A FIRST TEXTBOOK OF GROWING AND HEALING WITH LIGHT EMITTING DIODES (LED PHOTOBIOENERGETICS AND PHOTOTHERAPEUTICS) WITH ACCOMPANYING STUDIES AND RESOURCES

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fondly dedicated to my brother kurt, chet, serge and crellin
with many thanks to dr. robison and mr. forcier
katy, suresh
and a little boy i call "eaglewolf"
as well as everyone who has graciously allowed the use of their works to be reproduced here.

INTRODUCTION

LET THERE BE LIGHT!

I remember looking at people with a regular prism and found that they all had green around the nose if they were alive. So one morning, after getting involved with grow lights that were beginning to be offered with light emitting diodes, I woke up knowing these would also be good for us (and other biological organisms) as well as disinfective and for many other modalities. LEDs are coming into their own as energy saving devices in a world challenged by diminishing oil reserves. My own house is 12 volt compliant with LED lighting that I hand-made and are bright enough to read by as well as soft as moon light when used as a night light. I also catch the rain.

Long ago I was with Crellin Pauling at the University of California, Riverside, and had access to a scintillator and spectrum photometer. Before my freshman classes began I picked up "Bioenergetics" by, I think, Lehninger. There I was introduced to mitochondria and the "Kreb's Cycle" (Krebs fell out of favor, I suppose, because he was the researcher who later brought us laetrile) wherein ADP (adenosine Di-phosphate) becomes ATP (a tri-phosphate) and then back to ADP as a basic energy transfer system in physiology. Bernard Jensen, Ph.D., in "The Chemistry of Man" reminds one that phosphorus is luminescent - it glows in the dark - and is known as the light bearer in natural healing, is in lecithin which makes up part of the phospholipid membranes of cells, and that bone phosphorus comes from vegetable matter and brain and nerve phosphorus derive from animal sources.

Phosphorus balances calcium, iodine and magnesium forming bonds with iron, potassium, sodium, magnesium and calcium as a phosphate, is insoluble in water and is non-conductive.

Phosphorus is in white blood cells, every nucleus, solid tissue and bone and cells need phosphorus to divide normally, oxygenate tissues and participate in the regulation of osteosis. It is necessary for acetylcholine and stimulation of wakefullness. It is influenced by vitamin C and the last thirty years has shown the electron transfer mechanism to be used in several other subsystems of energy exchange or free radical quenching. The ability of phosphorus to take light and move it into bioenergy and to luminesce in response to biostimulation is the crux of understanding photobiology.

"Phosphorus was discovered in 1669 by a German alchemist, Henning Brand, in the course of his search for the Philosopher's Stone. Brand heated the residue left on evaporation of urine..... (Greek ...phosphoros, giving light...Phosphorus vapor is tetratonic ... P4 ...at high temperatures the vapor is dissociated slightly, forming some diatomic molecules....P2...White phosphorus ...slowly changes to... red phosphorus, in the presence of light or on heating...iodine, ... serves as a catalyst."

Red phosphorus is more stable than white which catches fire at 40 degrees centigrade but **oxidizes slowly giving off a white light** (phosphorescence).
...
Several other allotropic forms of the element are known. One of these, *black phosphorus,* is formed from white phosphorus under high pressure. It is still less reactive than red phosphorus, and is the stable form of the element (standard enthalpy, relative to white phosphorus, -43 kJ mole to -1; that of red phosphorus -18 kJ mole to -1)."

pp 198-199

"Phospholipids are important substances that are present in every tissue ... especially

in cell membranes and the sheaths on nerves ...On hydrolysis they yield fatty acids, an alcohol, phosphoric acid, and a nitrogen-containing compound. Lecithin is formed in the body from fats. It is a good emulsifying agent and probably helps in carrying fats to the tissues.

"Cell membranes, such as the membranes of the red blood cell, consist of about equal amounts of lipids and proteins. There is also a small amount ... of polysaccharide, which is combined with polypeptide chains in the form of glycoproteins.

The principal properties of a membrane are largely determined by the nature of the phospholipids in it. The molecules for these substances carry electric charges and hydrogen-bond-forming groups at one end, and consist of hydrocarbon chains at the other end. The polar ends are hydrophilic, and form the surfaces of the membrane, whereas the hydrocarbon ends, which are rejected by aqueous phase, extend toward other hydrocarbons. A double layer is formed, about 8 nm thick, ..."

"... the ends of the chains move rather freely, so that the structure approximates tha of a liquid in the middle portion of the membrane and of a crystal toward the surfaces. "

pp 534-536

Chemistry, copyright Linus Pauling1947-1970, Pauling and Pauling1975 (Freeman)

In structural chemistry many molecules cannot be described completely by valence theory and so several potential configurations of a molecule are known as the **theory of resonance**. (One potential consequence of this is that one DNA strand codes for proteins and the other strand repairs mistakes!) It is reasonable, in light of the foregoing, that the iodine catalyst implicates the thyroid and basal metabolic rates with a photobiologic effect wherein the p2-p4 electromagnetic inductance enfolds, through oscillative displacement, an electromagnetic component of the photon. Oxidation causes luminescence and reduction the opposite. This appears to be an engine to pass quanta of light to biological systems, particularly in light of phosphorus' unique bonding with iron, potassium, sodium, magnesium and calcium.

Phosphorus can have an oxidation potential between -3 and +5. Phosphoric acid is essential part of DNA and RNA, coenzymes, ADP/ATP and other vital biological functions. Two phosphorus/oxygen bonds in juxtaposition are the energy rich bonds in ATP and PO4 has 4 resonance structures. A lot of energy is stored in the phosphorus bond, swings quickly between resonance states and finally converts an electron to a photon and back.

Could the muscles on the skeleton be the energy for this engine as a results of the bones piezo-electric effect to being bent/stressed?

This is essentially just an extension of Einstein's seminal "Photo-Electric Effect".

Modern physics was boosted into quantum mechanics when Max Plank couldn't get a black body to explode from heating by ultraviolet light - or any other light. In quantizing the re-emitted energy his observations gave the quantity as h (hot body but not exploded body):

"The German physicist Max Planck (1858-1947) ... discovered that ... the hot body cannot emit or absorb light of a given wavelength in arbitrarily (sic) small amount , but must emit or absorb a certain quantum of energy of that wavelength. ... light quanta or photons - ... pointed out by Einstein ... supports this concept."

"... the light energy of wavelength lambda absorbed or emitted by a solid body ... (is) .... proportional to the frequency v ... (equal to the speed of light divided by wavelength lambda):

$$E=hv$$

E is the energy of light

v is frequency

h is **Plank's constant** - **the basis of quantum theory** - is **0.66252 x 10-33 J s**

"the units h, J s, have the dimensions of energy x time, ..."

"In 1887 the German physicist Heinrich Hertz (1857-1894), who discovered radio waves, observed that a spark passes between two metal electrodes at a lower voltage (less resistance km) when ultraviolet light is shining on the electrodes than when they are not illuminated. ... J. J. Thompson (discovered) in 1898 that negative electric charges are emitted by a metal surface on which ultraviolet impinges. ... called the **photoelectric effect** ... visible light falling on a zinc plate does not cause the emission of photoelectrons, whereas ultraviolet light with a wavelength shorter than 350 nm does cause their emission. The maximum wavelength effective is called the photoelectric threshold. ... the alkali metals are especially good .... sodium is about 650 nm ... ."

"It was discovered that the **photons are emitted with extra kinetic energy, depending on the wavelength of the light**. ... If the collecting electrode is given a slight negative potential, which requires work to be done on the electrons to transfer them from the emitting metal to the collecting electrode, the flow of photoelectrons to the collecting electrode is stopped if the incident light has a wavelength close to the threshold, but it continues if the incident light has a wavelength much shorter than the threshold wavelength. By increasing the negative charge on the collecting electrode the potential difference can be made great enough to stop the flow of electrons. ... explained by Einstein in 1905, ... when the light is absorbed by the metal all of the energy on one photon is converted into energy of a photoelectron. However, the electron must have a certain amount of energy to escape from the metal. ... Ei (the energy of ionizing the metal). The remaining energy is kinetic energy of the

photoelectron. The Einstein photoelectric equation is

hv=Ei=1/2(m)(v)2 ..."

m is mass of electron

v is its velocity

"... **the energy of the light quantum, hv, is equal to the energy required to remove the electron from the metal, Ei, plus the kinetic energy imparted to the electron**, 1/2mv2. ... the energy quanta ... is measured by measuring the potential difference, V, which is necessary to keep the photoelectrons from striking the collecting electrode; the product of the potential difference V and the charge of the electron, e, is the amount of work done against the electrostatic field, and when V ... prevent(s) the electron from reaching the collecting plate ..

eV=1/2(m)(v)2 ..

eV=hv - Ei

V=hv/e - Ei/e ..."

page 67-71

With this you can calculate the retarding voltage to stop photoelectric effect.

so the amount of energy in 650 nm is the speed of light divided by frequency

v=3x10 to 8th/650 x 10 to -9=**4.62 x 10 to 14th cycles per second** or hertz

energy of photon (**0.6625 x 10 to -33 J s**) times the above (4.62 x 10 to 14th Hz) =

0.306 x 10 to -18 J

then **retarding voltage** would be minimal for 650 nm but twice as much for 325 nm (0.612 x 10 to -18th J) half used to eject electron from metal and the other half is kinetic energy of electron.

eV=0.306x10 to -18 J

V= 0.306 x 10 t0 -18 J/ 0.612 x 10 to -18 C (sic)=1.91 volt

to prevent flow in sodium photoelectric cell illuminated with wavelength 325 nm.

Chemistry, copyright Linus Pauling1947-1970, Pauling and Pauling1975 (Freeman)

and in Albert Einstein Relativity
The Special and General Theory
Crown Pub., NY 1961 (original 1916):

"Even though classical mechanics does not supply us with sufficiently broad basis for the theoretical presentation of all physical phenomena, still we must grant it a considerable measure of "truth", since it supplies us with the actual motions of heavenly bodies with a delicacy of detail little short of wonderful. ... In the general laws of nature which have been formulated with reference to K, the magnitude and direction of the velocity .... would necessarily play a part. We should expect, for instance, that the note emitted by an organ pipe placed with its axis parallel to the direction of travel

would be different from that emitted if the axis of the pipe were place perpendicular to this direction. Now in virtue of its motion in orbit round the sun, our earth ... If the principle of relativity were not valid we should therefore expect that the direction of motion of the earth at any moment would enter into the laws of nature, and also that the physical systems in their behavior would be dependent on the orientation in space with respect to the earth. For owing to the alteration in direction of the velocity of revolution of the earth in the course of a year, the earth cannot be at rest relative to the hypothetical system Kzero throughout the whole year. However, the most careful observations have never revealed such anisotropic properties in terrestrial physical space, *i.e.* a physical non-equivalence of different directions. This is very powerful argument in favour of the principle of relativity."
Pages 12-15

and so phase angle illumination has to have significance! (also debunks the string theory and it's dimensions as there has to be an equivalence of directions).

Part II The General Theory of Relativity XVIII Special and General Theory of Relativity

"The basal principle, which was the pivot of all our previous considerations, was the *special* physical relativity of all *uniform* motion. (from page 18 ... If, relative to K, K' is a uniformly moving co-ordinate (Eucledian/Galilean - km) system devoid of rotation, then natural phenomenon run their course with respect to K' according to exactly the same laws as with respect to K. This statement is called the *principle of relativity* (in the restricted sense).) ... every motion must be considered as relative motion. .... motion here taking place in the following two forms, both of which are equally justifiable : (a) the carriage is in motion relative to the embankment. (b) The embankment is in motion relative to the carriage. In (a) the embankment, and in (b) the carriage, serves as the body of reference in our statement of the motion taking place. ...A particle left to itself and sufficiently far removed from all other particles moves uniformly in a straight line. With reference to K (Galilean reference body) the laws of nature were to be as simple as possible. But, in addition to K, all bodies of reference K' should be given preference in this sense, and they should be exactly equivalent to K for the formulation of natural laws, provided that they are in a state of uniform rectilinear motion and non-rotary motion with respect to K ... In this sense we speak of the *special* principle of relativity, or special theory of relativity.
pages 59-61

"In contrast to the electric and magnetic fields, the gravitational field exhibits most remarkable property, which is of fundamental importance for what follows. **Bodies which are moving under the sole influence of a gravitational field receive an acceleration, *which does not in the least depend either on the material or on the physical state of the body.*** ...

According to Newton's law of motion ... (Force)=(inertial mass)x(acceleration)
where the "inertial mass" is the characteristic constant of the accelerated body.
If now gravitation is the cause of the acceleration, we then have

(Force)=(gravitational mass) x (intensity of the gravitational field),
where the "gravitational mass" is likewise a characteristic constant for the body.
From these two relations follows:

(acceleration)=
(gravitational mass)/(inertial mass) x (intensity of the gravitational field)

... **The *gravitational* mass of a body is equal to its *inertial* mass**. ... **The *same* quality of a body manifests itself according to circumstances as "inertia" or as "weight" (lit. "heaviness"). ...**
pages 63-65

"We thus have good grounds for extending the principle of relativity to include bodies of reference which are accelerated with respect to each other, and as a result we have gained a powerful argument for a generalised (sic) postulate of relativity.

"We must note carefully that the possibility of this mode of interpretation rests on the **fundamental property of the gravitational field of giving all bodies the same acceleration, or, what comes to the same thing, on the law of the equality of inertial and gravitational mass.** ..." "The suspended body experiences a downward force in the gravitational field, and this is neutralized by ... gravitational mass of the suspended body"."... we see that our extension of the principle of relativity implies the *necessity* of the law of equality of inertial and gravitational mass."
pages 66-68

Albert Einstein Relativity
The Special and General Theory
Crown Pub., NY 1961 (original 1916)

Recognizing that gravitational mass is sometimes inertial and vice versa is the way our elders approached the force in place and the force as it acts afar. Einstein then recognizes, with Newton, that special relativity and the laws of motion did not answer all of the forces, which is even more mysterious when the reference body is rotating uniformly, but that general relativity holds for every body whatever its state of (relative) motion(s).

It seems that light masquerades as electricity in matter and that the four dimensions of light - electromagnetic rotation (to be addressed in the second edition and incorporating the bioelectric and electromagnetics of the therapy described by Robert Becker), direction (wave) and horizontal stability (particle...particularity) and time become three dimensions (wave-particle-time) enformed in matter (an electromagnetically stable package or sheath which acts as a push-pull on bond angles and bubbles off, as mass displaces water, or captures "electrons" where bioenergy is stored for transfer.)

This is most dramatically reflected in the relativity thought experiment known as the "Einstein-Rosenberg Rigid Rotating Disk Paradox" : like an old vinyl record on a spindle, some point "A" circles the center at some speed while a point "B" at the edge of the disk is not only traversing a greater distance but also moving faster than point "A" while, in this example, conserving centripetal and centrifugal forces due to the rigidity of vinyl but if the push-pull of electromagnetic forces is uneven but balanced a kind of energetic "Brownian motion" may ensue. Is this how momentum is conserved? Is this how gravity works...not bending but push-pulling effects in a balance of awesome dynamic proportions? At what point does "B" fly away or collapse into point "A"?). We have to have conservation of matter and energy with angular momentum conservation of electromagnetic rotation although we can channel the entropy into useful work before it "grounds out".

E can be angular momentum and the mass of Einstein is a coefficient of mass/ masslessness and light is at least a squared squared (and probably higher) speed as the angular momentum is conserved in toto and light is the phenomenon of wave (photon) jump as momentum equalizes.

E=mc2

is based on motion (light speed) or lack thereof and its relation to mass.
Both E and angular momenum are conserved in the universe.
Mass increases/decreases relative motion thus dark matter is only dark in that it isnt light right now but explains apparent simultanaeity as displacement of momentum in an active matrix equilibria.
It is relative luminesence that balances energy conservation both kinetic/potential and angular momentum-not speed but homeostasis or, more appropriately, dynamic equilibrium-Isn't gravity the same as matter bound angular momentum? Aren't these the "gears" of "primum mobilium" and the cause of non-uniform accelerations? Angular momentum creates and destroys mass and redistributes "heaviness".
....

Scientific American August 2005 at 57:

“In general, the magnetoresistance of layered systems is significantly higher than that of a system not built in layers, ... magnetoresistance or tunneling magnetoresistance ... depend on the electrons’ spin s (their angular momenta)... torque that can switch a layer’s magnetism from one direction to another.”

Scientific American June 2005 at 55 :

Addressing the fine structure of the gravitational constant (k = 6.673 x 10 to -31) we have the wavelength, lambda, equal to the square of the electric charge (mass of the electron being 9.10938188 x 10 to -31) divided by 2 **x “empty space”** hc (1/137.0359.....pretty cumbersome!) “... **the fact is that all elementary particles arise as quanta of a corresponding quantum field. ... All fields have a property called spin, an intrinsic quality of angular momentum** ...” at 42. It is estimated that 4-5% of

mass in universe from energy of motion (quarks and gluons). So, it would seem we are in a 2:3 ratio of gravity to mass to explain energy and angular momentum!

Particles that interact with Higgs field (**boson** has spin zero with electron spin @ 1/2 and photon (force) spin of 1) **behave as if they have mass**, proportionate to the strength of the field time the strength of the interaction affect of weak force. see Ibid 44. Dark matter, up to 70% of universe, from gravitational effects? I think angular momentum effects is more descriptive.
The masses of the quarks themselves, however, and also the mass of the electrons are entirely caused by the Higgs field. Ibid 46

The electromagnetic particle is a photon!

In classical physics angular momentum, a property of spinning, is a balance of centripetal and centrifugal force - an imbalance tending to one or the other and can be the mechanism of instantaneous change in the universe more as a function of Archimedian displacement theory rather than the bending theory of modern physics. Should the balance fail the spinning will expand or contract ...or transfer "displacement" as rearrangement of the whole.

Quantum physics postulates up and down spinning as well as all sorts of intermediary properties to what is a process of oscillation displacement by light which is in a dynamic stasis that is ready to transfer kinetic energy from or to light. Oftentimes small differences such as the difference in spin from center to edge creates a quantum engine to redistribute angular momentum.

