87 to 1/31/91, Vanderbilt University, under Contract N00014-87-C-0146 with the Office of Naval Research, developed a Free-Electron Laser Center, and carried out a program of research in medicine, biology, and materials science. The accomplishments of this effort are described in the final report.

The program may be divided into six projects, as follows:

I. **Free-Electron Laser Construction and Operation** under the direction of C. A. Brau, Department of Physics, College of Arts and Science. The objective of this effort has been to commission the free-electron laser and the optical beamline required to distribute the laser beam to the laboratories. Although the company supplying the free-electron laser ceased operations before the laser was completed, the installation and commissioning was completed by Vanderbilt personnel.

II. **Mechanisms of Photon-Induced Damage in Optical Materials**. The effort may be further subdivided into three efforts:

A. **Desorption of Atoms and Molecules from Surfaces**, under the direction of N. H. Tolk, Department of Physics, College of Arts and Science,

B. **Nonlinear Effects of Lasers**, under the direction of R. F. Haglund, Department of Physics, College of Arts and Science.

C. **Interaction of Lasers with Defects in Optical Materials**, under the direction of R. A. Weeks, Department of Materials Science and Engineering, School of Engineering.

III. **Picosecond Spectroscopy of Biopolymers**. Under the direction of G. S. Edwards, Department of Physics, College of Arts and Science, the objective of this research has been to examine the vibrational and electronic spectroscopy of important biopolymers, including DNA and RNA.

IV. **Nonthermal and Selective Effects of Free-Electron Laser Irradiation of Tissue**. Under the direction of R. H. Ossoff, Department of Otolaryngology, School of Medicine, the objective of this research has been to examine the effects of lasers on tissue, including the effect on wound healing.

V. Free-Electron Laser Based Studies of Biomembrane Dynamics and Drug Interactions. Under the direction of S. Fleischer, Department of Molecular Biology, College of Arts and Science, the objective of this research has been to understand the dynamics of membrane proteins, and how they are related to membrane function.

VI. Free-Electron Laser Applications in Neurosurgery. Under the direction of R. J. Maciunas, Department of Neurosurgery, School of Medicine, the objective of this effort has been to study the interaction of pulsed lasers with brain tissue, and to develop the techniques of stereotactic laser neurosurgery.

The close collaboration of researchers from so many Schools and Departments of the University has been a major factor in the success of the program, and will continue to be so as the program continues and expands to embrace researchers from around the world.

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Vanderbilt University Free Electron Laser Center
for Biomedical and Materials Research
N00014-87-C-0146

V. C

October 1987 - December 1990
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DYNAMIC CHARACTERISTICS OF BIOMEMBRANES AND MEMBRANE PROTEINS
J. Oliver McIntyre and Sidney Fleischer

Our long-range research interest is to understand how the cell works in terms of its component organelles and membranes. We are studying cell function at several levels of organization, from the intact cell, to organelles, to membranes and to molecular components. The focus of our biophysical studies is on two **membrane proteins**. The first is the **calcium pump protein (CPP)** of skeletal muscle sarcoplasmic reticulum which is one of the extensively studied examples of a membrane ion pump due to its importance in cell and muscle physiology as well as being a model for understanding biological pumps generally. The second, **3-hydroxybutyrate dehydrogenase (BDH)**, is the best-studied example of a lipid-requiring enzyme. BDH has an absolute and specific requirement for the phospholipid phosphatidylcholine (PC) for enzymic activity.

Our pre-FEL research includes studies to characterize the **rotational motion** of membrane proteins as well as to measure **distances** between unique sites on membrane proteins with respect to one another and with respect to defined positions in the phospholipid bilayer. The biophysical methods employed are fluorescence lifetime and time-dependent anisotropy.

Preliminary studies have been carried out with the calcium pump protein, a transmembrane ion pump. The pump protein has been labeled at unique sites with either IAEDANS or FITC or EOSIN fluorescent probes and fluorescence lifetime and time-dependent anisotropy studies are in progress. We have also begun studies to characterize the dynamic properties of BDH reconstituted into phospholipid vesicles both in the presence and absence of activating phospholipid, i.e., PC. Initial studies were carried out using the fluorometry facilities at the Laboratory for Fluorescence Dynamics of the University of Illinois at Urbana together with Enrico Gratton, our collaborator. Studies are now in progress with equipment recently installed in the FEL facility at Vanderbilt. Multifrequency phase and modulation fluorometry, with excitation from a sync-pumped, mode-locked, cavity-dumped dye laser system, was used to obtain fluorescence lifetime and time-dependent anisotropy data for both the intrinsic fluorescence (tryptophan) of BDH as well as for BDH covalently derivatized with IAEDANS. Lifetime data were analyzed independently (two or three component analyses) as well as with a model-dependent global analysis. The data indicate that the tryptophan fluorescence of BDH exhibits three lifetime components which are similar in the presence versus absence of PC, suggesting that the environment and motional characteristics of the tryptophan of BDH are not modulated by PC. For the IAEDANS-BDH, three lifetime components were also detected in the presence of PC. With this probe, time-dependent anisotropy data for BDH in presence of PC yielded rotational correlation times of 2.2×10^{-7} sec and 4.7×10^{-9} sec. Studies are in progress to relate dynamic processes with functional state of BDH in the membrane. We are in the process of modifying the fluorometry facility in the FEL Center so as to enable both broad multifrequency detection and quantitation of more rapid dynamic processes as required for correlation of structure with function.

We anticipate that the broad tunability of the FEL especially in the UV and IR will enable studies including specific crosslinking of ligands to membrane components and detection of new resonances for study of lipid-protein interaction in biomembranes.

Strategic Defense Initiative Organization

Fourth Annual

Contractors' Meeting

on

Medical Free Electron Lasers

ABSTRACTS

September 22-24, 1989

Dallas, Texas

N00014-87-C-0146

October 1987 - December 1990

**Dynamic Characteristics of Biomembranes and
Membrane Proteins**J. Oliver McIntyre, and Sidney Fleischer
Vanderbilt University
Nashville, Tennessee

Our long-range research interest is to understand how the cell works in terms of its component organelles and membranes. We are studying cell function at several levels of organization, from the intact cell, to organelles, to membranes and to molecular components. The focus of our biophysical studies is on two **membrane proteins**. The first is the **calcium pump protein (CPP)** of skeletal muscle sarcoplasmic reticulum, which is one of the extensively studied examples of a membrane ion pump due to its importance in cell and muscle physiology as well as being a model for understanding biological pumps generally. The second, **3-hydroxybutyrate dehydrogenase (BDH)**, is the best-studied example of a lipid-requiring enzyme. BDH has an absolute and specific requirement for the phospholipid phosphatidylcholine (PC) for enzymic activity.