Truly, we stand on the shoulders of giants (with a few serendipitous events like varying and/or mixing the impurities in silicone diode design and production to get specific frequencies to fluoresce).

During 1999 to 2003 the University of Wisconsin Medical College (MCW) and the National Aeronautics and Space Administration (NASA) collaborated on several experiments to address problems with bone density, wound healing and other biological problems being faced by astronauts in zero gravity conditions (see Healthlink at the Medical College of Wisconsin or Dr. Whelan in Appendix 3). Their results are not only startling but, I believe, will issue in a new age of healing modalities.

The tricorder is here, we just need to construct it (them)-mine is more of a removable head flashlight/light saber affair with a multivibrator to power an array that individual LED wavelengths can be accessed and blended with a twist of the handle....my "tricorder" would be a central 635 nm 15 degree pulsing at 4 cps to enhance genetic repair surrounded by 634 nm 30 degree @ 8 cps immune support and phagocytosis enhancement with some yellow to assist lymph movement and of course the 660/670 nm hemoglobin helpers alternating with 830/880 . Perhaps even vitamin A/beta

carotene and vitamin C could be stimulated with some orange (I am sure that the Drs. Pauling would wonder if LEDs could enhance vitamin C) and some blue for disinfectant. Deeper stimulation with 880 nm. I suppose varying according to healing stage would be desirable. In fact, I have had healing from small infections within 24-48 hours with a small handheld commercial laser pointer in the appropriate range pulsed 10-20 times and feel that even the "flesh-eating" bacteria might be overcome with perhaps a combination of an ultra-violet, blue and red array. I would sure like to see a bacteriology lab and the technologies you are about to encounter.

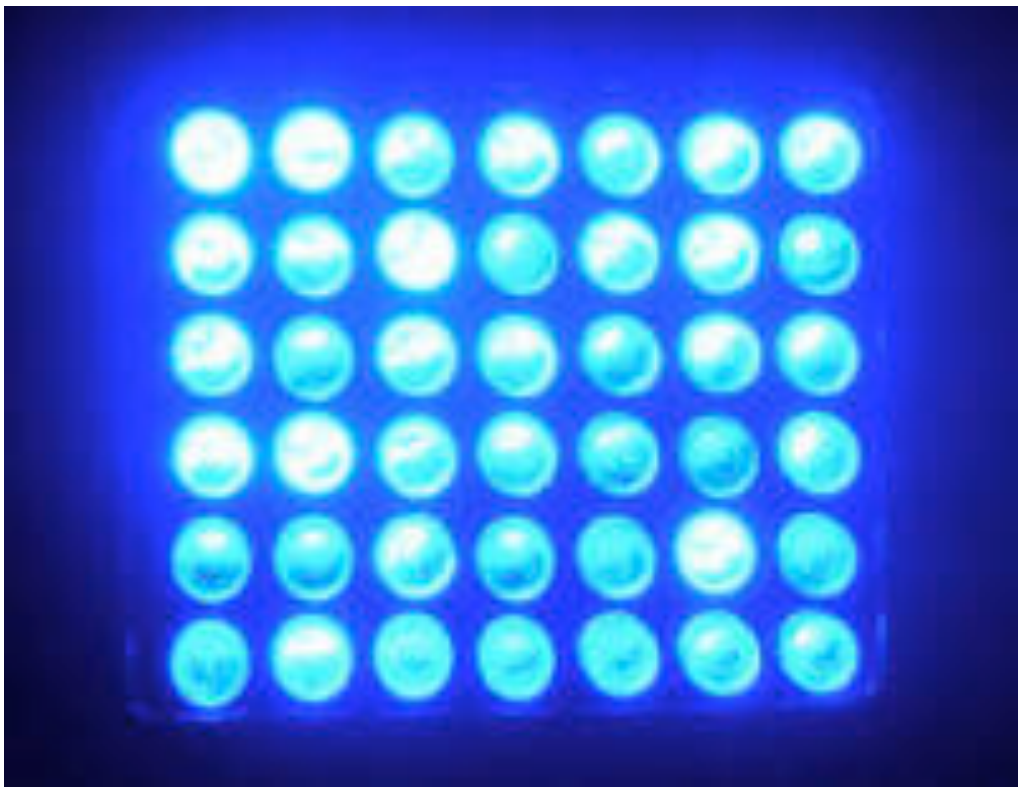
NASA found that 3 discrete wavelengths brought about the best healing. Given that the average LED is + or - 10-25 nanometers (nm) from it's peak emission, the concept of discrete is strained and it becomes understandable that three pulsed with a "peak wavelength" might really be broad coverage. Can't you see a toothbrush of fiber optic fibers and the blue lights (with some DNA repair reds) ? or grow lamps with "buglight" LEDs or filter shades to reduce attraction to insects? Water glasses that disinfect your water? (see http://www.guyotdesigns.com for a glasses with just white light LEDs) . UV flashlights to kill mold? A UV penlight to sterilize public facilities? UV is not just for forensics or checking for counterfeit bills any longer. Nano "bandaids" that have embedded arrays and get current from being on the skin? These bandages and wraps would have vitamin D enhancing UVB 282 nm (see "Townsend Letter" Feb-Mar 2006, Wysong, pg.73) on a low, constant power, probably in a surface mount 180 degree beamwidth in a 1:1 with 660nm hemoglobin enhancing, one-half of the red pulsing at 4 cycles per second and the other half on continuously. **Perhaps a musical pulse in 4/4 time would enhance the healing results.** Opioid stimulation would be necessary in severe burn situations.

It would be nice to see some grant money to develop ways to slow/halt/heal decubitis as I have many friends with various stages of paralysis but always the same problem with "bed sores" and other sores...a perfect population ready for wound healing LEDs!

I expect to see neckbands to enhance oxygen delivery to the brain in ischemic (oxygen starvation from stroke, infarct, etc.) at retail stores and anti-SARS/Bird masks with surface mount local sterile field devices. Eyecups and sleep masks for retinal repair and rejuvenation. Fertility may well be supported with light. Even fibromyalgia, lupus, multiple sclerosis, chronic fatigue and other maladies will be controlled with light treatments in one's home while sleeping, showering or other positive passive exposure opportunities. The possibilities are endless, right now! Some even inject chemical/silicon units that are photoactive and then activate with light in photodynamics. Perhaps beamwidth and beam angle studies will disclose more precise results unmasking the photons masquerading at the universal costume ball.

I offer this in the spirit of sharing and encouraging the development and deployment of all the newly available light technologies for the reduction of human suffering. I hope that anyone from grade school to post doc studies will be intrigued and find this a point of beginning their life long inquiry with some of the who, whats, where and even how to links provided herein. The why may never be adequately answered but I submit

that light is bioelectrically active in living matter and that matter is the angular momentum package that plays the ancient mayan ball game of " pass and get the (photo) electron in the hole". Live long and prosper!



Blue light array from Elixa http://www.elixa.com

Light therapy as a treatment alternative (from http://www.my-tronic.com) :

"...researchers look for ancient remedies and combine them with modern medicine. One of these ancient cures is light therapy defined as a mean (sic) to heal using visible light or UV light to treat a variety of medical conditions. ... in 1903 when the Danish Dr. Niels R. Finsen received the Nobel prize in Physiology of Medicine for his method of treating disease – especially lupus vulgaris, with UV light. He also treated smallpox using red light. Many other scientists before used light to cure, but he was in fact the first one to obtain astonishing results.

Today a well-known form of light therapy is UV – used to cure psoriasis. One form of treatment is the Goeckerman regimen, combining UV-B light with coal tar applied to the affected skin. This medical treatment is also known as phototherapy and it may be also used for other skin conditions such as eczema, lymphoma, pruritis, atopic dermatitis, and so on. As UV-B, UV-A is also used to treat psoriasis. The method is called PUVA and it combines psoralen (a drug) and ultraviolet-A.

Impacts of coloured light on specific disorders

Mental conditions were already treated with light therapy since the 1880s, when many hospitals around the world calmed their patients with blue light and stimulated others with red. It is already scientifically proved that coloured lights have different effects:

Red gives energy and strength, enhances sexuality, adds vitality and stimulates blood circulation. Orange invigorates the lymphatic system; yellow improves concentration and stimulates the circulatory system and skin; green heals and balances; blue reduces stress and violet calms the metabolic system and the nerves. Strobe light excites nerve endings (neural pathway report only changing stimuli). A non-colour strobe is recommended for children with learning difficulties and people with chronic pain. Colour strobe lights bring out deep-seated emotional problems."

Photobiology

From Wikipedia, the free encyclopedia
http://en.wikipedia.org/wiki/Main_Page

Photobiology is the scientific study of the effects of light on living organisms. The field includes the study of photosynthesis, photomorphogenesis, visual processing, circadian rhythms, and ultraviolet radiation effects. The major professional photobiology societies are the American Society for Photobiology, whose official journal is Photochemistry and Photobiology, and the European Society for Photobiology, whose official journal is Photochemical and Photobiological Sciences.

STUDY QUESTIONS AND IDEAS TO COGITATE DURING YOUR READING

1) WHAT DEVICE WOULD YOU MOST LIKE TO SEE FOR EITHER GROWING OR HEALING?

2) WHAT IS THE PHOTO-ELECTRIC EFFECT? IS IT RELATED TO BROWNIAN MOTION? HOW MIGHT ARCHIMEDES, IMHOTEP, ARISTOTLE OR LEONARDO DA VINCI EXPLAIN THE EFFECT?

3) HOW WOULD NEWTON AND EINSTEIN VIEW LEDS?

4) CONTRAST NEWTONIAN AND QUANTUM MECHANICS.

5) WHAT IS MITOCHONDRIA? WHAT DOES IT DO? WHY IS THIS IMPORTANT?

6) IS DNA STATIC? A RIGID TEMPLATE? A "SLINKY"?

7) WHY WOULD BEAM WIDTH BE IMPORTANT? BEAM ANGLE?

8) WHAT IS HARMONY, RESONANCE, PITCH, TIMBER, TEMPO?

9) HOW IS INTENSITY MEASURED? WHY IS THIS IMPORTANT? ARE THERE BETTER WAYS TO DESCRIBE?

10) WHAT IS TEMPERATURE OF 660 NM? COMPARATIVE MAGNITUDE?

11) EXPLAIN ELECTRON TRANSPORT WITH PHYSIOLOGICAL EXAMPLES.

12) WHAT IS ANGULAR MOMENTUM AND HOW IS IT CONSERVED?

13) WHAT IS A PRISM? WHAT IS A RAINBOW?

14) EXPLAIN THE ALPHA HELIX HYDROGEN BOND.

15) EXPLAIN THE DOUBLE HELIX WITH REFERENCE TO BONDING, REPAIR MECHANISMS AND METHYLATION.

16) WHAT IS A CATALYST?

17) HOW IS ENERGY STORED IN A CRYSTAL? WHAT IS A LATTICE? HOW MANY BASIC SHAPES ARE THERE FOR CRYSTALS ?

18) WHAT ARE THE RESONANCE FACTORS IN CRYSTAL GEOMETRY?

19) EXPLAIN THE ANATOMY OF THE EYE. THE SKIN.

20) ARE THERE ANY CONTRAINDICATIONS TO LIGHT THERAPY?

21) ANY CANCER WARNINGS OR USES?

22) DISCUSS LASER POINTERS, BEAM ANGLE AND THERAPY.

23) HOW DO PLANTS RESPOND TO MOLD? COULD MOLD BE CONTROLLED WITH LIGHT WAVELENGTHS? WHY?

24) EXPLAIN WHY PLANTS LOOK GREEN.

25) WHAT COLOR LIGHTS ARE NEEDED FOR PLANT GROWTH? IS IT DIFFERENT FOR BLOOMING?

26) EXPLAIN AND GIVE EXAMPLES OF WAVELENGTH AND DEPTH OF PENETRATION OF LIGHT. ARE ADVERSE PHYSIOLOGICAL REACTIONS POSSIBLE? BENEFICIAL? EXPLAIN.

27) HOW CAN LIGHT BE MAXIMIZED IN HORTICULTURAL APPLICATIONS?

28) GIVE SOME OF BLUE LIGHTS ACTION ON PLANTS. SAME FOR RED. GREEN. YELLOW.

29) WHY ARE CAROTENOIDS ORANGE?

30) WHAT IS PHYCOBILLIN AND IS IT OF ANY USE?

31) WHAT KIND OF YELLOW WOULD YOU USE TO DISCOURAGE BUGS? WHAT KIND OF FILTER? BOTH?

32) WHAT IS ANGIOGENESIS?

33) WHAT DOES HYPERBARIC MEAN? WHAT KIND OF THERAPY IS THIS AND WHY IS IT USED LEDS? WHAT COLOR LEDS?

34) WHAT IS TYPICAL BANDWIDTH OF LEDS USED IN HEALING? BEAM ANGLE?

35) WHAT IS PHOTODYNAMICS? PHOTOTHERAPEUTICS? PHOTOBIOENERGETICS? PHOTOMEDICINE?

36) WHAT IS THE AVERAGE WAVELENGTH OF CELL TISSUE? CYTOCHROME C?

37) WHAT IS ACCUPUNCTURE? ACCUPRESSURE? WHY WOULD YOU STIMULATE A POINT? SEDATE A POINT?

38) WOULD YOU TREAT WITH LIGHT THROUGH THE NAVEL? EXPLAIN.

39) WHAT IS A NANOBACTERIUM?

40) DO YOU THINK LEDS CAN BE USED AS AN ANTIBACTERIAL? ANTIVIRAL? GERMICIDE?

41) WHAT BEAM WIDTH WOULD YOU USE TO TREAT A RETINA? WOULD A LASER POINTER BE A GOOD TOOL FOR HEALING?

42) NAME SOME CONDITIONS OF THE EYE THAT MIGHT BE HELPED, AND HOW, WITH LIGHT THERAPY?

43) WHEN WOULD 880 NM LED LIGHT BE INDICATED?

44) HOW DOES LED LIGHT AFFECT FIBRINOGEN AND CLOTTING?

45) WHAT IS A JOULE?

46) WHY IS INTENSITY IMPORTANT? CAN YOU EXPLAIN WHY INTENSITY IS RELATED TO SQUARE CENTIMETERS?

47) WHAT IS CONSIDERED A HIGH DOSE OF LIGHT? A NORMAL DOSE? EXPLAIN.

48) ARE LIGHT CALCULATIONS AND ELECTRICAL CALCULATIONS SIMILAR? DIFFERENT? EXPLAIN.

49) WHAT LIGHT DAMAGES DNA? WHAT LIGHT REPAIRS DNA? DISCUSS.

50) EXPLAIN WHAT PULSING THE LIGHT DOES AS OPPOSED TO CONTINUOUS RADIATION.

51) CAN YOU USE TOO MUCH LIGHT? EXPLAIN.

52) WOULD PULSING TO A MELODY AFFECT THE OUTCOME? DISCUSS.

53) DESIGN A RESEARCH PROJECT THAT INCLUDES LIGHT AND IT'S BIOPHYSICAL AND/OR QUANTUM MECHANICAL INFLUENCES.

THE FOLLOWING QUESTIONS REFER TO THE APPENDICES AND ARE MORE ADVANCED IN SCOPE AND DEPTH

PATENTS

1) LOOK AT THE PORTIONS OF THE SOLAR OASIS PATENT:

A- WHAT KINDS OF LEDS ARE BEING USED?

B- WHAT COLORS? FREQUENCIES?

C- WHY ARE THE BEAM ANGLES VARIED?

D- WHY IS THERE NO YELLOW?

E- WHAT CHANGES WOULD YOU MAKE AND WHY?

F- HOW DO YOU FIGURE HOW FAR FROM THE PLANTS THE LIGHTS SHOULD BE?

2) WOULD MUSICAL PULSES BE SUPERIOR? DISCUSS.

3) WHAT IS AN ANTENNA AND HOW DOES IT WORK?

4) WHY WOULD A BIOLOGICAL ENZYME BE AFFECTED BY RED LIGHT? WHAT IS NIR?

5) IS IT BETTER TO HAVE WIDE COVERAGE OR PINPOINT LIGHT? USE EXAMPLES.

6) CONSIDERING BEAM ANGLE, BAN WIDTH AND INTENSITY WOULD PHASE ANGULATION BE USEFUL? WHY?

7) LOOK AT THE PATENTS BY DEW, ET AL., BARTELT, ET AL. AND ANDERSON, ET AL. : (THREE PATENTS RELATING TO HEALING)

A-HOW ARE THEY DIFFERENT? HOW ARE THEY THE SAME?

B- USING AS MANY PARTS OF THESE THREE PATENTS AS YOU CAN TRY TO CREATE A SYNTHESIS AND DESCRIBE YOUR DEVICE.

8) LOOK AT THE DOUKAS, ET AL., HASA, ET AL. AND THE TWO TRAUNER, ET AL. PATENTS : (PHOTODYNAMIC THERAPY)

A- RELATE SELECTIVE WAVE THERAPY TO GENERAL PHOTODYNAMIC MEDICINE.

B- DISCUSS INHIBITION AS A MECHANISM FOR HEALING AND DISCUSS THE INTERPLAY OF FEEDBACK MECHANISMS THAT ENHANCE OR RETARD.

WAVELENGTHS AND ORDERS OF MAGNITUDE

1) LOOK AT VITAMIN D AND BETA CAROTENE AND DESCRIBE THEM IN COMPARARTIVE TERMS AND TRANSLATE NANOMETERS OF ABSORPTION OR ADSORPTION TO TEMPERATION AND MOLECULAR/ATOMIC BOND STRENGTHS.

2) DISCUSS THE USE OF FIBER OPTICS WITH LEDS- BOTH PROS AND CONS.

3) DISCUSS THE 600-900 NM BAND RELATIVE TO BIOLOGICAL REPAIR.

BIOLOGICAL DISRUPTION.

4) DISCUSS MITOCHONDRIAL PROTEIN SYNTHESIS IN RELATION TO VIBRATIONAL PATTERN HEALING FROM COLOR IRRADIATION.

Comparison of Lamp Colors

Color Name	Warm White	Cool White	Daylight	Red	"Bug"	Green	Blue	Plant or Aquarium
Typical Color Temperature (in Kelvin's) or approximate Wavelength (in Nanometers)	3,000K to 3,500K	4,100K to 4,200K	6,000K to 7,000K	647nm to 700nm	585nm to 647nm	491nm to 575nm	424nm to 491nm	647nm to 700nm and 424nm to 491nm
Phosphor wavelengths commonly used in lamps	At least one phosphor from each of the Red, Green and Blue columns is used. The ratio of the phosphors used determines whether the produced light is perceived as Warm White, Cool White, or Daylight shades.			611nm (Red-Orange) or 658nm (Red)	626nm (Orange)	528nm or 546nm (Green)	450nm (Blue)	Mix of 611nm or 658nm and 450nm

GROWING

The action spectrum of photosynthesis in green plants has principle peaks in the blue and red regions. LED with response peaks in the UV-A (380 nm) and red (620 nm) regions and the red and blue fractions of the light continuum are the most valuable energy resources for plant life, and plants necessitate more red (625 to 675 nm) than blue (400 to 470nm). Yellow (525 nm) triggers some photosynthesis and IR influences seeds and UV color and scent.

The photosynthetically active radiation is the range of light wavelengths that stimulates photosynthesis. The principle photosynthetic pigments are chlorophyll a and chlorophyll b. The range of 400-700 nm is defined as the photosynthetic photon flux (PPF) and is specified in terms of moles per square meter per second.

How Light Colour Influences Plant Growth

"Blue light: plants react to the intensity of blue light. Lessening the blue light will cause poor growth – the strength of the radiation in any other part of the spectrum is not as important as the intensity of the blue, which shapes height and quality.

Red (660 nm) and infrared (730 nm) (also known as IR or far red) light: Intensifying the total of IR in relation with 660 nm red makes plants grow tall and thin. On the other hand if red is increased while IR diminished, plants will be short but thick. Plant reactions are not linear with the red/far red ratio and they can also vary in their response to red and far red light.

Ultraviolet light (UV): While overexposure is dangerous, small amounts of UV light can be beneficial for the flora. In many cases UV light is a very important cause for colours, taste and aroma. But UV-C and UV-B are believed to stop plant spread and this is why they have to be removed from the light under which plants are developed in green houses by UV stabilisers or glass. Removal of the UV up to 400nm is might be effective also in case of virus carrier insects (as insects see partly in UV).

Direct light from the Sun distributes the useful wavelengths only on special times of the day and in small quantum enough for a harmonious growth in some parts of the Earth, yet not enough on others.

...The researchers found our that blue and red light is essential for plant growth and, in general, a percentage of 8% blue LEDs and 92% red LEDs, both with the same frequency and relative intensity per LED, are enough for a harmonious evolution. Blue has a smaller influence than red however a percentage between 1% and 20% of blue light can be selected, depending on the plants and their growth requirements. ... LEDs are not the only ones efficient for growing plants: sulphur microwave lamps are the most efficient light sources known to man, that can generate as much light as the noonday sun, perfect for illuminating large-scale systems such as greenhouses. For smaller applications, such as indoor gardens, LEDs seem to be the right choice."
Mihaela Lica
http://searchwarp.com/swa7630.htm

MOLD, BACTERIA AND ULTRA-VIOLET

Mold is a particularly difficult problem (let alone where to introduce it!) as it has a spore as well as a fruiting stage so UV and other disinfectants may be the only way to control spores from becoming active-mold doesn't grow in direct sunlight. As mold is a primitive "plant" I put it at the beginning.

UV light is measured by wavelength with wavelengths from 100 to 200 nanometers, (2) UV-C at 200 to 280 nanometers, (3) UV-B at 280 to 315 nanometers, and (4) UV-A at 315 to 400 nanometers .200 to 280 nanometers is the most lethal wavelength for microorganisms with 264 nanometers being the peak germicidal wavelength, is known as the Germicidal Spectrum.

http://www.ext.colostate.edu/index.html :
Clean It - Dry It - Disinfect It:
Mold and Mildew Control

By Jane K. Frobose, Colorado State University
Cooperative Extension, Denver County
October 1, 1999

Molds produce mildew, a growing organism, gray to bluish-green. Molds grow in damp, warm, poorly aired and dimly lit areas. Eliminate growth factors and the problem can be kept to a minimum.

Generally, you can find mold anyplace where moisture or relative humidity levels are high -- wet or damp basements, for example. Here mold can grow on walls, floors or carpeting. Moisture from the earth can migrate through concrete walls and floors.

from Mold-help.org (http://www.mold-help.org/index.php):

"Airborne mycotoxins from can definitely destroy one's health. Sometimes, people are unaware that they are breathing mold spores and mycotoxins until they are very sick. Certain people have a minor allergic reactions to the non-toxic mold, but once you leave the affected area they most likely recover with few serious side effects. However, if they have been exposed to the dangerous molds such as Stachybotrys or Chaetomium, they could suffer from a myriad of serious symptoms and illnesses such as chronic bronchitis, learning disabilities, mental deficiencies, heart problems, cancer, multiple sclerosis, chronic fatigue, lupus, fibromyalgia, rheumatoid arthritis, multiple chemical sensitivity, bleeding lungs and much more.
...
Molds come in thousands of different varieties, but a few who are some of the offenders that invade our homes. Alternaria and Cladosporium are the molds most commonly found both indoors and outdoors throughout the United States. Aspergillus, Penicillium, Helminthosporium, Epicoccum, Fusarium, Mucor, Rhizopus, and Aureobasidium are also common. One of the mycotoxins, aflatoxin, is produced by the fungi Penicillium, Aspergillus flavus and Aspergillus parasiticus. Four different aflatoxins, B1, B2, G1 and G2, have been identified with B1 being the most toxic, carcinogenic and prevalent. Another very dangerous family of toxin producers is Fusarium. The toxins zearalenone, trichothecenes or moniliformin can be formed by various types of Fusarium including F. moniliforme, F. oxysporum, F. culmorum, F. avenaceum, F. equiseti, F. roseum, and F. nivale.

The most dangerous mold strains are: Chaetomium (pronounced Kay-toe-MEE-yum) and Stachybotrys chartarum (pronounced Stack-ee-BOT-ris Shar-TAR-um) as they have been proven to produce demylenating mycotoxins among others, meaning they can lead to autoimmune disease. Under certain growth and environmental conditions, both of these fungi release toxic, microscopic spores and several types of mycotoxins that can cause the worst symptoms which are usually irreversible such as neurological and immunological damage. Some of these natural mycotoxins include a very strong class known as trichothecenes. Trichothecenes are also produced by several common molds including species in the genera Acremonium, Cylindrocarpon, Dendrodochium, Myrothecium, Trichoderma, and Trichothecium. The trichothecenes are potent

inhibitors of DNA, RNA, and protein synthesis, and have been well studied in animal models because of concern about their potential misuse as agents of biological warfare, due to their ability to destroy human health (mentally and physically), and never appear in an autopsy. "

and

"...three natural ingredients that kill mold: tea tree oil (an essential oil found in most health food stores), grapefruit seed extract, and vinegar. There are pros and cons of each, but all three work. Vinegar is by far the cheapest. Tea tree oil is expensive, but it is a broad spectrum fungicide and seems to kill all the mold families it contacts. The problem is that it has a very strong smell, but that dissipates in a few days. Grapefruit seed extract is also expensive, but has no smell."

from http://www.care2.com/ (There are formulas on their site.)

Deactivation of organisms using high-intensity pulsed polychromatic light

Document: United States Patent 5900211

Abstract: A method of deactivating microorganisms in food products, packaging material, water, air, and other products involves illuminating the microorganisms using at least one short-duration, high-intensity pulse of broad-spectrum polychromatic light. In variations of this embodiment, the light has an intensity of at least 0.1 J/cm.sup.2, the pulse duration is from between about 10 nanoseconds and 10 milliseconds, and/or at least 50% of the at least one pulse's energy is transmitted in light having wavelength from between about 170 and 2600 nanometers. Advantageously, the microorganisms may be Cryptosporidium parvum oocysts, Bacillus pumilus spores or poliovirus.

Toshio Nishida, Hisao Saito, and N. Kobayashi
Physical Science Laboratory

Ultraviolet (UV) light is chemically active and has precise spatial resolution. Therefore, a compact and efficient UV light source will provide a variety of applications in the fields of chemistry, biology, environmental science, and optics. However, present UV light sources are power consumptive and quite large in size. Semiconductor UV light sources, such as light emitting diodes (LEDs), represent a solution to this problem. Nitrides containing aluminium (Al) have a band gap in the wavelength range between 200 nm and 360 nm.

[1] T. Nishida et al., Jpn. J. Appl. Phys. 37 (1998) L459.
[2] T. Nishida et al., Int. Conf. on Blue Laser and Light Emitting Diode 1998.
[3] T. Nishida and N. Kobayashi, Phys. Stat. Sol. A 176 (1999) 45.

and back to Wikipedia:
Lighting

The single most important (and expensive) factor for the indoor cultivator to consider is lighting.

Fluorescent lighting

(see Frank Duda IV work on fluorescents and other information http://nemesis.lonestar.org)

Fluorescent ballasts and bulbs are very inexpensive and much cooler and more efficient than incandescent bulbs. In cultivation, fluorescent lighting is useful for growing seedlings and rooting clones, because the light produced is very gentle (unlike HIDs, explained next), and won't burn young and/or sensitive plants. Fluorescents are available in 'warm' and 'cool' spectrums, with 'warm' providing more light in the red spectrum and 'cool' providing more light in the blue spectrum. Cultivators generally use 'cool' bulbs in order to encourage short internodes.

The best type of light to use for indoor cultivation is a High-intensity discharge lamp (HID). High intensity discharge lamps typically work by passing an electrical current through vaporized gas at high pressure, although low pressure sodium bulbs have gas at low pressure. There are many types of high intensity discharge bulbs, including mercury-vapor lamps, sodium vapor lamps, metal halide lamps, and conversion bulbs for metal halide and high pressure sodium.

The only high intensity discharge bulbs suitable are metal halide (MH) and high pressure sodium (HPS). There are bulbs available in many different wattages from 75 to 1500 watts.

All high intensity discharge bulbs require a special ballast to run, which is contained in a metal box, which grows warm and hums quietly when in use. A metal halide ballast contains a capacitor and a transformer, and a high pressure sodium ballast contains a capacitor, a transformer, and an ignitor. Recently, electronic ballasts have also become available.

Metal halide (MH)

Metal halide bulbs produce light that is strongest in the blue spectrum, technically about 4000 kelvins, or around 460 nanometers. Metal halide bulbs also come in various coated varieties intended to increase the red spectrum, but these are all

inferior to a high pressure sodium in the red spectrum.

Metal halide bulbs produce about 65-115 lumens per watt and last up to 12,000 hours. They are available in vertical (BU or BD), horizontal (HOR), and universal (U), which may be burned either vertically or horizontally.

Metal halide is an excellent bulb for vegetative phase of growth, as it encourages short internodes (distance between sets of leaves), and inhibits cell elongation, creating a shorter, stockier plant. Growers with a single ballast often purchase a high pressure sodium ballast, and use a metal halide conversion bulb (a metal halide bulb designed for a high pressure sodium ballast) during vegetative phase.

High pressure sodium (HPS)

High pressure sodium bulbs produce light strongest in the red spectrum, technically about 2,200 kelvins, or around 660 nanometers.

High pressure sodium bulbs produce less heat and more light than metal halide bulbs, producing 97-150 lumens per watt, and they last longer as well, up to 24,000 hours.

High pressure sodium bulbs are excellent bulbs for the flowering phase, and the choice of most growers who have only one bulb. High pressure sodium bulbs are an excellent choice for the reproductive phase of growth, as they trigger a greater flowering response in the plant, and simulate a more autumn-like light spectrum. A high pressure sodium conversion bulb, a high pressure sodium bulb designed to be burned in a metal halide ballast, can be used during the reproductive phase if a grower has a metal halide ballast.

If high pressure sodium is used for vegetative phase, plants will usually grow slightly more quickly, but will also have longer internodes, and may be taller.

LED grow lights

LED panel light source used in an experiment on plant growth by NASA.

Recent advancements in LEDs have allowed for the production of relatively cheap, bright and long lasting grow lights that emit only the colors of light required for plant growth. These lights are attractive to indoor-growers since they do not consume as much power, do not require ballasts, and produce a fraction of the heat of HID lamps.

The lamps consist of arrays of many wide-spectrum red and a few narrow-spectrum blue LEDs of specific wavelengths.

Light intensity

According to the inverse square law, the intensity of light radiating from a point source (in this case an HID bulb) is inversely proportional to the square of the distance from the source. So if an object twice as far away, it receives only 1/4 the light.

Reflectors

Reflectors are the most important aspect of maximizing light efficiency. They come in two main types, designed to hold a bulb either horizontally or vertically. Most horizontal reflectors can be fitted with glass and air-cooled to reduce grow-room temperatures, and allow the bulb to be placed closer to the plants, although the glass panel slightly reduces light output. Water cooled reflectors are also available, but are rarely used, as they are very expensive and significantly reduce light output.

* Vertical

Vertical reflectors are generally less practical than horizontal reflectors, as they are less efficient, although they are usually also less expensive. When a bulb is burned in a vertical position, most of the light is emitted sideways, and must be reflected downward towards the plant, which increases the distance that the light must travel.

Vertical reflectors are available in cone and parabolic dome shapes. Cone shaped reflectors are very inexpensive but also very inefficient, and are generally not used. If a vertical reflector is used, it is generally of the parabolic dome variety.

* Horizontal

Horizontal reflectors are much more efficient than vertical reflectors, and generally more expensive. Most growers use horizontal reflectors, as the cost of a more expensive reflector is offset by the savings of burning fewer lights to generate the same light intensity at plant level.

Horizontal reflectors are available in a variety of shapes, most of which are roughly trapezoid shaped, although "bat-wing" or "gull-wing" designs are also relatively common.

Light distribution

When growing with artificial light, the light intensity will be very uneven in the grow-room. The plants closest to the light source will receive far more energy (in the form of photosynthetically-active radiation) than plants far away from the source. Additionally, plants will grow towards the light source (this is known as phototropism). In order to address this, many growers simply move their plants around within the grow room in order to ensure that all plants are growing evenly. This is easily facilitated by placing

planters on casters.

Another option for the cultivator is to purchase a light mover. A light mover simply moves the light around within the grow-room, so that the plants will grow evenly without being moved, and also allows the bulbs to be placed closer to the plants. Light movers are available in two styles, linear and circular. Linear models have a motor which moves slowly along a rail in a straight line, suspended from which is a light. Circular models have a central motor which rotates two or three arms, from each of which is suspended a light. Circular movers generally allow the light to cover slightly more area.

"All Grow Lights Are Not Created Equal"

says Jonathan Cardinale of LED Gow Master, Global, the distributor for Solar Oasis, one of the new LED grow light companies. Solar Oasis (http://www.solaroasis.com/) is the only company with a patented array of LEDs for growing plants. These are available in my eBay store "Eco Shack" Other grow light resources are in appendix three.

(http://www.stores.ebay.com/EcoShack).

There is only 'one' LED light bar which comes with a single purchase... you can built or connect up to 8 LED light bars per power supply (every bar comes with a power supply or a power patch cord). The Kit shown below is a total of 8 LED light bars connected together with patch cords and a single power supply with a rack to hold them.

Press Releases

7/29/05 SolarOasis- SolarOasis- Announces Collaborative Research Effort With Biosphere Foundation and NASA

7/26/05 SolarOasis- Granted U.S. Patent 6,921,182

Press Release: July 26, 2005

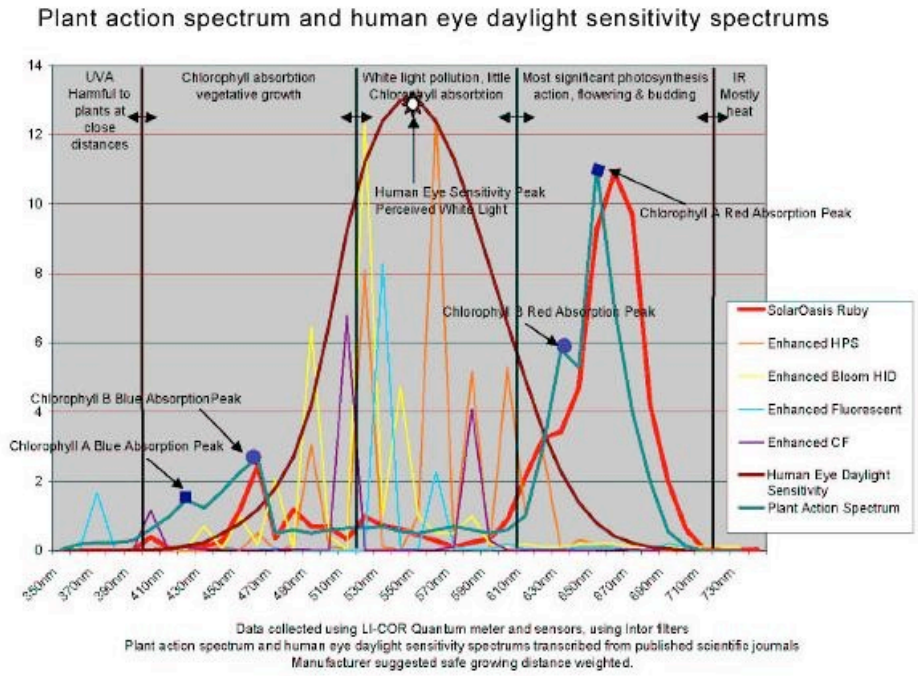
FOR IMMEDIATE RELEASE

SolarOasis- Granted U.S. Patent Number 6,921,182 For Ruby Gro-Bar Technology

Reno, Nevada – SolarOasis LLC announced today the granting of U.S. patent number 6,921,182 for its SolarOasis Ruby Gro-Bar technology, the first commercial LED grow

lighting technology available outside the laboratory.