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Interaction of Radiation with Cells and Cell Components

J. Tribble, J. Kozub, G. Edwards, K. Lane, A. Aly, and R. Ossoff,
Vanderbilt University

The first step of this study involved reducing the scattered light from a suspension of living cells in order to reveal their UV-Vis absorption characteristics. Low power optical density was measured for suspensions of different cell types as a function of refractive index of the suspending protein solution. The protein concentration yielding minimum optical density was found and UV-Vis absorbance spectra of the "clarified" suspension and of cells in normal saline were recorded. Based on these results, we are investigating wavelength dependent effects of pulsed radiation from an Nd:YAG pumped tunable dye laser. The first series of laser experiments, to determine bacteriostasis as a function of laser power and wavelength, correlate with absorption spectra.

Research supported by Vanderbilt FEL from L Reinisch

10 January, 1992

IV - M.

1. Fluorescence detection and identification in the management of acute otitis media.

Collaborators: Jay Werkhaven, M.D.
The Nemours Children's Clinic
Jacksonville, Florida

Jerri Tribble
Dept. of Physics
Vanderbilt University

Fluorescence Spectroscopy of Bacteria in Otitis Media, J.A. Tribble, J. Werkhaven and L. Reinisch, Applied Spectroscopy (in preparation).

Abstract

The management of otitis media in children continues to be hampered by two fundamental problems: The prevalence of bactericidal resistant species is increasing (frequent misdiagnosis lead only to enhancement of the growth conditions for resistant species); The identification of resistant species is time consuming, expensive and requires drainage of the middle ear. The fluorescence spectra of four strains of bacteria, commonly found in otitis media: *P. aeruginosa*, *S. aureus*, *B. catarrhalis* and *H. influenzae* has been measured. The excitation wavelength has been varied from 280 to 500 nm and the emission spectra measured. The fluorescence spectra are presented at two dimensional fluorescence finger prints. These fingerprints will ultimately be used to identify the bacteria remotely, and non invasively from otitis media.

Future Research Support:

(i) An Innovative Technology Research Grant to the National Institutes of Health is being prepared and will be submitted February 1, 1992.

(ii) The Deafness Research Foundation has been contacted. A proposal will be submitted before their annual due date in June, 1992.

(iii) We are in the initial stage of contacting the Whitaker Foundation for support.

2. Measurement of the membrane dynamics of the activated purple membrane.

Collaborator: J. Czégé
Uniformed Services University of the
Health Sciences
Bethesda, Maryland

The Effect of Triton X-100 on Purple Membrane as Measured by Changes in the Dynamics. J. Czégé and L. Reinisch, Photochem. and Photobiol (submitted, 1992).

Abstract

We have observed the light scattering transients arising from changes in the curvature of purple membrane fragments upon photoexcitation at pH 8.05 and 4.1 with and without treatment of Triton X-100. The low ionic strength room temperature suspensions are excited with 532 nm light pulses from a Nd:YAG laser (20 ns). The scattering of 320 nm light is monitored from 3 μ s to 1 s at scattering angles from 15° to 60°. We simultaneously measure the transient transmission changes at 320 nm. The transient light scattering signals change significantly with the addition of 0.02% Triton X-100 at pH 8.05 and 0.006% Triton X-1000 at pH 4.1. At these concentrations of Triton we observed maximal amplitudes in the transient changes of the scattered light intensity. At higher concentrations, the Triton solubilizes the protein and the scattering signals are completely attenuated. The transient transmission changes become severely distorted by the scattering changes in the Triton treated samples. We can explain these changes using our bent membrane model and assuming a greater initial curvature and an increased transient curvature change in the membrane fragments after the Triton X-100 is added. The amplitudes of the scattering changes as a function of the scattering angle from 15° to 60° agree with model calculations of the scattering amplitudes.

Future Research Support:

- (i) A R01 grant proposal has been submitted to the National Institutes of Health, 1 November, 1991.

Vanderbilt University Free Electron Laser Project for Biomedical and Material Research. Contract NOO14-87-C-0146. October 1987 to December 1990.

Nonthermal and selective effects of Free Electron Laser irradiation of tissue. Robert Ossoff, M.D., David L. Zealear, Ph.D., Debra Gonzalez, M.D. and Jerri Tribble, M.S. Department of Otolaryngology, Vanderbilt University Med Center, Nashville, TN.

Our ongoing and planned research efforts encompass several diverse projects. These come together under a common theme: an investigation of the laser's potential to affect biological tissue in selective and nonthermal ways.

Investigations into the area of laser tissue interactions have focused primarily on the problem of wound healing. We have used, and continue to use, a tri-level approach which involves experimentation on the whole animal (macroscopic), cellular (microscopic), and molecular levels. We have been active in defining the effect of laser wavelength, power and pulse structure on various aspects of the wound healing process. With this approach, we hope to refine the laser as an ablative and biostimulating instrument.

Our approach to the study of selective laser effects has begun with an investigation of the spectral properties of whole cells, subcellular organelles and macromolecules, and the extracellular matrix. Spectrophotometric analysis has allowed us to identify particular tissue chromophores with characteristic absorption peak profiles. The transfer of laser energy can be largely restricted to a particular chromophore, if the laser is tuned to match a distinguishing peak within the chromophore's spectral fingerprint. For example, in a study of laser bacteriostasis, enhanced kill of *E. coli* was demonstrated if the laser (Nanosecond Nd-YAG pumped tunable dye) was tuned to one of the two distinguishing absorption peaks for *E. coli*. The broad and continuously tunable features of the FEL will provide greater flexibility in targetting specific chromophores, in particular those possessing absorption peaks outside the range of conventional lasers.

Our interest in laser tissue ablation extends to deriving methods for the clean dissection and removal of diseased tissue while eliminating lateral thermal damage to healthy tissues. In theory, this may be achieved by using laser energy of the appropriate wavelength with minimal pulse duration for the diffusion of thermal energy. The effect of various irradiation paradigms on the degree of tissue ablation and wound healing over time is being investigated using conventional as well as short pulsed lasers (eg., excimer and tunable dye lasers capable of delivering nano or picosecond pulses). In a study of pulsed versus continuous carbon dioxide laser irradiation of tissue, crater profiles (e.g. depth, width) varied in a manner consistent with less nonspecific thermal damage resulting from pulsed irradiation. Laser paradigms found to minimize thermal tissue damage with these conventional lasers will guide our search for purely nonthermal tissue interactions with the FEL, clearly the best technology available for achieving this type of interaction.

Within the last ten years there have been numerous reports of the biostimulating effects of laser irradiation. Biostimulating effects are defined as nondestructive interactions which alter the structure and function of cells or extracellular matrix. Our own work has focused on the effect of sublethal laser irradiation on aspects of the wound healing process. These include: collagen synthesis, elastin synthesis, overall protein synthesis, fibroblast proliferation and wound tensile strength. Studies have been conducted at all three levels of investigation in our laboratory. At the macroscopic level, rat wounds withstood greater breaking tensions when exposed to sublethal laser irradiation. One possible explanation was increased collagen deposition in wounds as a result of laser biostimulation of protein synthesis by fibroblast cells. This biostimulating effect on fibroblasts has been demonstrated in our laboratory at the microscopic level (i.e. tissue culture) as revealed by polyacrylamide gel electrophoretic patterns. Preliminary studies at the molecular level in our laboratory have suggested that laser induced cross linking of ribosomal RNA may be the mechanism underlying the biostimulating effect. Using sublethal FEL doses at the appropriate wavelength we hope to develop the technology to change the behavior of cells in ways that would be clinically relevant and beneficial.

13 May 1988

MECHANISMS OF LASER-TISSUE INTERACTION AND CLINICAL APPLICATIONS

Glenn Edwards, Robert Ossoff, Al Ali, John Kozub, and Changle Liu
Progress Report, May 1988

As stated in the original proposal "the theme of this project is the targeting of electromagnetic energy into diseased tissue, with an emphasis on selective laser-tissue interactions matched to cellular characteristics that are unique to the diseased tissue. In order to establish selective methods of interaction, the first goal of the project is to characterize the frequency specific mechanisms of absorption by DNA, proteins, and lipids as a function of intensity and pulse duration. The second goal is to determine the lifetimes of the various excited states; this information will suggest parameters for ultrafast pulses that will maximize the nonthermal relative to the thermal effect. The third goal of this project is to refine these pulse parameters to optimize selective tissue interaction and thus produce the desired clinical effect."

During the past year we have undertaken a number of steps to initiate this research effort: the steps include assembling qualified personnel, developing a number of spectroscopic systems for pre-FEL and dual-beam FEL experiments, initiating pre-FEL experiments at Vanderbilt, and performing experiments at existing FEL centers. Progress for each of these steps will be addressed below.

Personnel. This research effort calls for interdisciplinary expertise and cooperation. Towards this end we have added a number of collaborators to the original investigators. Two graduate students in the Department of Physics and Astronomy are supported as research assistants by the contract and are working with Professor Edwards on problems that will evolve into their dissertation topics. Both of these students came to Vanderbilt to work in this research area. John Kozub has just finished his first year and is investigating the manner in which electromagnetic radiation interrupts the natural interaction of biopolymer systems. It is worthwhile to point out that John has been quite successful during his first year and has recently been nominated by the Graduate Program Committee to receive Vanderbilt's Lagemann Award presented to "the most promising entering or first-year graduate student in the Department of Physics and Astronomy." The other student is Changle Liu, an advanced graduate student, who left Purdue University to take advantage of Vanderbilt's graduate program in experimental biophysics. Changle joined the department only four months ago and is investigating the effect of pulsed radiation on biopolymer systems. In addition to these physics graduate students, Al Ali is a medical doctor working for Dr. Ossoff and in close collaboration with the physicists in developing protocols for laser-tissue interaction.

Equipment. During the past year we have exerted a significant effort to develop two different spectroscopic systems. One of these systems is the Hewlett Packard 8510

Network Analyzer. We have modified this spectrometer to allow an alternative calibration scheme that greatly enhances reproducibility and yields high precision measurements of biological solutions and tissue. This project required significant software/hardware development. For the past three months we have been measuring biopolymer solutions and cellular suspensions with good preliminary results. The second radiation source is a Quantel Nd:YAG pumped dye laser system that is to be used as a nanosecond or picosecond laser for both pre-FEL and dual-beam FEL experiments. Currently this laser is being integrated into a complete spectroscopic system for biophysical research. The laser arrived only a month ago and is currently operating at specification and is being modified for lasing in an extended wavelength regime.

In order to produce and characterize biopolymer samples we have developed a nearly complete laboratory of molecular biology in the first floor of the Department of Physics and Astronomy. Since early this spring we have been producing large quantities of purified DNA samples for spectroscopic investigation.

Pre-FEL Experiments. As mentioned above we have performed a number of preliminary experiments investigating the fundamental mechanisms of laser-tissue interaction. We have measured the infrared absorption properties of DNA which is an essential step in the series of experiments that will determine the lifetime of the various excitations. In addition, we have measured the ultraviolet scattering and absorption properties of cell suspensions with intriguing results in laser induced cell death.

During the summer we will extend these measurements beyond the preliminary stages.

During the summer we will begin a series of experiments investigating the effect of pulsed radiation of DNA protein interactions. We will monitor the effect of radiation induced changes on the cell and related clinical applications.

FEL Experiments. During the past year we have been planning a series of experiments for the far-infrared FEL at the University of California, Santa Barbara. The pulsed nature of the UCSB FEL, as enhanced by switching techniques, will be used to measure the homogenous and inhomogeneous nature of the broadening of DNA features in the infrared. This will allow us to determine the lifetime of these modes. Although a minimum lifetime has been determined by cw experiments, a more accurate estimate of the lifetime is necessary in order to design an exposure protocol that will optimize the resonant effect.

Publication

Vanderbilt University FEL Center for Biomedical and Materials Research, G.S. Edwards and N.H. Tolk, Ninth International FEL Conference, Williamsburg 1987. Proceedings to appear in Nuclear Instruments and Methods in Physics Research.

Vanderbilt University Free-Electron Laser Project
for Biomedical and Materials Research
Contract No. N00014-87-C-0146

Far-Infrared Vibrational Modes of DNA

C. Liu and G.S. Edwards
Department of Physics and Astronomy
Vanderbilt University

and

S. Morgan and E. Silberman
Department of Physics
Fisk University

Nashville, Tennessee

Raman and infrared activity of low-frequency (20 to 300 cm^{-1}) vibrational modes of naturally occurring nucleic acids and polynucleotides have been measured (1,2). Distinct bands are clearly resolved in poly(dA).poly(dT) but are only weakly seen in other sequences of both DNA and RNA. These results are discussed in terms of inhomogeneous line broadening of vibrational modes. These experimental results are well described by lattice dynamics (3,4). Temperature dependent experiments (1) indicate that these modes are nonlinear and calculations imply that they may play an important role in the localized "melting" of DNA that is required for transcription and translation.

The Vanderbilt FEL Center has recently installed a Raman laser system with enhanced stray-light rejection and throughput that promises improved resolution of the observed bands and a more thorough investigation of the line broadening process is underway. In addition to the investigation of Raman activity, both line broadening and non-linear processes may be probed directly by intense, far-infrared sources such as the UC-Santa Barbara FEL; preliminary experiments have been performed at this facility.