"This patent exemplifies SolarOasis' commitment to the continued advancement of solid state plant grow lighting," said Larry Capen, CEO of SolarOasis, noting that a number of additional patent applications have been filed based on SolarOasis research into LED grow lighting technology.



http://www.solaroasis.com

Pigments are colorful compounds.

Pigments are chemical compounds which reflect only certain wavelengths of visible light. This makes them appear "colorful". Flowers, corals, and even animal skin contain pigments which give them their colors. More important than their reflection of light is the ability of pigments to absorb certain wavelengths.

Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other autotrophs --organisms which make their own food using photosynthesis. In plants, algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. However, since each pigment reacts with only a narrow range of the spectrum, there is usually a need to produce several kinds of pigments, each of a different color, to capture more of the sun's energy.

There are three basic classes of pigments.

Chlorophylls are greenish pigments which contain a porphyrin ring. This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight.

There are several kinds of chlorophyll, the most important being chlorophyll "a". This is the molecule which makes photosynthesis possible, by passing its energized electrons on to molecules which will manufacture sugars. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in the plants. A third form of chlorophyll which is common is (not surprisingly) called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. The differences between the chlorophylls of these major groups was one of the first clues that they were not as closely related as previously thought.

Carotenoids are usually red, orange, or yellow pigments, and include the familiar compound carotene, which gives carrots their color. These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll. For this reason, they are called accessory pigments. One very visible accessory pigment is fucoxanthin the brown pigment which colors kelps and other brown algae as well as the diatoms.

Phycobilins are water-soluble pigments, and are therefore found in the cytoplasm, or in the stroma of the chloroplast. They occur only in Cyanobacteria and Rhodophyta.

Phycobilins are not only useful to the organisms which use them for soaking up light energy; they have also found use as research tools. Both pycocyanin and phycoerythrin fluoresce at a particular wavelength. That is, when they are exposed to strong light, they absorb the light energy, and release it by emitting light of a very narrow range of wavelengths. The light produced by this fluorescence is so distinctive and reliable, that phycobilins may be used as chemical "tags". The pigments are chemically bonded to antibodies, which are then put into a solution of cells. When the solution is sprayed as a stream of fine droplets past a laser and computer sensor, a machine can identify whether the cells in the droplets have been "tagged" by the antibodies. This has found extensive use in cancer research, for "tagging" tumor cells.

Phycoerythrin

Phycoerythrin is a red protein from the light-harvesting phycobiliprotein family, isolated from red, blue-green, and cryptomonad algae.

A strong absorption peak exists at about 566nm, and a strong emission peak exists at 575nm +/-10 nm. (I.e.: phycoerythrin absorbs blue light and reflects red light.)

Like all phycobiliproteins, phycoerythrin contains as chromophore (light-capturing part) a molecule that consists of an open chain of four pyrrole rings. This chromophore is known as phycoerythrobilin.

In algae, phycoerythrin is an accessory pigment to the main light-absorbing chlorophyll pigments responsible for photosynthesis. The light energy is captured by phycoerythrin and is then passed on to chlorophyll.

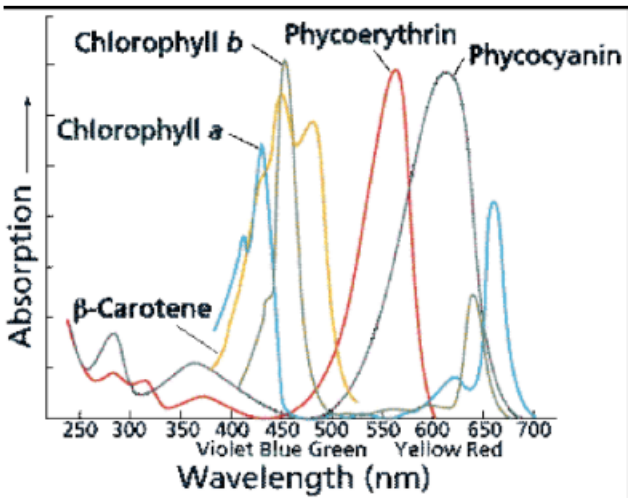
R-Phycoerythrin is useful in the laboratory as a fluorescence-based indicator for the presence of cyanobacteria and for labeling antibodies in a technique called immunofluorescence, among other applications.

What is phycocyanin?

* Blue accessory pigment attached to photosynthetic membranes

* Accounts for up to 20% of proteins in cyanobacteria

* Nitrogen storage molecule



Finally, indoor gardening enthusiasts can erase heat from their formula,

by adding new LED Grow-
Master Advanced Growth Arrays to their garden.

Emitting 100% Plant-Absorbed-Light, LED Grow-Master Gro-Bars use a patented blend of LEDs, to
produce only colors of light that plants need to grow.

Costing up to $135.00 per month to operate, high-wattage HID lighting systems produce only 25%
visible light, the rest is invisible heat, and only ½ an HIDs visible light is actually absorbed by plants
and used for photosynthesis.

“Plant Specific Lighting has much more to offer,” says Jonathan Cardinale, CEO of LED Grow-Master
Global. “Eliminating wasted light (Green/Yellow) and heat (Infra-Red) make LED Grow-Master Gro-
Bars the most efficient plant lighting systems on the market today, with 90% electrical savings, an
98% less heat, eliminating the need for large exaust fans.”

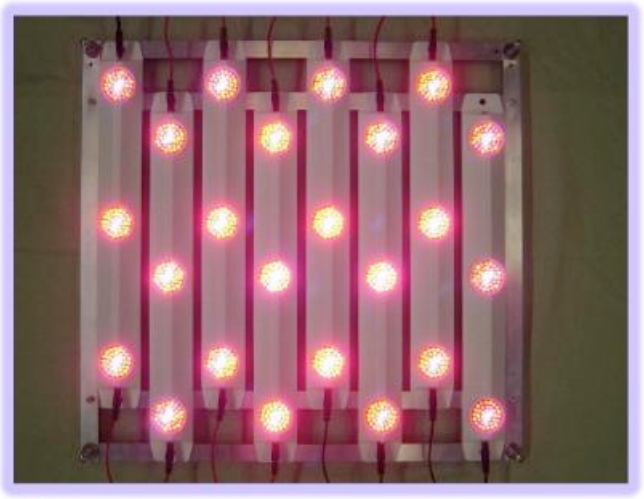
No Mercury, No Glass, No UV, and 7-10 years rated life make LED Grow-Master Lighting Systems the
“Greenest” horticultural lighting available, facing the world’s energy crunch head-on, with efficiency.

Low voltage UL listed power supplies, and advance circuitry make these systems safe for kids and
pets, opening the doors to classrooms, and home gardeners.

Using a patented and patent pending blend of LED colors designed and engineered by SolarOasis.

Easy to hang, and move LED Grow-Master Plant Lighting Systems allow for maximum control in your
garden, giving completely directional light eliminates the need for a reflector.

it looks red to us!



LED-Synthesis™

"Plant Specific Lighting"

8 LED Grow-Master™ Bars on SunRack™

Features:

- 8 LGM Bars (48 watts)
- 1-SunRack
- 1-AC/DC Adpter.
- 7-Array Adapt Chords
- Assembly Instructions
- 24"x24"
- Little Radiated Heat
- No wasted light
- Low Electrical Cost
- No Mercury,Glass,or UV
- Long Life
- Low-Voltage UL Power Supply
- Safe for kids and pets
- Solar/Wind/Battery Compatible
- 3'x3' Coverage
- 48 watts
- Pennies per day to opperate
- Proven Patented and patents pending
- Advanced Technology

In conclusion, LED-based illumination can enhance photosynthetic activity ensuring better plant morphology than attainable with high pressure sodium lamps.

AND WHAT ABOUT "BUG LIGHTS?"

FS923.pdf

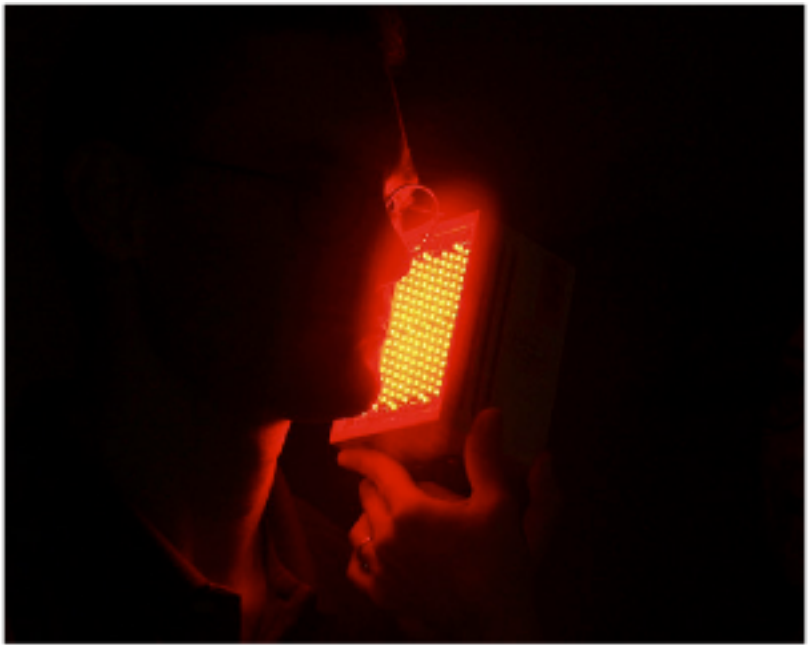
http://about.com
You have posed an interesting question, and I'm not certain I can give you a definitive answer. However, insects are attracted to white ceilings more than others as they are phototropic (attracted to a light source), and I would logically assume that creating a darker surface might be a deterrent. ...
I think the best way to handle the insect problem is to first replace the porch light bulb with a bug light. These yellow/amber bulbs emit a wavelength of light that is much less attractive, and may help to reduce the number of insects around the porch. ...

Eric

HEALING

LED technology was developed to enhance the growth of plant tissue in space by NASA's Marshall Space Flight Center and Quantum Devices Inc. of Barneveld, Wisconsin. LEDs have a similar physiological effect on human cells as they do on plant cells.
LEDs stimulate cytochromes in the body that increase the energy metabolism of cells. Cytochromes are part of the "electron transport chain" (see Krebs cycle above).

Note: "LED Light therapy has been deemed a nonsignificant risk by the FDA; thus, FDA approval for the use of LEDs in humans has been obtained. "
(Lager Engineering http://www.lagerengineering.com/home.shtml)



NASA photo
The LED center

photo Elixa http://www.elixa.com

LEDs are being studied in comparison to and in conjunction with hyperbaric oxygen therapy, a standard treatment in which the patient is placed in a pressurized oxygen chamber to stimulate new cell growth.

"So far, what we see in patients and what we see in laboratory cell cultures, all point to one conclusion," said Dr. Whelan. "The near-infrared light emitted by these LEDs seems to be perfect for increasing energy inside cells. This means whether you're on Earth in a hospital, working on a submarine under the sea, or on your way to Mars inside a spaceship, the LEDs boost energy to the cells and accelerate healing."

The Marshall Star Jan. 4, 2004: Light emitting diodes bring relief to young cancer patients front page (see also Dec.21, 2000

photo Elixa http://www.elixa.com

NASA scientific and technical information:
spinoff 2005
Lighting the Way for Quicker, Safer Healing
Health and Medicine
Originating Technology/ NASA Contribution

Who's to say that a little light can't go a long way? Tiny light-emitting diode (LED) chips used to grow plants in space are lighting the way for cancer treatment, wound healing, and chronic pain alleviation on Earth.

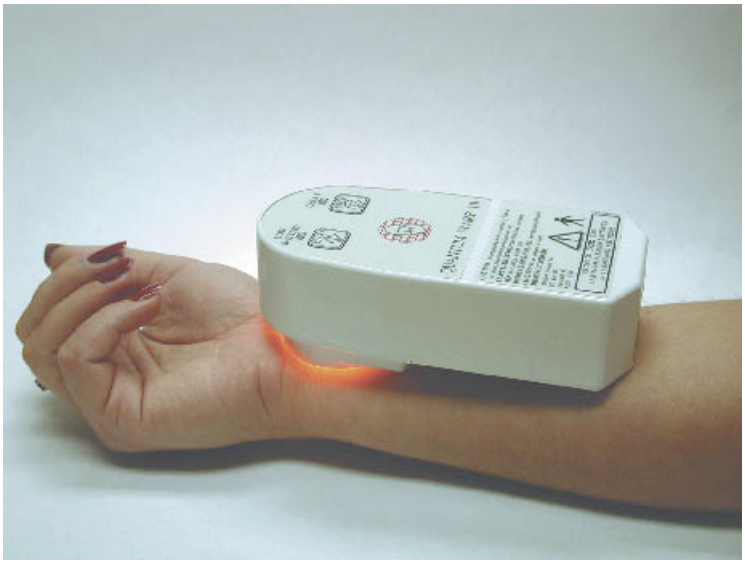
LED-ENHANCEMENT OF CELL GROWTH

Studies on cells exposed to microgravity and hypergravity indicate that human cells need gravity to stimulate
on Earth. An LED blanket device may be used for the prevention of bone
growth. As the gravitational force increases or decreases, the cell function responds in a linear fashion. This poses depth of near-infrared light penetration into human tissue has been measured significant health risks for astronauts in long-term space flight. The application of light therapy with the use of

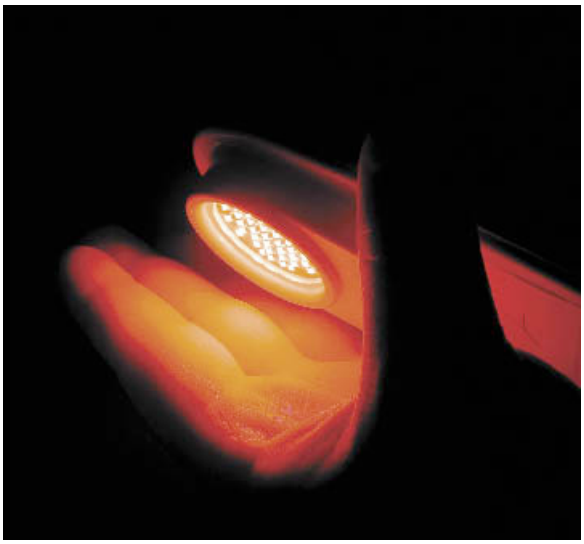
NASA LEDs will significantly improve the medical care that is available to astronauts on long-term space missions.

NASA LEDs stimulate the basic energy processes in the mitochondria (energy

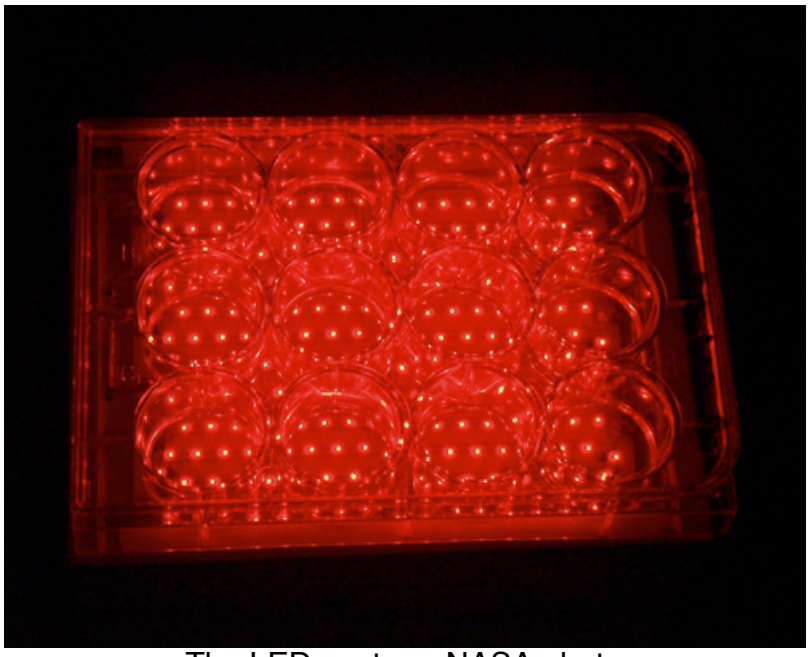
compartments) of each cell, between input and exit at the photon detector. The light is absorbed by particularly when near-infrared light is used to activate the color sensitive chemicals (chromophores, cytochrome systems) inside. Optimal LED wavelengths include 680, 730 and 880 nm and our laboratory has improved the
healing of wounds in laboratory animals by using both NASA LED light and hyperbaric oxygen. Furthermore, DNA synthesis in fibroblasts and muscle cells has been quintupled using NASA LED light alone, in a single application combining 680, 730 and 880 nm each at 4 Joules per centimeter squared.
CP552,Space Technology and Applications International Forum-2001,edited by M. S. El-Genk Muscle and bone atrophy are well documented in astronaut



top photo Scientific and Technical Information (STI) NASA - Quantum Devices, Inc WARP-10 device
and bottom photo Scientific and Technical Information (STI) NASA



THAT IS 680, 730 AND 880 light photons at wavelengths between 630-800 nm travel 23 cm



The LED center....NASA photo

...average wavelength of cell
tissue in the human body ranged between 600 nanometers and 720 nm;
660 is the mid-point. So in essence, the reason a 660 nm works better
than any other single frequency is because it is closer to the
resonant frequency of cell tissue. The other reason is that 660 nm
absorbs better in hemoglobin.
... The LED diffuses; the single frequency
laser does not. With this diffusion, the cell can actually be in
control of the treatment and shut off the molecules when it was done.
... single frequency light in a laser can stimulate DNA in damaged
cell tissue. They used a low power laser under 50-milliwatts because
higher laser can cut tissue.

AND THIS IS FASCINATING! :

THE MERIDIAN SYSTEM Acupuncturists discovered that single frequency
light could activate acupressure points. Pulse light could stimulate
it; continuous light could sedate the acupuncture points. But they
also discovered that light applied to a meridian end-point can
actually be traced flowing through the meridian to the organ
acupuncture points.
PENETRATING THROUGH THE BLOOD STREAM You can even get light into the
blood stream. One of the best ways is through your belly button,
because the aorta artery is behind the belly button. So if you insert
the light there for 20 minutes, every drop of blood in the body will
pass in front of the light, increasing the activity of your white
cells, red cells, B-cells and T-cells, so you can boost your whole
immune system.

and this is another reason iI like http://www.elixa.com real innovators!