1. J.W. Powell, G.S. Edwards, L. Genzel, F. Kremer, A. Wittlin, W. Kubasek, and W. Peticolas, Physical Review A 35, 3929 (1987).
2. C. Liu, G.S. Edwards, S. Morgan, and E. Silberman, submitted.
3. Y. Kim and E.W. Prohofsky, Physical Review B 36, 3449 (1987).
4. L. Young, V.V. Prabhu, E.W. Prohofsky, and G.S. Edwards, submitted.

Vanderbilt University Free-Electron Laser Project
for Biomedical and Materials Research
Contract No. N00014-87-C-0146

Pulsed-UV Induced Photochemistry of
RNA-Protein Complexes

J. Kozub and G.S. Edwards
Department of Physics and Astronomy

and

S. Northington and W. LeStourgeon
Department of Molecular Biology

Vanderbilt University
Nashville, Tennessee

It has recently been shown by Von Hippel and coworkers that nanosecond pulses of UV radiation can crosslink nucleic acids to proteins in a selective and wavelength dependent manner. In addition, it is well known that uv radiation can cause pyrimidine dimers. We have used a nanosecond Nd:YAG pumped dye-laser system with doubling and mixing crystals to generate 8 ns pulses of 263 nm radiation to investigate dimer formation in RNA and RNA-protein crosslinking in hnRNP, the RNA-protein complex that may play a role in post-transcriptional processing. We conclude that i) the energy required for uv damage to RNA is the same energy required to crosslink the C proteins of hnRNP. The events can be detected at 7 millijoules of UV irradiation. ii) C proteins specifically crosslink to RNA through uridine; and iii) UV light-induced site-specific termination of reverse transcription is due to uridine damage especially at sites with the potential to form pyrimidine dimers, i.e. UU > UC > C.

These studies imply that the mechanism of UV-induced protein-nucleic acid crosslinking is largely dependent on the photochemistry of specific nucleotides and on the nature of the protein-RNA interaction. Currently we are studying the effect of wavelength on the selectivity of induced crosslink and have evidence that suggests at least two crosslinking mechanisms are available. We plan to extend these results to investigate the possibility that different mechanisms will be optimized for picosecond pulses as available from the FEL being installed at Vanderbilt University.

IV - 6.

M. to 12:10 P.M.
15, KCCC

The design of the endoscope permits its removal without disturbing the catheter. This method requires minimal training and should be safe and fool-proof. Its clinical advantages will be reviewed.

10:42 A.M.

A New Endotracheal Tube for CO₂ and KTP/532 Laser Surgery of the Upper Aerodigestive Tract

ROBERT H. OSSOFF, MD, DMD, AL ALY, MD,
NICK HOUCHIN, AAS, and DEBRA GONZALEZ, MD,
Nashville, Tenn., and Burlington, Vt.

A new endotracheal (ET) tube has been developed for laser surgery of the upper aerodigestive tract with the carbon dioxide (CO₂) and potassium titanyl phosphate/532 (KTP/532) lasers. The tube itself is made of silicone, which is wrapped circumferentially with reflective, metallic tape by a special process that uses no adhesive backing. Teflon tape is wrapped over the reflective, metallic tape to prevent any mucosal injury during either intubation or extubation. This Teflon tape retracts out of the way during impact with either laser.

Because methylene blue crystals are present within the ET tube cuff insufflation line, inflating the cuff with saline will yield methylene blue-colored saline in the cuff. The tube is shipped sterile and ready for intubation; neurosurgical cottonoids are included in the package.

This tube has been tested extensively in our laboratory with the CO₂ laser, including microspot and the KTP-532 laser, and has been found to resist penetration and combustion with both wavelengths under normal operating conditions.

SCIENTIFIC
SESSIONS

August 1991
IV-K

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CO₂ Laser Micromanipulator Parallax Error Resolved

JAY A. WERKHAVEN, MD, JERRI TRIBBLE, and
ROBERT H. OSSOFF, MD, DMD, Nashville, Tenn.

Most current CO₂ laser micromanipulators for microlaryngoscopy experience the parallax aiming problem. This occurs when the beam mirror is offset below the optical path for the microscope, making use of the laser difficult, through small laryngoscopes or in pediatric patients. The newer "hot mirror technology" micromanipulators that are now available overcome this problem.

In addition to providing a laser beam coincident with the optical path, most offer much smaller spot sizes (250-micron diameter at 400-mm focal length), but all partially block some of the light available for illumination. To quantitate this, optical absorption spectra were determined for six hot mirrors. Clinical experience with more than 100 cases has demonstrated the advantages of these new mirrors to minimize mucosal thermal damage and give improved exposure for subglottic and pediatric laryngoscopy.

August 1990

IV-J.

9:00 A.M.

**A Comparison of Vocal Fold and Skin Fibroblast
Elastin Production in Tissue Culture**DEBRA A. GONZALEZ, MD, DAVID L. ZELEAR, PhD,
J.M. DAVIDSON, PhD, and ROBERT H. OSSOFF, MD, DMD,
Nashville, Tenn.

The biomechanical properties of the vocal cord allow for its vibration, lengthening, and shape change during voice production. Histologic studies have shown that the vocal fold mucosa is primarily a connective tissue, and that elastin is prominent within the lamina propria. Because the only known function of elastin is to provide elastic recoil to tissues, we suspect vocal fold elastin is a necessary feature for normal voice production. Experiments were performed in order to determine if a structure requiring high elasticity for normal function—such as the vocal fold—contains fibroblast cells distinguished in their elastogenic capacity.

Vocal cord and skin fibroblasts were explanted and maintained in tissue culture. An enzyme-linked immunoassay was used to quantify the soluble tropoelastin (TE) secreted by early passage cells into the tissue culture media. Comparison of dog vocal fold and pig skin elastin production revealed that the vocal fold cells produced 150,000 molecules TE/cell/hour, which was 2 to 4 times greater than skin fibroblast TE production. TE production by both cell types was up-regulated by treatment with 1.6 μ M hydrocortisone (increased 120% by skin cells and 53% vocal fold cells). These models were initially chosen because they closely resemble human tissues: vocal cords of dogs and human beings have similar histologic features, whereas the skin of pigs and human beings is noted to be biochemically similar. Despite the attempt to individualize each assay by using competing antigen (alpha elastin) purified from the same species, we were concerned about the accuracy of an inter-species comparison. Comparing elastin production by fibroblasts taken from vocal cord and skin of the dog, vocal fold cells produced twice as much TE as skin cells.

In order to assess the functional significance of cells exhibiting high elastogenic activity, fibroblast elastin synthesis was compared in two species differing in their vocalization behavior. Dog vocal fold cells produced 5 times more TE (>100,000 molecules/cell/hour) than pig vocal fold cells.

Vocal fold fibroblast cell cultures represent a valuable tool for the study of vocal fold connective tissues. The finding that dog vocal fold cells produce significantly more elastin than skin cells supports the idea that elastin is an important component within the vocal fold mucosa. The finding that dog vocal fold cells produce significantly more TE than pig vocal fold cells correlates well with published comparative histology showing less elastin within pig vocal fold mucosa, and suggests that vocal fold fibroblasts grown in culture exhibit levels of elastogenesis that are representative of their physiologic activity in vivo.

(Supported in part by the Veterans Administration, Genentech, Inc., and NIH grants AG 06528 and GM 37387 [J.M.D.] and the SDIO Office of Naval Research [R.H.O.])

An Investigation of Laser-Induced Protein Synthesis by Cultured Fibroblasts

DEBRA A. GONZALEZ, MD, JEFFREY M. DAVIDSON, PhD,
DAVID L. ZEALEAR, PhD, and ROBERT H. OSSOFF, DMD, MD,
Nashville, Tenn.

While lasers have been conventionally used in medicine as ablative or photocoagulating instruments, there is growing evidence in the literature that laser energy may also be used in subtle ways to modulate cellular metabolic activity and wound repair. The purpose of this investigation was twofold: (1) to determine whether sublethal laser irradiation can alter the pattern of overall protein synthesis by cells in culture and (2) whether the observed changes in protein synthesis are caused by nonspecific thermal mechanisms or to specific photochemical events related to the spectral characteristics of the irradiation.

Cultured human skin fibroblasts were exposed to either sublethal Nd:YAG laser irradiation or heat shock at 42° C. Laser power densities and heat intensities used were determined to be sublethal by a dye exclusion-viability assay. After laser irradiation or heat shock, cells were incubated in media containing ³⁵S-methionine and time allowed for the incorporation of radioactivity during protein synthesis. Radiolabelled proteins were separated from unincorporated amino acids in the cell lysates and incubation media by TCA precipitation. Equal numbers of precipitable counts per minute were loaded onto SDS-polyacrylamide gels. After electrophoresis, autoradiography was performed in order to detect bands of newly synthesized radioactive proteins. The media were processed separately from the cell lysates in order to analyze the pattern of secretory proteins in isolation. These represented the extracellular matrix proteins that participate in wound healing. Proteins within the cell lysates represented cytoplasmic proteins, whose synthesis has been shown to be induced by heat shock and other stresses.

Preliminary results indicated that sublethal Nd:YAG irradiation, delivered continuously at power densities ranging from 500 to 1750 Joules/cm², was capable of inducing changes in the type and quantity of synthesized proteins by fibroblasts. Autoradiography of the cell lysates revealed at least one new protein induced by either laser irradiation or heat shock. This protein band had a molecular weight of 85 to 89 kilodaltons. Within the media fractions, increased synthesis of a 60-kilodalton molecular weight protein was observed after laser irradiation, but not after heat shock at 42° C.

These observations suggest that both thermal and spectral components may play a role in the alteration of protein synthesis by cultured fibroblasts. However, laser photochemical effects may more readily influence synthesis of those proteins secreted by cells during wound healing.

(Supported by the SDIO Office of Naval Research)

tendons were consistently smaller in size than controls ($P < .001$). Ipso facto, tendons treated at each dose level developed nearly twice the tensile stress of control tendons ($P < .001$). Ultimate tensile strength, energy absorption capacity and strain did not differ between treated and control tendons ($P > .10$) and no dose dependent effects were observed in the biomechanical characteristics of treated tendons. Electron microscopy revealed that unlike control tendons, the fibroblasts and collagen fibrils of laser treated tendons were mostly aligned in their longitudinal axis. These findings demonstrate for the first time that laser biostimulation modulates the ultrastructure and biomechanics of healing tendons.

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A STUDY OF DE-NOVO PROTEIN SYNTHESIS BY LASER IRRADIATED HUMAN NEUTROPHILS. D.A. Gonzalez¹, J. Hurtig¹, J.M. Davidson², C.K. Broadley², R.L. Hoover², R.H. Ossoff¹.

¹ Dept. of Otolaryngology and ² Dept. of Pathology, Vanderbilt University Medical Center, Nashville, TN.

There is growing interest in the biostimulating effects of laser irradiation. With the advent of laser angioplasty and the introduction of techniques for laser purification of banked blood, we are interested in the effects of sublethal laser energy on blood cells, particularly those involved in inflammation. The purpose of this investigation was: 1) to determine whether sublethal Nd:YAG laser irradiation can alter the pattern of protein synthesis by human neutrophils in short-term culture and 2) whether the observed changes in protein synthesis are due to nonspecific thermal mechanisms or to specific photochemical events.

Human neutrophils were isolated from whole blood, maintained in short-term culture and exposed to either heat (42° C, 60 min) or to sublethal continuous Nd:YAG irradiation. Sublethal doses, defined using a dye exclusion viability assay, ranged from 500-2000 joules/cm². After laser irradiation or heat shock, newly synthesized proteins were labelled in vitro with ³⁵S-methionine. TCA precipitation of proteins, polyacrylamide gel electrophoresis and autoradiography were performed in order to detect bands of newly synthesized proteins.

Autoradiography revealed a new protein band with a molecular weight of 40 kilodaltons that was induced by laser irradiation but not by heat shock. Increased synthesis of 21 and 26 kilodalton proteins was also noted following laser irradiation but not following heat shock. These data suggest that neutrophil protein synthesis can be altered by sublethal Nd:YAG irradiation in a non-thermal way. Similar experiments using picosecond pulsed Nd:YAG irradiation are under way.

Supported in part by the SDIO Office of Naval Research (RHO), by the Veterans Administration, Genentech, Inc., and NIH grants AG 06528 and GM 37387 (JMD) and NIH grant HL-36526 (RLH).

THE VANDERBILT UNIVERSITY
FREE-ELECTRON LASER X-RAY FACILITY

Perry A. Tompkins, Weiwei D. Andrews, Charles A. Brau and James A. Waters
Department of Physics and Astronomy
Vanderbilt University

Frank E. Carroll, David R. Pickens, Ron R. Price and Carlton F. Roos
Department of Radiology and Radiological Sciences
Vanderbilt University Medical Center

We wish to submit this abstract to the conference on
X-Ray Detector Physics and Applications
(Oral).