Journal of Clinical Laser Medicine & Surgery
Effect of NASA Light-Emitting Diode Irradiation on Wound Healing
Dec 2001, Vol. 19, No. 6: 305-314
Harry T. Whelan, MD
Department of Neurology, Medical College of Wisconsin, Milwaukee, Wisconsin;
Naval Special Warfare Group TWO, Norfolk, Virginia; NASA - Marshall Space Flight
Center, Alabama...
James Caviness, MD
Submarine Squadron ELEVEN, San Diego, California

Objective: The purpose of this study was to assess the effects of hyperbaric oxygen
(HBO) and near-infrared light therapy on wound healing..... LED produced

improvement of greater than 40% in musculoskeletal training injuries in Navy SEAL team members, and decreased wound healing time in crew members aboard a U.S. Naval submarine. LED produced a 47% reduction in pain of children suffering from oral mucositis. Conclusion: We believe that the use of NASA LED for light therapy alone, and in conjunction with hyperbaric oxygen, will greatly enhance the natural wound healing process, and more quickly return the patient to a preinjury/illness level of activity. This work is supported and managed through the NASA Marshall Space Flight Center-SBIR Program.
This paper was cited by:
Survivorship and Mortality Implications of Developmental 670-nm Phototherapy: Dioxin Co-exposure
Ronnie L. Yeager, Jill A. Franzosa, Deborah S. Millsap, Jinhwan Lim, Stephen S. Heise, Phoebe Wakhungu, Harry T. Whelan, Janis T. Eells, Diane S. Henshel
Photomedicine and Laser Surgery. Feb 2006, Vol. 24, No. 1: 29-32

Mary Ann Liebert, Inc

Journal of Clinical Laser Medicine & Surgery
Effect of NASA Light-Emitting Diode Irradiation on Molecular Changes for Wound Healing in Diabetic Mice
Apr 2003, Vol. 21, No. 2: 67-74
Harry T. Whelan, MD
Department of Neurology, Medical College of Wisconsin, Milwaukee, Wisconsin
Conclusion: We believe that the use of NASA light-emitting diodes (LED) for light therapy will greatly enhance the natural wound healing process, and more quickly return the patient to a preinjury/illness level of activity. This work is supported and managed through the Defense Advanced Research Projects Agency (DARPA) and NASA Marshall Space Flight Center-SBIR Program.

Mary Ann Liebert, Inc

US Patent 6692517 B2

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population. Urol Res 2004;32:190–5.

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NB=nanobacteria

Mitochondrial Signal Transduction in Accelerated Wound and Retinal Healing by Near-Infrared Light Therapy

Janis T. Eells, Margaret T.T. Wong-Riley, James VerHoeve, Michele Henry, Ellen V. Buchman, Mary P. Kane, Lisa J. Gould, Rina Das, Marti Jett, Brian D. Hodgson, David Margolis, & Harry T. Whelan
Department of Health Sciences – Clinical Laboratory Sciences Program

Photobiomodulation by light in the red to near infrared range (630-1000 nm) using low energy lasers or light-emitting diode (LED) arrays has been shown to accelerate wound healing, improve recovery from ischemic injury in the heart and attenuate degeneration in the injured optic nerve. Recent evidence indicates that the therapeutic effects of red to near infrared light result, in part, from intracellular signaling mechanisms triggered by the interaction of NIR light with the mitochondrial photoacceptor molecule cytochrome c oxidase. We have demonstrated in primary neuronal cells that NIR-LED photo-irradiation increases the production of cytochrome oxidase in cultured primary neurons and reverses the reduction of cytochrome oxidase activity produced by metabolic inhibitors. We have also shown that NIR-LED treatment prevents the development of oral mucositis in pediatric bone marrow transplant patients. Photobiomodulation improves wound healing in genetically diabetic mice by upregulating genes important in the promotion of wound healing. More recent studies have provided evidence for the therapeutic benefit of NIR-LED treatment in the survival and functional recovery of the retina and optic nerve in vivo after acute injury by the mitochondrial toxin, formic acid generated in the course of methanol intoxication. Gene discovery studies conducted using microarray technology documented a significant upregulation of gene expression in pathways involved in mitochondrial energy production and antioxidant cellular protection. These findings provide a link between the actions of red to near infrared light on mitochondrial oxidative metabolism in vitro and cell injury in vivo. Based on these findings and the strong evidence that mitochondrial dysfunction is involved in the pathogenesis of numerous diseases processes, we propose that NIR-LED photobiomodulation represents an innovative and non- invasive therapeutic approach for the treatment of tissue injury and disease processes in which mitochondrial dysfunction is postulated to play a role including diabetic retinopathy, age-

related macular degeneration, Leber's hereditary optic neuropathy and Parkinson's disease.

Photobiology:
endoscopes with LED lasers?
photoreactive nanoparticles?

Basic research, investigating how light interacts with molecules, cells, organisms, and people.:
WHAT IS PHOTOMEDICINE?

Photomedicine is a broad topic, discovered in antiquity ... It covers any setting in which light is part of the treatment, diagnosis, cause or prevention of a condition important to human health.

Over the past twenty years, Wellman Center for Photomedicine has had many successes, benefiting millions of people around the world. Wellman has developed

* light-activated drugs to treat cancer and to prevent age-related blindness
* target-"smart" surgical lasers for skin and eye diseases
* live tissue microscopes to guide treatment or replace biopsies
* catheters which image the coronary arteries
* detectors for early or pre-cancer diagnosis
* light-activated rejoining of tissue after trauma or surgery
* ways to enhance wound healing and influence the immune system

The opportunities for progress in photomedicine are even greater now than when the lab began twenty-seven years ago.

The work of Wellman researchers generally falls into four areas of photomedicine: Pulsed Laser Treatments, Diagnostics and Imaging, Photosensitization , and Skin Photobiology.

The Laboratories at Massachusetts General Hospital were founded by Dr. John A. Parrish in the 1970's as one of the first academic research laboratories in the world devoted to the study of the roles of light in human biology and medicine.
http://www.massgeneral.org/wellman/welcome.asp

Focus: November 4, 1994 - Cancer: RED LIGHT FOR METASTATIC CANCER
Cancer: RED LIGHT FOR METASTATIC CANCER Angiogenic inhibitor stops secondary tumors.. Cancer Fighters: (l to r) Marsha Moses, Micheal O'Reilly, Lars Holmgren, Usha Tedrow, Judah Folkman,

http://focus.hms.harvard.edu/1994/Nov4_1994/Cancer.html

Light Emitting Diodes for Plant Growth Also Activate Tumor-Treating Drugs
Light Emitting Diodes (LEDs) -- developed for NASA Space Shuttle plant growth experiments -- may help treat cancerous brain tumors in children through activating light-sensitive drugs used to treat tumors.

Experiments indicate that when special tumor-fighting drugs are illuminated with LEDs, the tumors can be more effectively destroyed than with conventional surgery. The light source, consisting of 144 of the tiny diodes, is compact and mechanically more reliable than lasers and other light sources used to treat cancer. The entire light source and cooling system is only the size of a medium suitcase.

Dr. Harry Whelan of the Medical College of Milwaukee, WI, has obtained FDA approval to use the LED probe for the treatment of children's brain tumors on a trial basis. Dr. Whelan's therapy involves injecting the patient's bloodstream with a drug called Photofrin II. Photofrin II attaches to the unwanted tissues and permeates into them, leaving the surrounding tissues unaffected. Dr. Whelan then places the new solid-state LED probe near the affected tissue to illuminate the tumor and activate the Photofrin II drug. Once activated by the light, the drug destroys the tumor's cells, leaving the normal brain tissues virtually untouched.

The LED probe can be used for hours at a time and remains cool to the touch. The entire LED unit can be purchased for a fraction of the cost of a laser.

NASA Release 97-259, November 6, 1997

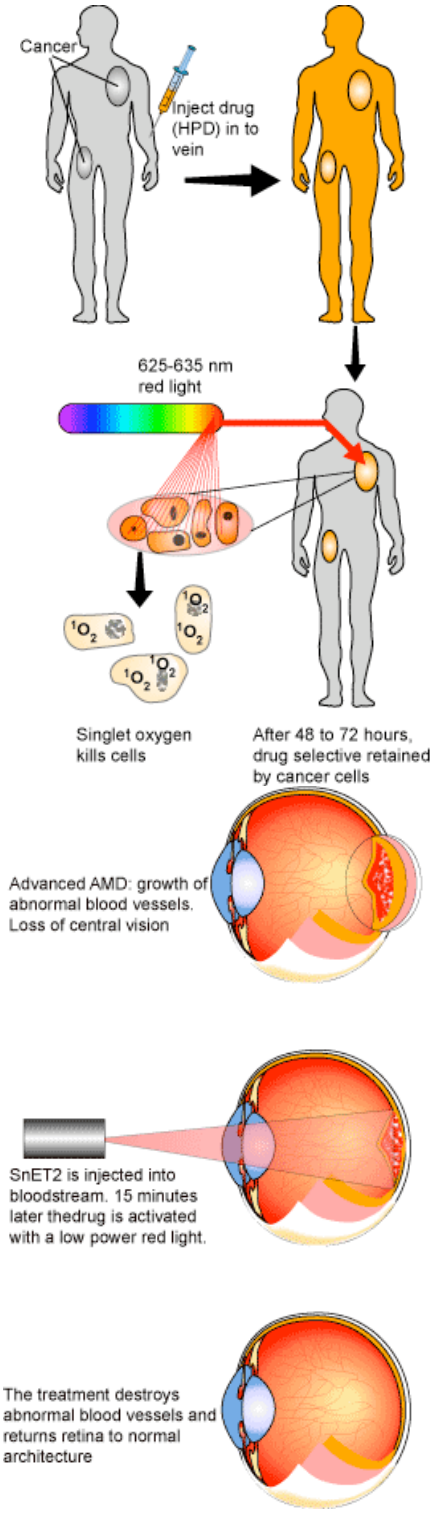
http://www.bioteach.ubc.ca/index.htm

What is Photodynamic Therapy (PDT)?

Photodynamic therapy (PDT, also called photoradiation therapy, phototherapy and photochemotherapy) is a unique, minimally invasive treatment that specifically targets diseased cells3. It is a relatively safe way to treat a variety of diseases, with little damage to healthy cells surrounding the diseased cells. The theory behind PDT has been around since the turn of the century: in 1900, Raab noticed that treating living tissues with certain compounds rendered them more sensitive to damage and death when they were exposed to light2. In the 1970's, these chemicals, known as photosensitizers, began to be used for therapeutic purposes2.

In photodynamic therapy, an inactive molecule called a photosensitizer is injected into the patient, where it circulates in the body and is allowed to accumulate in diseased cells. A photosensitizer is generally a kind of tetrapyrrole molecule (also known as a porphyrin) that absorbs energy from light and uses this energy to enable

chemical reactions to take place



http://www.bioteach.ubc.ca/index.htm

Medical College of Wisconsin

Light Emitting Diodes for Plant Growth Also Activate Tumor-Treating Drugs
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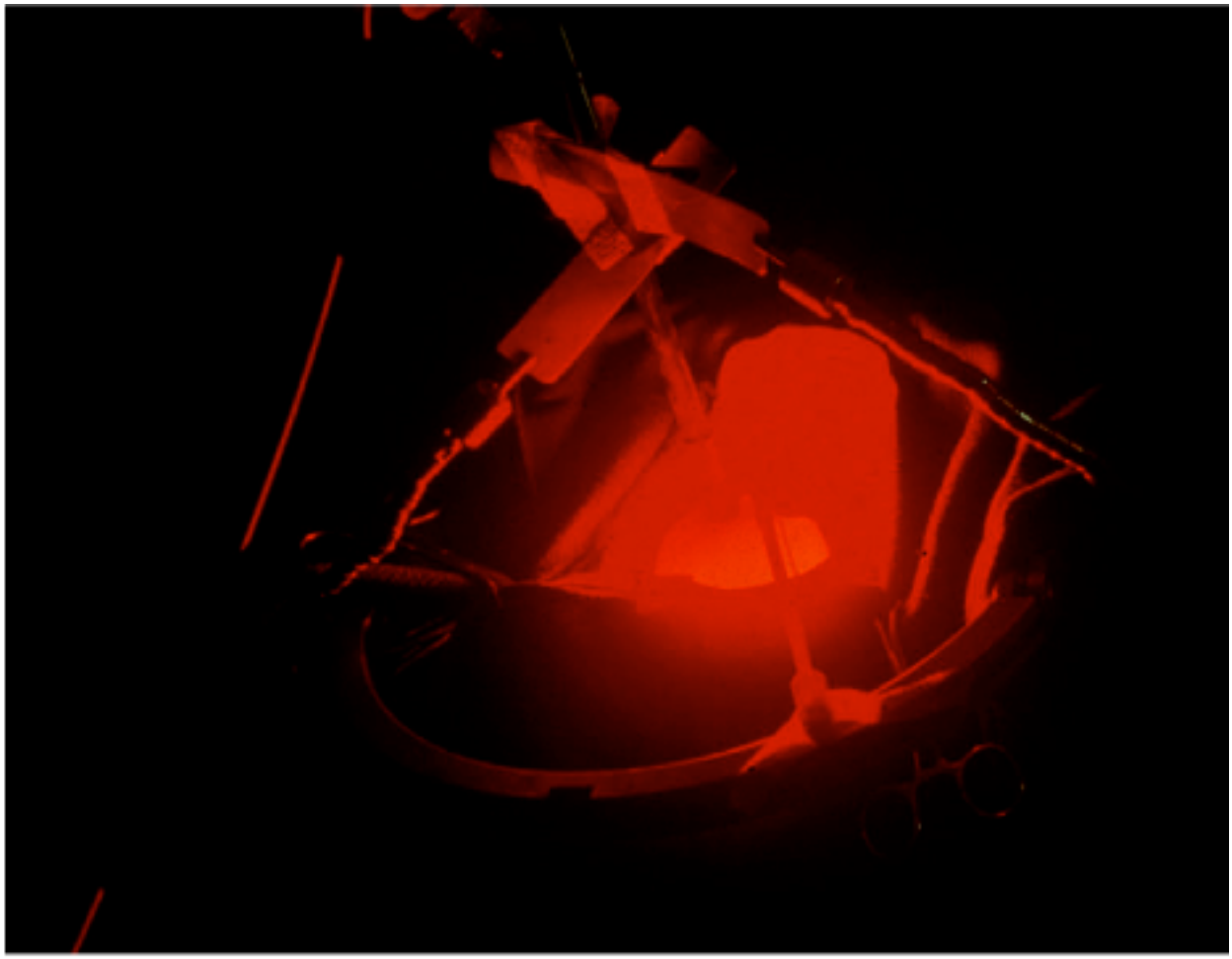
Dr. Harry Whelan of the Medical College of Milwaukee, WI, has obtained FDA approval to use the LED probe for the treatment of children's brain tumors on a trial basis.
from American Society for Gravitational and Space Biology http://asgsb.indstate.edu



Dr. Whelan

see also http://www.bmb.leeds.ac.uk/pdt/index.html

We now have ample clinical evidence that in contrast to other wavelengths, the 830 nm laser light produces specific beneficial biological reactions that are not produced by other wavelengths. This unique ability to stimulate greater response is the key to 830nm laser therapy.

The light from the laser distributes in the tissue in the shape of a ball or an egg . This is dependent on the wavelength of the light. Short wavelengths give a smaller and fairly round ball-shaped distribution, while longer wavelengths give a more egg-shaped distribution. The shorter 632 nm (HeNe) visible red wavelength... is more readily absorbed by blood and skin surface components, thereby limiting its tissue penetration.

The longer 830nm (GaAlAs) invisible infrared wavelength... is not as easily absorbed, therefore it has significantly greater depth of penetration.

This study has confirmed that responses to LLLT are dose, power output and wavelength-dependent. High dose (5 J/cm2) IR laser resulted in increases in plasma b- endorphin levels over the duration of the study suggests that localized, peripheral phototherapy of trigger points can induce cumulative activation of central hormonal /opioid pathways capable of regulating immune function. It is acknowledged that power density may have resulted in the fact that neither low dose nor high dose near monochromatic red light (660 nm) was found to be capable of eliciting significant changes in blood biochemistry.

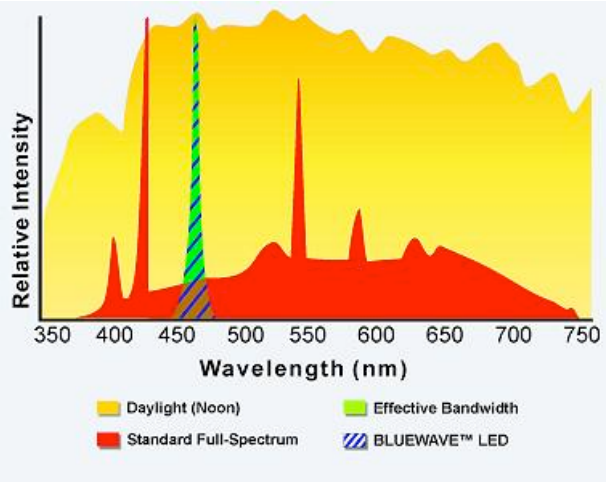
private communication from http://www.vibranthealth.com

The human cell functions at 630-640 nanometers (NM). It has been documented that DNA replication occurs at 635 NM, and cellular phagocytosis (cellular housecleaning or detoxification) happens at 634 NM, and that injured neurons (nerve cells) heal best at 635 NM.
... after DNA has sustained pyrimidine dimer damage due to ultra violet light exposure, subsequent exposure to blue light not only results in repair of the DNA dimer damage but also, as if by magic, causes other seemingly unrelated cellular repair to take place.
Raum & Zeit, "The Effect of Light and Color on Human Physiology," Prof. Dr. hc Orm Bergold M.D.