The Vanderbilt University Free-Electron Laser Program is developing the capability to create near-monochromatic X-Rays for Medical Imaging and other purposes. For this experiment we feed-back the normal infrared FEL light to collide with the electron beam. This causes Compton backscattering of the incident photons which create X-rays. These X-rays cannot feed a X-ray laser, but they have a collimated intensity and tunability which will make them highly suitable for medical imaging. This paper is particularly focussed on the X-Ray beam transport to be used with this experiment. This transport must collimate the X-Ray beam and re-direct it to match a beam chase located in the vault ceiling at a 40 degree angle to the electron beam axis. A brief description of the creation mechanism and X-Ray beam properties are included.

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Production of Near Monochromatic X-rays by
The Vanderbilt Medical Free Electron Laser
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The creation of a powerful, tuneable, monochromatic X-ray source would herald a new and exceptionally diverse generation of diagnostic medical and material imaging modalities.

The production of 2 micron photons within the Vanderbilt Free Electron Laser by an electron beam of high quality and high peak current, offers an opportunity to use the residual electron beam that is normally "discarded" from the FEL and make it collide with its own generated photons to create tuneable, nearly monochromatic X-rays by Compton backscatter.

Once the electron beam has exited the FEL, it will be transported using bending and focusing magnets to an interaction zone whose axis is aligned with a 12" PVC beam pipe which penetrates the MFEL building structure from the FEL vault to a shirtsleeves environment target room situated on the upper floor of the facility. The electrons will interact with the extremely intense photon flux of the FEL and other conventional lasers. Compton backscattering will generate a cone of intense narrow bandwidth X-rays which will be transported via the PVC beam pipe to a tissue or material sample in the target room.

Monte Carlo algorithms have been used to model the energy and angles of the X-ray beams that will be produced. The initial configuration of this device will generate photons up to 17.9 Kev, although higher energies are easily obtainable through the use of frequency doubling techniques, alterations of electron beam energy, and the utilization of various conventional lasers as well. The photon flux will be sufficient to allow us to perform in vivo and in vitro trace element analysis, near-monochromatic radiography and three-dimensional imaging in a fashion never before possible.

The initial X-ray experiments will include analysis of excised tissues, followed by attempts to detect malignant breast lesions implanted in nude mice. Initial proofs are to be followed by application of this new high contrast/low dose imaging modality to detection, analysis and treatment of breast lesions in humans.

This work has been supported in part by a Grant from the Eastman Kodak Corporation Health Sciences Division.

Vanderbilt University Free-Electron Laser Project
for Biomedical and Materials Research
Contract No. N00014-87-C-0146
October, 1987 - December, 1990

The Vanderbilt University Medical Free-Electron Laser Program

C. A. Brau
Vanderbilt University, Nashville, TN

The Vanderbilt University MFEL program comprises a broad variety of activities ranging from fundamental science to clinical applications. The activities span six departments in three schools of the University, including the Departments of Physics, Molecular Biology, Materials Science, Otolaryngology, Radiology, and Neurosurgery. The compact arrangement of the campus and the anticipation of working together in a single facility have made it possible to establish unique collaborations on innovative experiments.

To support these activities, the University has constructed a \$2.6 million, state-of-the-art laboratory building adjacent to the Medical School, the Physics Department and the Engineering School. A FEL is under construction by Sierra Laser Systems, to produce tunable, high-peak-power, pulsed radiation in the wavelength region from 2 to 10 microns. Construction of the free-electron laser is on schedule, and the machine should be in full operation in the Spring of 1990. Approximately 600 square meters of laboratory space is available on the floor above the vault, including two animal operating rooms. This space is now in use for experiments with conventional lasers, in preparation for the availability of the FEL.

At the present time, the laser applications projects underway include seven separate, but related efforts:

- (1) Surface physics: Laser-induced desorption and selective molecular bond-breaking on surfaces, which are important processes in laser-induced damage to optical materials as well as in the nonthermal interaction of lasers with biological materials.
- (2) Materials science: Mechanisms of infrared laser-induced damage in glasses and optical fibers, which are of importance to the application of FELs and other infrared lasers in medicine and other fields.
- (3) Biophysics: Nonlinear spectroscopy of large biomolecules such as DNA, which is important for biological processes such as the localized melting of DNA during transcription.
- (4) Molecular biology: Dynamics of membranes, especially ion transport mechanisms such as the calcium pump protein.
- (5) Wound healing: Nonlinear interactions of lasers with tissue, with the objective of understanding the selective, nonthermal effects of short-pulse laser radiation on collagen formation, fibroblast proliferation, and so on.
- (6) Neurosurgery: Fundamental studies of the nonlinear interaction of laser radiation with brain tissue, as well as the development of stereotactic imaging for neurosurgical procedures.
- (7) X-Ray imaging: Generation and application of monochromatic X-Rays in the region from 5 to 20 keV formed by Compton-backscattering infrared radiation off the electron beam from the FEL.

**THE FREE ELECTRON LASER:
A PHOTON FACTORY FOR SCIENCE AND MEDICINE**

RICHARD F. HAGLUND, Jr.
Department of Physics and Astronomy and
Free-Electron Laser Project in Biomedical and Materials Research
Vanderbilt University, Nashville, TN 37235

The free-electron laser (FEL) is a new kind of coherent light source based on a marriage of laser and high-energy accelerator technology. Vanderbilt's recently-funded FEL Project in Biomedical and Materials Research is an interdisciplinary collaboration between the Schools of Medicine and Engineering and the College of Arts and Science, whose goal is to bring together researchers both from Vanderbilt and other institutions who are interested in studying the electronic interactions of FEL photons with everything from atoms and molecules on surfaces to living tissue.

In this tutorial talk, I shall describe the basic operating principles of free-electron lasers in a qualitative way; summarize the program goals for our FEL project, including collaborative efforts with "outside" users; and finally consider three applications in which Vanderbilt researchers expect to use FEL photons in ways not possible with conventional lasers: studies of laser-induced surface physics and chemistry, especially in beam delivery systems for high-power ultra-short pulse infrared lasers; excitation of the normal modes of DNA and other biologically important macromolecules to study drug action, cross-linking and metabolism; and use of non-thermal laser-tissue interactions to reduce scarring and tissue damage in arthroscopic and other kinds of laser surgery.

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PHOTOINDUCED RIBONUCLEIC ACID CROSSLINKING USING VISIBLE AND INFRARED WAVELENGTHS. Michael D. Kyzer, Jerri A. Tribble, Walter-G Wrobel, Robert H. Ossoff. Vanderbilt University Medical Center, Department of Otolaryngology, Nashville, TN.

Efforts to study the effect of high intensity, short duration laser radiation on cell metabolism have yielded preliminary evidence of RNA crosslinking induced by radiation of visible (532nm) and infrared (1064nm) wavelengths. This is interesting both from a photochemical mechanistic and from a clinical point of view. Cytoplasmic RNA was exposed to 35 picosecond pulses (at 10Hz) from a Q-switched, mode locked Nd:YAG laser at power densities from 2.3×10^{13} to 7.5×10^{13} at 532nm and from 1.1×10^{13} to 5.4×10^{13} Watts/m² at 1064nm, at a constant total dose of 10 J. Irradiated and control samples were eluted through agarose gels under nondenaturing and denaturing conditions. No difference was observed between the control and irradiated samples on the nondenaturing gel, giving

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evidence that fragmentation and intermolecular crosslinking was minimal. On the denaturing gel, however, samples irradiated at both wavelengths showed distinctly similar mobility shifts suggesting a possible multiphoton mechanism. Further investigations are underway to study the power and wavelength dependence of RNA crosslinking induced by this spectral region.

• 150 Scientific Sessions—Tuesday

Otolaryngology—
Head and Neck Surgery

10:45 A.M.

An Investigation of the Potential for Laser Nerve WeldingM. KORFF, MD, D.L. ZEALAN, PhD, M. SCHWABER, MD, and
R.H. OSSOFF, DMD, MD, Nashville, Tenn.

Suture repair of a severed nerve is cumbersome, presents a focus for infection and neuroma formation, and does not always produce adequate stump alignment. An alternative method of nerve repair involves the use of the laser to effect epineural welding. In the present study, two factors were investigated to elucidate the potential of the laser in nerve anastomosis: first, the initial strength of the bond created, and second, the long-term damage inflicted on the nerve by the laser.

Experiments were performed to determine the appropriate laser energy using the Laserscope KTP/532 and Sharpian

1060 CO₂ lasers on intact rat sciatic nerves. Significant damage was detected with both lasers at 1.6 watts, as shown by decreases in the nerve compound action potential (CAP). Thirty pulses of 0.05-second duration were used to approximate the type of exposure required for effective epineural welding. At energies less than or equal to 1 watt, acute damage was minimal. However, significant CAP decreases were observed 1 week postoperatively in these animals. This effect was most likely caused by nerve entrapment, since epineural shrinkage was noted during the initial laser irradiation. In subsequent studies, this problem was alleviated by limiting circumferential exposure to 180 degrees. The axonal damage and epineural effects created by the KTP/532 laser were more variable than those observed with the CO₂ laser, making the CO₂ a safer laser for nerve welding.

The low breaking force generated by the laser anastomosis using 1-watt pulses indicated that a larger surface area of adhesion was necessary. A layer of subcutaneous tissue was wrapped around the apposed sciatic nerve ends and laser welded to the epineurium a few millimeters back from the anastomotic site using the CO₂ laser. This produced a higher breaking strength than with epineural welding alone. The laser irradiation was performed around 180° to maintain adequate stump alignment while allowing for nerve swelling. Chronic studies are under way to determine the quality of regeneration of subcutaneous tissue wrapped and laser anastomosed nerves as compared to suture anastomosed nerves.

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11:00 A.M.

**Investigation of the Wavelength Dependence of
Laser Induced Bacteriostasis**

JERRI A. TRIBBLE, MS, JOHN A. KOZUB, BS,
GLENN S. EDWARDS, PhD, AL ALY, MD, and
ROBERT H. OSSOFF, MD, Nashville, Tenn.

The wealth of literature recently addressing the question of which medical lasers are best for affecting particular biologic responses has prompted us to develop a method to study wavelength dependence of laser-induced biologic effects. The first step in this investigation was to measure the absorption spectra of living cells. By suspending the cells in a medium that matched their refractive index, the dominant scattering background was significantly reduced. Limiting this background revealed underlying absorption peaks that were characteristic of this cell type, providing a spectral window to which a laser could be tuned for optimal effect. Our model bacteria, *Escherichia coli* Cla, was observed to have a strong absorption peak near 268 nm, and weaker maxima near 350 nm and 426 nm. We found that maximal laser-induced cell death occurred when the laser was tuned to wavelengths corresponding to peaks within the bacteria's spectral fingerprint. The laser used for this study was an Nd:Yag pumped tunable dye laser delivering nanosecond pulses, tunable over the UV-visible range.

This two-phase study has since been extended to another Enterobacteriaceae, *Serratia marcescens* Nima, which has shown strong absorption peaks at 268 nm and 538 nm, with weaker absorption features near 390 nm and 510 nm, in agreement with the literature. Laser experiments with this bacteria are underway. While this investigation represents a model study of correlations between absorption characteristics of living cells and wavelength-dependent laser-induced biologic effects, it also has significant clinical implications for a novel approach to bacteriostasis.

(Supported by the Strategic Defense Initiative Organization program for free electron laser research)

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A PRELIMINARY STUDY OF THE EFFECT OF CO₂ LASER RADIATION ON CULTURED ENDOTHELIUM AND ON THE ADHESION OF NEUTROPHILS TO THESE IRRADIATED CELLS. Alaly, Caroline Kerr, Richard Hoover, Robert H. Ossoff. Vanderbilt Universtiy, Nashville Tennessee.

Polymorphonuclear leukocytes (neutrophils) and vascular endothelial cells interact with each other for a variety of physiologic and pathologic reasons. The initial step in an inflammatory response is increased neutrophil adhesion to the endothelium. The purpose of this study is to determine whether CO₂ laser radiation affects neutrophil-endothelial cell adhesion and thus modify the inflammatory response. Initially the effect of irradiation on endothelium integrity was determined, using a ⁵¹Cr release assay. Endothelial cells (from bovine lung microvasculature) were grown to

confluence in 6.4 mm diameter wells and labelled with ⁵¹Cr. The cells were irradiated with CO₂ laser energy in the range 0.34 - 10.00 watts/cm². The amount of ⁵¹Cr released from the cells, as an indicator of cell injury, was measured 1, 24, 48 and 72 hours after irradiation. Power densities of 2.31 watts/cm² and above, when delivered to the cells, caused a significantly greater amount of ⁵¹Cr release compared to that from non-lased, control cells (p < 0.01; Student's t-test). ⁵¹Cr release, as a result of power densities of 1.44 watts/cm² or below, was no different from that of controls.

Using the same laser energies as above, the effect of CO₂ radiation on neutrophil-endothelial adhesion was measured. Endothelial cells were grown to confluence in 11 mm diameter wells and irradiated. ⁵¹Cr-labelled neutrophils were then added to the cultures 1 and 6 hours after irradiation. The ⁵¹Cr-neutrophils were left to attach for 45 minutes, excess neutrophils removed and the remaining ⁵¹Cr measured and used as an index of neutrophil attachment. Preliminary results indicate that adhesion of neutrophils to endothelial cells is not affected up to 6 hours after lasing. Further study will determine whether adhesion is altered up to 24 hours post radiation.