"Blue light" is a therapy in the 430nm to 450nm light spectra range, that blue light range, 400nm to 500nm, corresponds to the absorption and action spectra of many extremely vital biomolecules.

http://www.electronichealing.co.uk/sad_golite.htm has lights for seasonal affective disorder (SAD) as does http://www.elixa.com :

"The specific bandwidth of light that is responsible for treating SAD and related circadian rhythm disorders ranges from 446 - 477 nm (nanometers), and is up to 5 times more effective than other wavelengths. This spectral graph shows that only Apollo's patented BLUEWAVE™ technology precisely matches this specific bandwidth.



"Patent-pending BLUEWAVE™ is the result of ten years of research with medical universities and the National Institutes of Health (NIH). Other companies may tout the benefits of this new research. Only Apollo has participated in this research and we are the only company to produce lights that provide 100% of the effective blue light." http://www.electronichealing.co.uk/sad_golite.htm

Y. Omata 1, J.B. Lewis 2, S. Rotenberg 2, P.E. Lockwood 2, R.L.W. Messer 2, M. Noda 1, S.D. Hsu 2, H. Sano 1, J.C. Wataha 1 *
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*Correspondence to J.C. Wataha, Medical College of Georgia, Augusta, GA 30912-1126, USA

Funded by:
Medical College of Georgia
Ministry of Funding, Japan

Abstract
Blue light from dental photopolymerization devices has significant biological effects on

cells. These effects may alter normal cell function of tissues exposed during placement of oral restorations, but recent data suggest that some light-induced effects may also be therapeutically useful, for example in the treatment of epithelial cancers. Reactive oxygen species (ROS) appear to mediate blue light effects in cells, but the sources of ROS (intra- versus extracellular) and their respective roles in the cellular response to blue light are not known. In the current study, we tested the hypothesis that intra- and extracellular sources of blue light-generated ROS synergize to depress mitochondrial function. Normal human epidermal keratinocytes (NHEK) and oral squamous cell carcinoma (OSC2) cells were exposed to blue light (380-500 nm; 5-60 J/cm2) from a dental photopolymerization source (quartz-tungsten-halogen, 550 mW/cm2). ... Collectively, the data support our hypothesis that intra- and extracellularly generated ROS synergize to affect cellular mitochondrial suppression of tumor cells in response to blue light. However, the identity of blue light targets that mediate these changes remain unclear. These data support additional investigations into the risks of coincident exposure of tissues to blue light during material polymerization of restorative materials, and possible therapeutic benefits.
J Biomed Mater Res, 2006
Received: 8 May 2005; Revised: 30 June 2005; Accepted: 5 July 2005
Wiley InterScience

http://www.elixa.com also addresses the issue of SAD and other interesting research in blue light therapy.

Of course, dermatologists have been early adopters of skin healing technologies.

see entire brochure at
http://www.medsurgeadvances.com

The red-light effect on facial appearance may be due to a bit of short-term "lifting", because it activates adenosine tri-phosphate (ATP) in skin cells - which is how it helps with wound healing as well - and accelerates their production of collagen...add UVB for vitamin D!

Then there is :

Date: Thu Mar 16, 2006
Subject: Re: [Therapeutic-Laser_Therapy]

...
You are totally right re some of these trade show performances. It seems there are still a huge group of "snake oil" salesmen in the USA. If the patient is clothed then even the underlying principle behind the original snake oil is missing!

These people should be exposed for the frauds that they are.

The power density of a 5mw line of 6" or 155mm is probably in the vicinity of 0.002 Joules/cm2 which in terms of normal therapeutic application of at least 12 Joules/cm2 for a larger muscle area, would take 6,000 times longer than a typical 300mw therapeutic Laser.

It's ridiculous.

Kerry

Kind Regards
Kerry Tume
M.Ac.F. M.I.L.A. ACONT
NAALT WALT LIA
http://health.groups.yahoo.com/group/Therapeutic-Laser_Therapy/ from http://health.dir.groups.yahoo.com/dir/Health___Wellness

MostDermatologists routinely use ultraviolet light as adjunctive therapy in the treatment of some acne, psoriasis or other refractive skin conditions. Other uses are, of course, disinfection and sterilazation.
(see above UV discussion under "mold")

Research at MCW:
http://www.mcw.edu/whelan/index.html

Abstract: Space light-emitting diode (LED) technology has provided medicine with a new tool capable of delivering light deep into tissues of the body, at wavelengths which are biologically optimal for cancer treatment and wound healing. This LED technology has already flown on Space Shuttle missions, and shows promise for wound healing applications of benefit to Space Station astronauts, and in Special Operations.

This work is supported and managed through the NASA Marshall Space Flight Center - SBIR Program.

Here are some of the full-length articles in Space Tech. & App. Int'l. Forum - 2001 (vol. 552:35-45),
1999 (vol. 458:3-15) & 2000 (vol. 504:37-43).

* Schmidt, M.H. Meyer, G.A. Reichert, K.W. Cheng, J. Krouwer, H.G. Ozker, K. Whelan, H.T. " Evaluation of photodynamic therapy near functional brain tissue in patients with recurrent brain tumors" Journal of Neuro-Oncology 67: 201-7, 2004.
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* Eells, J.T. Henry, M.M. Summerfelt, P. Wong-Riley, M.T.T. Buchmann, E.V. Kane, M. Whelan, N.T. Whelan, H.T. "Therapeutic photobiomodulation for methanol-induced retinal toxicity" Proceedings of the National Academy of Sciences.100(6): 3439-44,2003.
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* Whelan, H.T. Connelly, J.F. Hodgson, B.D. Barbeau, L. Post, A.C. Bullard, G. Buchmann, E.V. Kane, M. Whelan, N.T. Warwick, A. Margolis, D. "NASA Light-emitting diodes for the prevention of oral mucositis in pediatric bone marrow transplant patients" Journal of Clinical Laser Medicine & Surgery.20(6):319-24,2002.

Research at WARP:
(Quantum Devices, Inc.)
http://www.warp-heals.com/clinical_research.htm

Research at Medsurge:
http://www.medsurgeadvances.com/index.php

* Clinical Research Seeks to Optimize LED Blue Light Therapy for Acne
Intra- and extracellular reactive oxygen species generated by blue light
* Response of Spider Leg Veins to Pulse Diode 810nm Laser: A Clinical Histological and Remission Spectroscopy Study
* Using a Non-Uniform Pulse Sequence Can Improve Selective Coagulation With a Nd:YAG Laser (1.06nm) Thanks to Met-Hemoglobin Absorption: A Clinical Study on Blue Leg Veins
* Hair Removal with an Athos Nd:YAG 3.5 ms pulse laser: a 3-month clinical study

* Nonablative Remodeling: Clinical, Histologic, Ultrasound Imaging, and Profilometric Evaluation of a 1540 nm Er:Glass Laser
* Nonablative Laser Skin Resurfacing using a 1540 nm Erbium Glass Laser: A Clinical and Histologic Analysis
* Removing benign tumors of the skin with an Er:Glass 1540nm
* Treatment of Neck Lines and Forehead Rhytids with a Nonablative 1540nm Er:Glass Laser: A Controlled Clinical Study Combined with the Measurement of the Thickness and the Mechanical Properties of the Skin
* Treatment of Leg Telangiectasia with a 532 nm KTP Laser in Multiple Mode

APPENDIX ONE

PATENTS
http://www.uspto.gov/patft/index.html

United States Patent 6,921,182
Anderson, Jr. , et al. July 26, 2005
Efficient LED lamp for enhancing commercial and home plant growth

Abstract

A first set of orange LEDs with a peak wavelength emission of about 612 nanometers, a second set of red light emitting LEDs with a peak wavelength of about 660 nanometers, and blue light LEDs. Two beam spreads, 15° and 30°, were provided for both the 660 nm LEDs and 612 nm LEDs. When directed perpendicularly upon tops of the plant leaves, 10% light transmission occurred through the leaves for the 30° LEDs, and 80% light transmission for the 15° LEDs. Thus, fully 50% of the orange/red spectrum primarily used for photosynthesis was transmitted through the upper leaf canopy, making it available to support photosynthesis in leaves below. LED lamps are positioned at varying distances from the growing plants for controlling plant growth rates that vary with these distances, thereby to control plant inventory, because growth of plants can be greatly slowed to preserve them during periods of slow sales.
Inventors: Anderson, Jr.; William Grant (Fallbrook, CA); Capen; Larry Stephen (Oceanside, CA)
Assignee: SolarOasis (Reno, NV)
Appl. No.: 437159
Filed: May 13, 2003

Current U.S. Class: 362/231; 362/230; 362/800; 362/805
Intern'l Class: F21V 009/00
Field of Search: 362/231,230,227,228,805,800,2 315/185.S,200.A, 312,316,149,150,324
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6602275 Aug., 2003 Sullivan.
6688759 Feb., 2004 Hadjimichael.
2004/0109302 Jun., 2004 Yoneda.

Primary Examiner: Vo; Tuyet Thi
Attorney, Agent or Firm: Burns; Ian F.
Claims

1. A lamp for facilitating plant growth comprising:

(a) a first set of orange light emitting diodes (LEDs) having a peak wavelength emission of about 612 nanometers (nm); and

(b) a second set of red light emitting diodes (LEDs) having a peak wavelength emission of about 660 nm.

2. The lamp of claim 1 wherein about half of th LEDs of the first and second set of LEDs have a beam spread angle of about thirty degrees and the remaining half of the LEDs of said first and second set of LEDs have a beam spread angle of about fifteen degrees.
...
4. The lamp of claim 1 wherein said LEDs are arranged in serial strings of LEDs of mixed light emitting wavelengths.

5. The lamp of claim 1 wherein the total light output of th first set of LEDs is about half the total light output of the second set of LEDs.
...
8. The lamp of claim 5 wherein said LEDs are arranged in serial strings of LEDs of mixed light emitting wavelengths.

9. The lamp of claim 1 including a third set of LEDs emitting blue light.

(b) a first set of orange light emitting diodes mounted in the housing and having a peak

wavelength of about 612 nanometers;

(c) a second set of red light emitting diodes mounted in the housing and having a peak wavelength of about 660 nanometers; and

(d) a third set of blue light emitting diodes mounted in the housing and having a peak wavelength of about 465 nanometers, wherein the first, second and third sets of light emitting diodes in combination output light that stimulates plant growth....

21. The lamp of claim 20, wherein for every orange light emitting diode there are two red light emitting diodes.

22. The lamp of claim 20, wherein for every blue light emitting diode there are twelve red light emitting diodes.

23. The lamp of claim 20, wherein for every blue light emitting diode there are six orange light emitting diodes.

...
27. The lamp of claim 20, wherein the first set of orange light emitting diodes comprise 24 orange light emitting diodes, the second set of red light emitting diodes comprise 12 red light emitting diodes and the third set of blue light emitting diodes comprises 2 blue light emitting diodes.

28. The lamp of claim 20, wherein the first set of orange light emitting diodes promote the creation of carotenoids in plants.

29. A lamp for facilitating plant growth, the lamp adapted to be connected to a power source, the lamp comprising:

a plurality of strings of light emitting diodes, each string of light emitting diodes having alternating light emitting diodes chosen from the group consisting of:

(a) orange light emitting diodes having a peak wavelength around 612 nanometers and a beam angle of 30 degrees;

(b) orange light emitting diodes having a peak wavelength around 612 nanometers and a beam angle of 15 degrees;

(c) red light emitting diodes having a peak wavelength around 660 nanometers and a beam angle of 30 degrees;

(d) red light emitting diodes having a peak wavelength around 660 nanometers and a beam angle of 15 degrees; and

(e) blue light emitting diodes having a peak wavelength around 465 nanometers and a beam angle of 30 degrees.

30. The lamp of claim 29, wherein for every orange light emitting diode there are two red light emitting diodes.

31. The lamp of claim 29, wherein for every blue light emitting diode there are twelve red light emitting diodes.

32. The lamp of claim 29, wherein for every blue light emitting diode there are six orange light emitting diodes.

33. The lamp of claim 29, wherein the power source is 24 volts direct current.

...
40. A lamp for facilitating plant growth comprising:

(a) orange light generating means for generating orange light having a wavelength of about 612 nanometers;

(b) red light generating means for generating red light having a wavelength of about 660 nanometers;

(c) blue light generating means for generating blue light having a wavelength of about 465 nanometers; and

(d) the orange, red and blue light generating means being adapted in combination to output light frequencies that stimulate plant growth.

...

BACKGROUND OF THE INVENTION

The present invention relates to the field of growing plants.

For decades scientists have delved ever deeper into the inner workings of plants, and particularly into those processes which are driven by the chemical capture of light energy. At the same time, research into new methods for converting electricity into light of particular wavelengths has led some engineers to try to produce artificial lighting which promotes plant growth. Until recently this has meant modifying energy inefficient "white light" sources to produce more light at wavelengths known to promote plant growth and health. This hybrid technology, in which the bulk of the light from these augmented "plant grow lights" can't be used efficiently by plants, has dominated the market for four decades.

While electricity was abundant and cheap, these "old school" plant grow lights, based mainly on HID, high pressure sodium, or fluorescent style lamps, were acceptable despite their imperfections. But they still have many shortcomings. They typically convert only 10-15% of electrical energy into light, and only a very small portion of that light can be used by plants. Some of them, particularly the HID lamps, emit short wavelength UV light which is damaging to both the plants being grown under them and the people tending the plants. All of these lamps generate waste heat which must be eliminated to prevent damage to the plants they illuminate, adding to their operational cost. They contain environmentally damaging metals, are fragile, and have a short operating life.

...
Its preferred power source is the subject of our copending utility patent application Ser. No. 10/397,763 filed Mar. 26, 2003 and entitled USE OF TRACK LIGHTING SWITCHING POWER SUPPLIES TO EFFICIENTLY DRIVE LED ARRAYS.

A key part of our research involved the determination of which light frequencies or wavelengths would produce superior plant growth results. Each plant pigment absorbs light at one or more specific wavelengths. The areas of peak absorption for each pigment are narrow, and the measurements made with pigments concentrated in a test tube are different than those done on living plants. The wavelength of the light used determines it's energy level, with shorter wavelengths having greater energy than longer wavelengths. Thus each absorption peak, measured by the wavelength of light at which it occurs, represents an energy threshold that must be overcome in order for the process to function.

There are many peaks of light absorption in the pigments found in plants, and ideally it would be best to match them each with the most appropriate LED. But this is not practicable because of the limited desired area available in the lamp being designed, and because LEDs are not available in every wavelength of the spectrum. The compromise is to see what LEDs are readily available and match them, as well as one is able, to groups of closely matched pigment absorption peaks, while striving to meet the minimum requirements of plants for healthy growth.

Our patent searches turned up U.S. Pat. Nos. 5,278,432 and 5,012,609, both issued to Ignatius et al., who suggest LED plant radiation very broadly within bands 620-680 or 700-760 nm (red) and 400-500 nm (blue). After a year and a half of research, we settled on three more specific light wavelengths that produced the best plant growth results.

660 nanometers (nm) is the wavelength that drives the engine of the photosynthetic process. The 680 nm wavelength is perhaps closer to the peak absorption wavelength of one of the two chlorophylls found in higher plants. However, at 680 nm you miss completely the absorption curve of the second chlorophyll, and furthermore the output curve of a 680 nm LED has a fair amount of light output above 700 nm, which is known

to cause unwanted morphological changes to plants. LEDs of 680 nm output are also rare in the marketplace, making them relatively expensive. Our choice of a 660 nm first wavelength component is a compromise wavelength commonly used in plant growing research, which supplies energy to both types of chlorophyll without emitting enough light above 700 nm to adversely affect plant growth.

...
Our second 612 nm wavelength component was selected not to promote photosynthesis, but to match one of the peaks of the carotenoids. As noted in "Influence of UV-B irradiation on the carotenoid content of Vitis vinifera tissues," C. C. Steel and M. Keller (http://bst.portlandpress.com/bst/028/0883/bst028883.htm), "carotenoid synthesis . . . is dependent upon the wavelength of visible light, and is diminished under yellow and red filters."

By providing the orange 612 nm light, we not only promote creation of carotenoids, which are required for plant health, but also add a little to photosynthesis, since the carotenoids pass their absorbed energy to chlorophyll. Carotenoids are required for plant health due to their ability to absorb destructive free radicals, both from solar damage and from chlorophyll production, whose precursors will damage plant tissue in the absence of the carotenoids. During research we found that, beneficially, test plants turned a deeper green, i.e. produced more chlorophyll, with the addition of our 612 nm light component. This ability to increase a plant's chlorophyll content with this specific light wavelength is an important aspect of our invention.

Blue light of about 465 nm, this wavelength being non-critical, is strongly absorbed by most of the plant pigments, but is preferably included as the third component in our lamp to support proper photomorphogenesis, or plant development. Any LED near this wavelength will work as well, but the 470 nm LEDs are commonly available and less expensive than many other blue LEDs.

Regarding the proper proportion for each wavelength, it is known, from independent laboratory research, that a blue/red proportion of 6-8% blue to red is optimal. In sunlight the blue/red light proportion is about 30%, but this is not required by plants. More than 8% blue light provides no additional benefit, but adds to the cost of the device since blue LEDs are among the most expensive to manufacture. In our device we include about 8% blue light, which is near optimal for plant development while offering the greatest cost savings. Our research showed that best results were obtained when the output of the 612 nm orange LEDs in our device was added to the output of the 660 nm red LEDs when calculating our most desired blue/red proportion.
...
To improve the manufacturability of our circular lamp, it proved better to use LED strings that mixed wavelength, i.e. instead of putting the 660 nm LEDs into their own strings, we use strings that contain both 660 nm and 612 nm LEDs, and in one string use all three wavelengths. Normally this isn't done because it offers a greater potential for having a "current hogging" LED alter the string's designed operating characteristics. Current hogs can be a problem even when all of the LEDs in a string

are of the same wavelength and manufacture, but when the string is composed of a mixture of wavelengths the chances of having this problem are increased. LED strings of mixed wavelength are to be used when the supplied voltage and current is tightly controlled.