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KTP LASER-INDUCED MODULATION OF THE WOUND HEALING PROCESS
Michael D. Kyzer, Al S. Aly, and Robert M. Ossoff, Vander-
bilt University Medical Center, Nashville, TN 37232

Castro, et. al., have reported the Nd:YAG mediated altera-
tion of fibroblast collagen synthesis *in vitro* without con-
comitant cellular damage. This project investigates a sim-
ilar process in an *in vivo* rat system using the KTP laser
(532 nm). Sprague-Dawley rats were anesthetized with ket-
amine and pentobarbital (intraperitoneally), and subsequently
their dorsal epidermis was shaved. The exposed epidermis
was then cleaned with a moist cloth to remove residual mel-
anin pigment which possesses a significant extinction coef-
ficient at 532 nm. The rats were then mounted on a plexi-
glass translation stage whose rate of horizontal movement
was fixed at 0.11 cm/s; the defocused KTP beam (dia. 1 cm)
was directed at the rats' dorsum, and the translation was
of sufficient duration to produce two discrete 3.0 cm ipsi-
lateral strips. One of these strips was produced with a 2W
beam and the other with a 3.5W beam. A scalpel was then
used to create an incision down the center of each lased
strip; matched control incisions of identical length were
made on the contralateral side. The incisions were each
closed with four skin staples, and the tensile strength of
the wounds was measured acutely, and on days 3, 7, 14, and
23 postoperatively.

The results show that the KTP laser effected the alteration
of normal wound healing biology in such a manner as to ac-
celerate the initial formation of wounds having supranormal
tensile strength. Indeed, preliminary data indicate that
these hypertensiometric wounds supercede the strength of
their matched controls by 32% at day 3 (n=5 for each time
point), presumably secondary to augmented fibroblast colla-
gen deposition at the wound site. The rate of collagen
synthesis in the irradiated tissue will be determined by a
³H-hydroxyproline assay specific for collagen production.
Furthermore, the distribution of fibroblast activity will
be investigated by an *in situ* immunohistochemical localiza-
tion technique which utilizes a radioactive probe specific
for collagen messenger RNA. The present results already
suggest the potential role of the laser in modulating the
process of scar formation.

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A HISTOLOGIC COMPARISON OF SUPERPULSE CO₂ AND CHOPPED CONTINUOUS WAVE CO₂ LASER TISSUE EFFECTS

Jay Werkhaven, Vanderbilt University, Nashville, Tn.

Bruce R. Maddern, Nemours Clinic, Jacksonville, Fla.

David Kardatzke, University of Pittsburgh, Pittsburgh, Pa.

David M. Harris, Wenske Laser Center, Chicago, Ill.

Superpulsing a carbon dioxide laser beam is an option on many medical lasers. The observation of less charring at the impact site is observed clinically but no quantitative study has been done to examine the range of effects of the various parameters of superpulse (i.e. pulse duration [PD] and repetition rate [RR]).

Quantitative histologic measurements on an animal model (depilated rat skin) were performed using a Coherent XL-55 CO₂ laser. The available range of superpulse parameters were a pulse duration of 100 - 900 microseconds and repetition rate from 100 -900 Hz (RR dependent upon PD). Nineteen sets of superpulse variables were paired with chopped continuous wave pulses of the same average power to tissue. The zone of thermal coagulation for each impact was measured.

Eighteen of nineteen superpulse parameters demonstrated less thermal coagulation than the continuous wave impact. Most were significant at the $p < 0.05$ level (controlling for the comparison-wise error rate).

The advantage of less charring and less thermal coagulation suggests superpulse is clinically useful, especially in areas such as the glottis where minimal thermal effect is desired.

Supported by a grant from The Children's Hospital of Pittsburgh and Coherent Medical Lasers, Palo Alto, Ca.

each larynx was studied under 50 different conditions. The G.C.I. and true vocal efficiency were then calculated for each combination of airflow rate, width and tension. Linear regression analysis of data obtained from ten larynges proved G.C.I. to have a highly statistically significant linear relationship with vocal efficiency ($p < 0.001$, $R^2 = .95$). When sound intensity was expressed in dB rather than Watts/cm², the competence index did have a statistically significant relationship with vocal efficiency. These results confirm that G.C.I. (expressed in Watts/cm² ÷ cc/sec) is a simple clinical measure for assessing vocal efficiency of the larynx.

234 VOCAL CORD FIBROBLAST ELASTIN PRODUCTION IN TISSUE CULTURE. *D.A. Gonzalez¹, R.R. Nair³, R.H. Ossoff¹, J.M. Davidson^{2,3}, Depts. of ¹Otolaryngology and ²Pathology, Vanderbilt Univ. Medical Ctr., Nashville, TN; ³V.A. Medical Ctr., Nashville, TN 37232

The biomechanical and anatomical properties of the vocal fold, an elastic tissue, are thought to influence voice production in man; however, there is little information in the literature concerning the biochemistry of connective tissues within the vocal cord. A series of experiments was performed to detect and quantify vocal cord fibroblast elastin production. The effect of mechanical and chemical stimuli were also investigated in order to identify potential changes in elastogenesis.

Cultures of adult canine vocal cord fibroblasts were established by explanting, and an enzyme linked immunosorbent assay was used to quantify the soluble tropoelastin (TE) secreted by 2nd-3rd passage cells into the culture medium. Control cell populations were found to synthesize large quantities of elastin precursor, greater than 115,000 molecules TE/cell/hr. Treatment with ascorbic acid suppressed TE synthesis whereas treatment with hydrocortisone (1.3 uM) and transforming growth factor-B (TGF-B, 10ng/ml) led to significant increases in TE production by 28% and 91% respectively. Cells were subjected to mechanical stretch in order to simulate the stimulus of vocal fold movement during phonation. When cells were stretched (5%, 0.5 Hz, Flexercell[®]) for a period of 48 hours they synthesized 23% more TE than the stationary controls. Stretched cells pretreated with either hydrocortisone or TGF-B also demonstrated further significant increases in TE production (37% and 29% respectively) noted after 24 hrs of stretching when compared to similarly treated stationary controls.

This study demonstrates the successful isolation and culture of a vocal cord fibroblast cell strain, and will provide a tool for further study of the extracellular matrix components that may be responsible for the biomechanical properties and functional correlates of this unique structure. We report the discovery that vocal cord fibroblasts synthesize significant amounts of elastin. The finding that these cells respond to hormonal and mechanical stimuli by increasing elastin production may have clinical implications for minimizing loss of vocal cord elasticity in the injured or aging larynx.

Supported in part by the Veterans Administration, Genentech, Inc., and NIH grants AG 06528 and GM 37387 (JMD) and the SDIO Office of Naval Research (RHO).