Regarding prior art found during our searches, the mounting and plug in of an LED array light module in a MR-16 or the like fixture is disclosed in Lys U.S. Pat. No. 6,340,868 in FIGS. 20 and 21. Lyes teaches the use of these LED array modules for accelerating plant growth; see FIGS. 92A and 92B. Lys also teaches in FIG. 22 the use of a 24 volt DC module for energizing three LED strings connected in parallel. Lowrey U.S. Pat. No. 6,504,301 discloses an MR-16 outline package for a mixed wavelength LED arrangement; other lighting packages such as MRC-11 etc. are mentioned in his specification col. 7. Okuno U.S. Pat. No. 4,298,869 discloses a conventional lamp screw in fixture for three parallel LED strings of two volt LEDs supplied by 19.5 volts. The concept of placing the LEDs very close to the plants as they generate little heat is taught in col. 1 of U.S. Pat. No. 6,474,838.

BRIEF SUMMARY OF PREFERRED EMBODIMENTS OF THE INVENTION

We finally found that the proportion of twelve red 660 nm LEDs plus six orange 612 nm LEDs and one blue 470 nm LED was optimal. Our preferred circular lamp can hold two of these optimal sets of LEDs, resulting in a device with twenty-four red 660 nm LEDs, twelve orange 612 nm LEDs, and two blue 470 nm LEDs. Also, we used mixed LED strings that contained both types of LEDs for enhanced wavelength mixing.

We used LEDs having two beam spreads of 15° and 30°, in equal proportions, for both the 660 nm LEDs and 612 nm LEDs. When directed perpendicular to the upper surface of mature cotton plant leaves, we found that a quantum light sensor placed below the leaf registered 10% light transmission for the 30° LEDs, and 80% light transmission for the 15° LED, and fully 50% of the orange/red spectrum primarily used for photosynthesis was transmitted through the upper leaf canopy, making it available to support photosynthesis in leaves below.

...

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

Five light wavelengths commonly known to match the absorption peaks of plant pigments were identified: 430 nm (blue, near ultraviolet), 450 nm-470 nm (blue), 570 nm (lime green), 610 nm (orange), and 660 nm (red). Our experimental efforts in turning theory into practice to select the best components, was anything but straightforward, and has taken the better part of a year to bring to its current level of development. Our final test results have allowed us to eliminate the 570 nm lime green LED. This left us with the following mix in our preferred embodiment: 12×660 nm (Red), 30° beam angle spread;
12×660 nm (Red), 15° beam angle spread;

6×612 nm (Orange), 30° beam angle spread;
6×612 nm (Orange), 15° beam angle spread; and
2×465 nm (Blue), 30° beam angle spread; all as shown in FIGS. 1, 2, and 2A.

We finally determined that the superior results we were seeing were not cause by the 570 nm green LEDs, and our results were substantially improved using the wavelength mix shown above. The number of variables we were testing made it difficult to isolate the exact effects caused by the different light wavelengths used, and it has only just become apparent that the 570 nm light wavelength was superfluous.

...
The graph of FIG. 3 shows a solid line curve 20 of the wavelength distribution output of our invention in its preferred embodiment compared to the absorption spectrum curve 24 of chlorophyll A and absorption curve 22 of chlorophyll B 22, the wavelengths which most efficiently drive photosynthesis range between 600 nm and 700 nm, which closely matches the output peaks of our invention. Even though chlorophyll has its strongest absorption in the blue wavelengths, these wavelengths are very inefficient for driving the photosynthetic processes. The small amount of blue in our invention, is not used to drive the photosynthetic process, but is instead used to promote proper plant morphology. Thus, our final LED wavelength mix covers the absorption peaks for both chlorophyll A and chlorophyll B. The 465-470 nm LEDs also supply energy to the two chlorophylls, as well as the carotenoids, but inefficiently. The main purpose of the 465 nm light is to support photomorphology, promoting a short, compact growth pattern, broad leaves, and thick stems. The amount of blue light (460 nm to 470 nm) provided is optimally 6% to 8% of the provided amount of orange/red light within the 600 nm to 700 nm range. Sunlight is approximately 30% light in the blue portion of the spectrum, but it has been shown by university researchers that amounts higher than 8% provide no additional benefit.

As shown in FIG. 1, our preferred circular lamp embodiment contains thirty-eight LEDs, as follows: 12 narrow beam angle red LEDs labeled r, 12 wide beam angle red LEDs labeled r-, 6 narrow beam orange LEDs labeled o, 6 wide beam orange LEDs labeled o-, and 2 wide beam blue LEDs labeled b.

The circuit of FIG. 1 for driving the LEDs includes regulated 24 volt DC power source 10 that supplies three strings often LEDs, 12, 14, and 16, and one string of eight LEDs 18. Each string contains a mix of the LED wavelengths and beam spreads used in the invention, denoted 'r' for 15° beam spread 660 nm (narrow beam red), 'r-' for 30° beam spread 660 nm (wide beam red), 'o' for 15° beam spread 612 nm (narrow beam orange), 'o-' for 30° beam spread 612 nm (wide beam orange), and 'b' for 30° beam spread 465 nm (wide beam blue).

...
LEDs are manufactured to emit light with a particular viewing angle, or beam spread.

Typically the narrower the beam spread the higher the light pressure or intensity produced, and vice versa. If the beam spread is too narrow, the light from adjacent LEDs may not overlap, leaving gaps in the illumination area. For a plant growing light this would not be appropriate. Conversely, if the beam spread is too wide, the illumination area will be too large, covering areas beyond the plant's leaf canopy, so a great deal of light will be wasted. We selected LEDs which would, in our preferred embodiment for general use, provide a circle of illumination approximately 10-12 inches wide at a distance of ten inches from the light source. Since our preferred embodiment is smaller than 3"; in diameter, 100% illumination coverage of many size areas for commercial use and in the home is possible.

Growers employing artificial light sources for growing plants are cautioned to use fluorescent lighting only for seedlings, and to switch to High Intensity Discharge or High Pressure Sodium lamps after the plants are 12"; to 18"; tall. Fluorescent lighting is preferred because of its lower energy cost, but it has such a low light output that none of the light striking the upper leaf canopy can penetrate to the lower leaves, causing spindly growth. HID and UPS lights produce adequate light to penetrate a number of layers of leaf canopy, but at a much higher energy cost. The high temperature of HID and HPS lighting (the quartz envelope of the bulb exceeds temperatures of 1500° F.) is also more dangerous for the immature stems and leaves of seedlings.
...
As shown in FIG. 4, a wide beam LED 26 directs its light beam 28 onto the upper surface of a leaf 38. Measurements made at a point below the leaf 30 show only 10% of the light passes through the leaf to be available to the leaves below. A narrow beam LED 32 directs its light beam 34 onto the upper surface of leaf 38. In this case, measurements made at a point below the leaf at 36 show 80% of the light passes through the leaf to be available to the leaves below. When used in a 1:1 mix of wide to narrow beam LEDs, approximately 50% of the supplied light is available to the lower levels of the plant canopy. More specifically, we used two beam spreads, 15° and 30°, in equal proportions, for both the 660 nm LEDs and 612 nm LEDs. When directed perpendicular to the upper surface of mature cotton plant leaves, we found that a quantum light sensor placed below the leaf registered 10% light transmission for the 30° LEDs, and 80% light transmission for the 15° LEDs. Using our fully functional protoype described above, we found that fully 50% of the orange/red spectrum, primarily used for photosynthesis, was transmitted through the upper leaf canopy, making it available to support photosynthesis in leaves below.

These beam angles may vary somewhat depending on the distance of the plants from the lamps. For example, the lamp may be mounted upon the ceiling of a home and directed at a plant on a table. In this case the angles will be reduced from 30/15 degrees but the preferred ratio of beam angles of two to one will remain. Where the lamp is directly mounted upon an aquarium tank having plants therein for example, the beam spread angles could be increased rather than decreased.

At a distance of ten inches from a plant, the distance at which our tests were conducted, the lamp of FIGS. 1 and 2 produced a circle of light 10-12 inches in

diameter. If a plant is placed below the lamp, only a part of the plant is within the circle of light and the rest of the plant is outside, the portions of the plant outside the light would be expected to grow taller and bend towards the light. As seen in our research, this undesired result did not happen with plants grown under our lamp. Instead, the portions of the plant outside the circle of light simply stopped growing but remained healthy. It appears that if a portion of a plant receives sufficient blue light at 470 nm, undesired stem elongation is inhibited for the entire plant. Our invention provides this effect, which can be useful in commercial plant growing applications where plants placed along the periphery of the illumined area may be only partially beneath the light. As long as a plant is at least partially illuminated by one of our lights it will remain healthy without showing the morphology typical of under-illuminated plants (strong phototropism and unwanted stem elongation).

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Inventory Control by Adjusting Plant Growth Rate

It is known that the amount of 470 nm blue light reaching a plant affects its morphology, i.e. a low amount of 470 nm light produces longer stem internodes, while a larger amount of 470 nm light produces shorter stem internodes. It is also known that because LED lighting is much cooler than conventional plant lighting sources, an LED-based plant light can be placed much closer to a plant than a conventional plant light, with a resulting increase in light intensity falling on the plant's leaves. We found that plants tend to grow to within an inch or so of the light, slowing as they approach the lamp (i.e. the stem internode length continues to decrease as the light intensity increases when the plants grow closer to the light source), until they nearly stop growing when within an inch or so of the lights. This is an important feature of our invention for commercial plant growing operations, where plants which overgrow their pots can't be sold and are typically discarded. Thus, this feature of our invention would allow a commercial greenhouse to maintain their plants at their optimum size for an extended period simply by lowering the lights to a point near the tops of the plants.

...

As shown in FIG. 7-A, at time A, the light source 52 over the first plant 62 is lowered close to the plant, while the light source 52 over the second plant 60 is not. As shown in FIG. 7-B, at time B, which may be several weeks later, the first plant 62 shows little change in size, while the second plant 60 has grown considerably during the same time period. The difference is the greatly increased amount of 470 nm blue light reaching the first plant 62, which shortens the internode stem length, thus keeping it short. This feature will allow commercial plant growers to "hold" the size of plants, if necessary, until they can be shipped. Otherwise, they would overgrow their pots and be spoiled. The resulting inventory control is of course of great importance in running a plant growing business.

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* * * * *

Dew , et al. August 8, 1989

Laser healing method and apparatus

Abstract

The method and apparatus of the invention use a beam of laser emitted optical energy to effect wound closure and reconstruction of biological tissue. In response to input as to tissue type and thickness, a computer determines the output power, exposure time and spot diameter of the emitted beam to control the application of optical energy to produce thermal heating of biological tissue to a degree suitable for denaturing the tissue proteins such that the collagenous elements of the tissue form a "biological glue" to seal immediately and/or to reconstruct the tissue being heated. In a given embodiment, the computer directly controls output power of the laser by regulating the laser's input current, and limits exposure time by deactivating a shutter mechanism when the determined time is reached. Beam diameter is manually set in response to a displayed value for a sliding scale that controls the working distance between the end of a fiber optic beam guide and the tissue site.

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Related U.S. Patent Documents

Application Number	Filing Date	Patent Number	Issue Date
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Field of Search: 128/397,303.1 219/121L,121LA,121LB,121LS

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Parent Case Text

This application is a continuation-in-part of copending application Ser. No. 539,527 filed Oct. 6, 1983, now U.S. Pat. No. 4,672,969.
Claims

What is claimed:

1. Apparatus for the automatically controlled application of optical energy in the reconstruction of biological tissue to cause the formation of a proteinaceous framework from denatured protein in the vicinity of the biological tissue, the framework approximating the biological tissue to be reconstructed, said apparatus comprising:

...
13. Apparatus as in claim 1, wherein said optical energy source has a wavelength between approximately 1.2 and 1.4 micrometers.

...
15. Apparatus as in claim 1, wherein said optical energy source is a Nd:YAG laser operated at a secondary wavelength of 1.32 micrometers.

16. Apparatus as in claim 15, further comprising an auxiliary optical energy source that produces an auxiliary beam of optical energy which has a wavelength which is substantially absorbed in biological tissue.

17. Apparatus as in claim 16, wherein said auxiliary optical energy source comprises a helium neon laser.

18. Apparatus for the automatically controlled application of optical energy in the reconstruction of biological tissue to cause the formation of a proteinaceous framework from denatured protein in the vicinity of the biological tissue, the framework approximating the biological tissue to be reconstructed, said apparatus comprising:

a laser optical energy source for producing a beam of optical energy having a wavelength between approximately 1.2 and 1.4 micrometers;

an optical fiber guide for directing said beam of optical energy to a spot on a biological tissue to be reconstructed, said guide means having a distalmost end remote from said optical energy source and from which said optical energy is emitted; and

means operatively connected to said optical energy source, and responsive to a user input signal representative of a characteristic of the tissue, for generating corresponding appropriate settings for the output power, exposure time and spot diameter of said beam, and for controlling the beam in accordance therewith, to cause the amount of optical energy delivered by said source to said tissue to be within a tissue nondestructive range bounded by a minimum rate at which the tissue forms a collagenous substance and a maximum rate at which water in the tissue would boil; whereby proteinaceous components of the tissue are denatured.

...
20. A method for the automatically controlled application of optical energy in the reconstruction of biological tissue to cause the formation of a proteinaceous framework from denatured protein in the vicinity of the biological tissue, the framework approximating the biological tissue to be reconstructed, said method comprising the steps of:

entering an input signal into a computer representative of a characteristic of tissue to be reconstructed;

using said computer to determine suitable parameters, based on stored data, of an optical energy beam to be delivered to the tissue site at an energy level within a tissue nondestructive range bounded by a minimum rate at which the tissue forms a collagenous substance and a maximum rate at which water in the tissue would boil, whereby proteinaceous components of the tissue are denatured; and

using said parameters to control the power, exposure time and diameter of emission of an optical energy beam from an optical energy source to the tissue, thereby maintaining the energy delivered by said beam to the tissue at said energy level.
Description

BACKGROUND OF THE INVENTION

This invention relates generally to methods and apparatus for closing wounds and more particularly, to a method and apparatus for applying optical energy to biological tissue whereby the tissue is converted to a collagenous, denatured protein substance which joins severed tissues and closes wounds.

Historically, suturing has been the accepted technique for rejoining severed tissues and closing wounds. Suturing has been achieved with a surgical needle and suturing thread, and more recently, with a variety of polymeric or metallic staples. The intended function of sutures is to hold the edges of the wounds against one another during healing so as to reduce discomfort, pain, scarring, and the time required for healing.

It is a problem with known suturing systems that since they are applied intermittently along a wound, they permit gaps in the wound between sutures to remain open thereby accepting dirt and bacteria. Moreover, in addition to producing a relatively high risk of infection and tissue rejection, such gaps between sutures are eventually filled in by keloid, which results in disfiguration and scarring. In addition, inflammation often results from the foreign body presence of the suture material.

It is an additional disadvantage of conventional sutures that they may slip in an axial direction thereby permitting relative motion between the tissues which are desired to be joined, and may loosen before the healing process has advanced sufficiently to maintain a tight closure of a wound. Thus, sutures must frequently be removed and replaced, thereby requiring multiple visits to a physician. There is a need, therefore, for a wound closure system which is uniform throughout the length of a wound.

A variety of cauterization and cryogenic techniques have been developed to reduce the flow of blood in an open wound, or a surgically-induced incision. Generally cauterization is achieved by using intense heat to sear and seal the open ends of the tissues, such as vessels and capillaries. In known cauterization systems, heat is generated by resistance heating of a metallic probe which is subsequently applied to the tissue to be cauterized. Alternatively, undesired blood flow is discontinued by applying a cryogenic temperature which freezes the tissue. More recently, the medical field has utilized high intensity optical energy generated by one or more lasers to achieve cauterization which limits blood flow. In such known laser systems, the optical energy is applied in sufficient quantity to sear or burn the vessels. Laser cauterization is illustratively described in U.S. Pat. No. 4,122,853 to Michael R. Smith. These techniques, however, destroy the surrounding tissue leading to longer healing times, infection, and scarring.

Recent advances in the state of the art have produced cauterization with the use of ultrasonic energy which is converted to mechanical vibrations through a knife. Such a rapidly vibrating knife simultaneously cuts and closes off severed vessels. A system of the ultrasonic vibrational type is described in U.S. Pat. No. 3,794,040 which issued to Balamuth. In the known system, ultrasonic energy is applied to create heating of the vessels desired to be cauterized above room temperature, but below a temperature at

which such vessel would sear. The heat thus produced causes hemostasis, by denaturing of the proteins in the tissue to form a collagenous substance which performs as a glue to achieve the closure or bond. This technique, however, has not gained widespread use for delicate surgery because it requires bringing a vibrating probe into contact with the tissue to be affected. Morever, ultrasonic energy is nonpreferentially absorbed and affects all of the surrounding tissue.

Optical energy generated by lasers has been applied in recent times to various medical and surgical purposes because the monochromatic and coherent nature of the light generated by lasers has been shown to have absorbency characteristics which vary with the nature of the illuminated tissue. Thus, for a given tissue type, the laser light may propagate through the tissue, substantially unattenuated, or may be almost entirely absorbed. Of course, the extent to which the tissue is heated, and ultimately destroyed, depends on the extent to which it absorbs the optical energy. It is generally preferred that the laser light be essentially transmissive in tissues which are desired not to be affected, and absorbed by the tissues which are to be affected. For example, when using lasers in fields which are wet with blood or water, it is desired that the optical energy not be absorbed by the water or blood, thereby permitting the laser energy to be directed specifically to the tissues desired to be affected. Such selective absorption also permits substantial time saving during an operation by obviating the need for cleaning and drying the operating field.

It is a further known advantage of a laser system that the optical energy can be delivered to the tissues desired to be operated upon in a precise location and at predeterminable energy levels. The precision with which the laser energy can be directed is enhanced by its ability to be guided by known thin optical fibers which permit the optical energy to be utilized within a body without requiring large incisions or to be inserted into the body through an endoscope. The optical fibers which conduct the laser-generated optical energy for performing the operation can be combined with other optical fibers which conduct light in the visible range, and further optical fibers which are of the image-transmissive type such that a surgeon may view and control an operation which is occurring within a body.

Ruby and argon lasers which are known to emit energy in the visible portion of the electromagnetic spectrum have been used successfully; particularly in the field of ophthalmology to reattach retinas to the underlying choroidea and to treat glaucoma by perforating anterior portions of the eye to relieve intraocular pressure. The ruby laser energy has a wavelength of 0.694 micrometers and, thus, appears red. The argon laser emits energy at 0.488 and 0.515 micrometers, thus, appearing blue-green. The ruby and argon laser beams are minimally absorbed by water, such as tissue water, but are intensely absorbed by the blood chromagen hemoglobin. Thus, the ruby and argon laser energy is poorly absorbed by nonpigmented tissue such as the cornea, lens, and vitreous humor of th eye, but is preferentially absorbed by the pigmented retina where it can then exert a thermal effect.

Another type of laser currently in surgical use is the carbon dioxide (CO.sub.2) gas

laser which emits a beam which is intensely absorbed by water. The wavelength of the CO.sub.2 laser is 10.6 micrometers and therefore lies in the invisible, far infrared region of the electro-magnetic spectrum. Reference to FIG. 1A shows that the absorption of energy by water in this part of the spectrum is so great that it is absorbed independently of tissue color by all soft tissues having a high water content. Thus, the CO.sub.2 laser makes an excellent surgical scalpel and vaporizer. Since it is so completely absorbed, its depth of penetration is shallow and can be precisely controlled with respect to the surface of the tissue being operated upon. The CO.sub. 2 laser is frequently used for neurological surgery where it is used to vaporize or coagulate neural tissue with minimal thermal damage to underlying tissues.

The fourth commonly used type of laser is the neodymium doped yttrium-aluminum-garnet (Nd:YAG) laser. The Nd:YAG laser has a predominate mode of operation at a wavelength of 1.06 micrometers in the near infrared region of the electromagnetic spectrum. As discussed in copending application Ser. No. 539,527, the Nd:YAG emission at 1.06 micrometers wavelength is absorbed to a greater extent by blood than by water making it useful for coagulating large bleeding vessels. The Nd:YAG at 1.06 micrometers laser energy has, for example, been transmitted through endoscopes to treat a variety of gastrointestinal bleeding lesions, such as esophogeal varices, peptic ulcers, and arteriovenous anomolies.

It is characteristic of all of these known uses of laser energy that the tissue thus exposed is destroyed by searing, charring, or vaporization. It is therefore an object of this invention to utilize laser energy either to heal or reconstruct tissue, rather than to destroy tissue.

It is also an object of this invention to replace surgical sutures or staples in wound closures by a technique which creates an immediate seal of the severed tissue, is faster, requires minimal surgical manipulation of tissue, reduces possibility of infection, and minimizes scarring.

It is another object of this invention to use the body's own tissue elements to form a seal or a bond between severed elements of tissue.

It is still another object to use electro-optical energy to form a collagenous bonding tissue which is similar in composition to the tissue from which it is produced.

It is yet a further object of the invention to provide wound closure and reconstruction, inter alia, of the following tissues: skin, nerve fiber, vascular tissues, reproductive tissue structures such as vas deferens or fallopian tubes, gastrointestinal tract, eye tissues, and tendons.

It is also a further object of the invention to provide the wound closure and reconstruction of the above-identified tissues quickly, with little or no scarring, and with minimal risk of infection.

It is a still further object of the invention to use laser energy having a low absorbance in a bloody or wet field to increase the utility of the laser within the normal operating fields.

It is still another object of the invention to utilize a laser energy which is not preferentially absorbed by either blood or water, thereby enabling a low temperature thermal effect to be produced upon a desired tissue with deeper penetration and with substantially reduced risk of damaging neighboring tissues.

It is also another object of the invention to provide a laser apparatus which is automated and portable for effecting closure of wounds and reconstruction of tissues.

SUMMARY OF THE INVENTION

The foregoing and other objects are achieved by this invention which provides a method and apparatus for the controlled application of optical energy to convert biological tissue into a collagenous substance for facilitating healing and wound closure. In accordance with the invention, responsive to an input signal representative of a characteristic of the tissue for which closure is sought, the parameters of a generated beam of optical energy guided to the area of the intended juncture are controlled to cause the amount of optical energy delivered to the tissue in the vicinity of the wound to be within a tissue nondestructive range that causes the tissue to be converted to a denatured proteinaceous collagenous substance which forms a biological glue that closes the wound.

The intensity of the optical energy is controlled such that the rate at which such optical energy is absorbed by the tissue in the vicinity of the wound and converted into thermal energy is within a tissue nondestructive range bounded by a minimum absorption rate at which the tissue is converted to a collagenous substance and a maximum absorption rate above which the water contained in the tissue wound boil.

In accordance with the invention, a beam of optical energy is produced by a source, illustratively a laser, having a wavelength selected such that the optical energy is propagated without substantial attenuation through water and/or blood, but is absorbed in the biological tissue desired to be repaired. Such substantially unattenuated transmission through water and blood simplifies surgical procedures by obviating the need for operation in a dry, clean field. The arrangement is further provided with a guide, such as a flexible optical fiber, for directing the beam of optical energy to the wound in the tissue. Moreover, the arrangement is provided with means for controlling the parameters of the beam so that the delivered energy is controlled to remain at a level above which the tissue in the vicinity of the wound is converted to the collagenous substance, but below a level at which water in the tissue being repaired would boil.

In an embodiment of the invention, described in greater detail below, the optical energy source is constituted by a Nd:YAG laser which is tuned or is tunable to 1.32 microns. Beam intensity control is provided by circuitry that regulates the laser power source. The flexible optical fiber is provided with a shutter and timer on a foot or hand operated switch to regulate exposure time. The optical fiber is provided with a hand-piece that includes a sliding scale which sets beam spot size at the tissue by establishing the working distance between the beam emitting end of the hand-piece and the tissue being operated on. In response to input information on tissue type and thickness, a microprocessor establishes the parameters for the beam intensity control circuitry, shutter timer and hand-piece scale required to achieve the proper energy level for tissue welding.

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DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Biological tissue comprises cell layers in a protein framework for tensile strength. All proteins are amino acids which have side chains which are dissolvable either in water or fat. Naturation is a process wherein the amino acids fold over, always in the same configuration for each protein type, when the protein leaves the interior of a cell and is confronted with tissue water. In such case, the hydrophobic portion of a side chain folds to the interior of the molecule. The proteinaceous components of the tissue can be unfolded or denatured by the application of heat.

As stated in copending application Ser. No. 539,527, it has been discovered that application of optical energy to biological tissue, in a nondestructive amount sufficient to generate enough heat to denature the proteinaceous components, can be used to cause the body's own tissues to substantially reproduce the prior tissue structure at a wound or severed tissue site. In particular, energy from an optical energy source, such as a laser, can be applied to bring the temperature of biological tissue somewhere above room temperature, but below the boiling point of water; preferably above 45 degrees centigrade and particularly to about 60-70 degrees centigrade. Collagen, a major source of protein in the body, is denatured by application of such energy in such a way as to go into solution and form a "biological glue" to seal a lesion, anastomize a severed vessel, or reconstruct damaged tissue. When the source of heat is removed, the proteins begin to re-nature and form an approximate replication of the prior tissue structure. As the body heals, the so-called "biological glue" will be reabsorbed and replaced by natural tissue.

The application of heat, to form a collagenous seal to immediately close a lesion or anastomize a severed vessel accelerates healing time, leaves little or no scarring, preserves the tissue, and avoids inflammation and/or infection caused by the inclusion of foreign suture material in a wound.

Optical energy of a particular wavelength is converted to heat in tissue which absorbs energy at that wavelength. As detailed in copending Ser. No. 539,527, it was

discovered that optical energy having a wavelength of 1.2 to 1.4 micrometers is relatively unattentuated in both water and blood and, so, is particularly advantageous for use as an optical energy source for the formation of a "biological glue" in order to effect repair of gastrointestinal tract tissue, close skin wounds (whether originating accidentally, intentionally or through biological processes), and repair and reconstruct tissue such as reproductive tissue, tendons, and vascular tissue, provided the intensity, exposure time and spot size of the beam at its point of incidence on the tissue are controlled to keep the energy absorption by the tissue within the desirable range. A suitable wavelength is obtainable using a commercially available Nd:YAG laser configured to generate optical energy at a wavelength of about 1.32 micrometers.

FIG. 1 illustrates a surgical system for achieving tissue welding in accordance with the invention. The system has a source of optical energy, laser 20, which is preferably of the Nd:YAG crystalline variety wherein an yttrium-aluminum-garnet (YAG) rod is doped with neodymium (Nd) ions as the active light-producing element. Such a laser 20 includes a resonant cavity for amplifying the emitted light and pumping means, such as a dc Krypton arc lamp, for supplying energy to create a population inversion of the normal energy state of Nd ions. The population inversion results in the stimulated emission of light according to well-known known laser principles.

Absent any tuning of the laser cavity, Nd:YAG lasers will emit light at a fundamental dominant wavelength of 1.06 micrometers. Such lasers also emit light at a secondary wavelength of approximately 1.32 micrometers. Proper utilization of this secondary mode in laser operation requires the dominant emission, which has a greater amplitude than the secondary emission, to be suppressed. Typically, peak power output at this secondary emission level is 20-30% of the continuous wave peak power output at the dominant level. It is the secondary wavelength that is utilized in the method and apparatus of the invention.

As readily understood by persons skilled in the art, laser 20 includes a power supply circuit for activating the pumping arc lamp and cooling means for cooling the laser. A suitable Nd:YAG laser for use in this invention is produced by Control Laser Corporation, Orlando, Fla. 32809.

A lens 21 is provided to focus the emerging coherent light beam from laser 20 into an optical fiber 22. Lens 21 may comprise a system of lenses. Optical fiber 22 can be of any known type, which efficiently transmits the desired wavelength. Optical fiber 22 provides a flexible conduit for guiding the optical energy from the laser into a hand-piece or wand 23 which is manipulable by the physician. A shutter 24 is located, preferably, between laser 20 and lens 21. Hand-piece 23 contains a shutter switch 25 which controls release of the laser energy and which may be actuated by either the hand or the foot of the operator. A timer 26 is provided to control the shutter and, thereby, the duration of energy exposure. Hand-piece 23 may include a lens (not shown) for focusing or defocusing the beam.

Advantageously, hand-piece 23 includes means to enable the physician to set the working distance between the tissue to be irradiated and the distalmost end of the optical fiber or lens. In an illustrative embodiment, as shown in FIG. 2, a sliding scale 27 which cooperates with a protective case 28 on the end of optical fiber 22 controls the working distance, and hence, the diameter of the beam spot. As shown in FIG. 2, the divergence of the beam is used to control the beam diameter as the distance between the distalmost end of the fiber 22 and the tissue is increased or decreased.

For a given suitable optical wavelength and mode or beam geometry, the following electro-optical parameters require proper adjustment for each type of tissue: output power, time exposure and beam spot size. In particular, the thermal effects on the tissue can be controlled by proper selection of the electro-optical parameters. Power density measures the energy concentration of the applied light beam and is typically expressed in watts per square centimeter area of the applied beam spot. Power density is directly related to the amount of heat that will be produced at a given absorptivity. Radiant exposure, expressed in joules per square centimeter, is a measure of the power density multiplied by the exposure time. If the wavelength of the applied beam is poorly absorbed, more heat can be generated by increasing the time of tissue exposure to the applied beam. Laser output power and beam spot size selections affect the power density; overall radiant exposure is affected by power density and time exposure selections.

Suitable means for control of the power output of laser 20 is provided by a control unit 40, described further with reference to FIG. 3, below. Optical output power detector 41 is provided for initial calibration of the beam of laser 20 at start-up and a second detector 42, which always receives a portion of the beam of laser 20 by means of a beam splitter 43, is provided for continuous monitoring and feedback adjustment of the laser 20 output. The power delivered to the tissue surface should be maintained under 10 watts for purposes of tissue reconstruction by laser 20 as described herein. The object is to deliver a specific amount of energy per volume of tissue. For a given spot size, which is related to the volume of tissue exposed, there are many combinations of power output and time exposure which will deliver equivalent amounts of energy. To-wit, power delivered to the tissue typically ranges between 1 and 4 watts; although power delivered could go as high as 10 watts if the time exposure were reduced commensurately.

In the lowest order transmission mode, TEM.sub.oo specifically, a more concentrated beam results which can be used for cutting purposes at higher power output or for achieving very small beam spot size for tissue reconstruction. In the alternative, multimode transmission can be used for tissue reconstruction, but the beam spot size can not be as finely focused as the TEM.sub.oo mode. However, if the beam is defocused, less power is delivered per unit area.

As will be understood, the selection of the various electro-optical parameters for each

tissue type is made as a result of skill and experience; but is determinable without undue experimentation by one of ordinary skill in the art.

In a particularly advantageous embodiment, data relating to appropriate settings of electro-optical parameters for various tissue types can be coded on a computer memory device, such as floppy disc or programmable read-only memory computer chip. The functions of control unit 40 and timer 26 can be computer controlled to adjust automatically the power level, and time exposure and display the proper spot size upon input of tissue type and the operating conditions by the physician or surgeon.

The system of FIG. 1 also includes a marker laser 30, illustratively a low-power helium-neon laser, which is coaligned with the infrared beam of laser 20. Laser 30, however, can be of any type which emits radiation in the visible range of the electromagnetic spectrum. The power rating of the helium-neon marker laser 30 is between 1-5 Watts. Marker laser 30 can be arranged so that its focal point coincides with that of the main operating laser 20 by any known technique.

As an optional feature, in order to permit the use of the laser apparatus of FIG. 1 on very thin tissue or tissue upon which only surface heating is desired, such as epineurium of nerve tissue, an auxiliary source of optical energy 50 can be incorporated into the apparatus to emit radiation having a wavelength which is intensely absorbed by biological tissue. A carbon dioxide laser, of any known type, would be a suitable auxiliary source. Source 50 is also preferably arranged so as to have its output beam coincide with the beam from marker laser 30.

I should be further pointed out that provision can be made for permitting selection of the 1.06 micrometer wavelength of the Nd:YAG laser 20 by means which are well known in the art for the purposes of tissue coagulation and wound hemostasis, as desired.

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In an illustrative embodiment, the apparatus of FIG. 1 is used for skin closure at a lesion site. The tissue edges of the lesion are brought into close approximation by manual manipulation, for example. Hand-piece 23 is positioned above the lesion at such a distance as to produce the desired beam spot size. The power, time exposure and spot size are set so as to heat the tissue above 45 degrees centigrade, but below the boiling point of water (100 degrees centigrade). Typical spot sizes range from 0.1 mm to 1.0 mm for levels of power delivered to the tissue ranging from 1 to 5 watts, and time durations ranging from 0.05 to 5.0 seconds. When the optical energy of laser 20 at 1.32 micrometers is released on the lesion site, with the electro-optical parameters adjusted as hereinabove suggested, the tissue at the lesion site is heated to a temperature sufficient to cause denaturization of the tissue proteins to the depth necessary to reconstruct the tissue in the lesion irrespective of whether the operating field is bloody or wet. The electro-optical parameters are set and controlled in response to input as to tissue type and thickness. Parameters may be specified for the

reconstruction of many soft tissues such as vascular structures, tendon, vas deferens, fallopian tubes, gastrointestinal tract, dura, and sclera. With an appropriately controlled modification of the level of power delivered to the tissue under repair, cartilage and tympanic membranes can also be repaired in accordance with the invention described hereinabove.

The above-described embodiments are provided for the purpose of illustration and are not to be construed as limiting. Other modifications and adaptations can be made by those of skill in the art without departing from the spirit and scope of the invention. In particular, the laser energy can be transmitted to the patient treatment site by an articulated arm with mirrors or it can be transmitted to the interior of a patient by endoscope. Moreover, materials other than neodymium-doped crystalline yttrium-aluminum-garnet can be used as a lasing medium to generate optical energy at the desired wavelengths.

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United States Patent 4,895,154
Bartelt , et al. January 23, 1990
Electronic stimulating device for enhanced healing of soft tissue wounds

Abstract

An electronic stimulating device for wound healing and, more particularly, for enhancing healing of soft tissue wounds. The device includes a plurality of signal generators for generating output pulses suitable for enhancing healing of soft tissue wounds by application of the output pulses through electrodes to the soft tissue to be healed. Two pairs of active electrodes are included along with a pair of return electrodes. Control signals from a pulse generator control generation of the output pulses and a digital timer is included for causing treatmet for a predetermined period of time. The intensity, polarity, and rate of the output pulses can be varied by rotation of control knobs on the front panel of the device or, alternately, can be effected by a series of switches located on the front panel of the device, with varying intensities of output being indicated by brightness varying light emitting diodes.
Inventors: Bartelt; James T. (Longmont, CO); Owens; Alan R. (Longmont, CO)
Assignee: Staodynamics, Inc. (Longmont, CO)
Appl. No.: 159863
Filed: February 19, 1988

Current U.S. Class: 607/50 ; 607/66; 607/72
Current International Class: A61N 1/32 (20060101)
Field of Search: 128/419R,421,422,423R,783
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Primary Examiner: Jaworski; Francis
Assistant Examiner: Manuel; George
Attorney, Agent or Firm: Harris; Robert E.

Claims

What is claimed is:

1. An electronic stimulating device for enhancing healing of soft tissue wounds, said device comprising:

signal generating means providing a stimulating signal suitable for enhancing healing of soft tissue, said stimulating signal having a peak current not greater than about 40 ma;

electrode means connected with said generating means for receiving said stimulating signal therefrom, said electrode means being adapted to be positioned contiguous to said soft tissue wound to be healed;

return electrode means adapted to be positioned adjacent to said soft tissue wound to be healed; and

control means connected with said signal generating means for controlling application of said stimulating signal to said soft tissue wound, said control means including timer means for causing application of said stimulating signal to said soft tissue wound for a predetermined time period.

2. The device of claim 1 wherein said control means includes pulse generating means for generating control pulses for application to said signal generating means for controlling application of said stimulating signal to said soft tissue, said timer means being connected with said pulse generating means.

3. The device of claim 2 wherein said timer means includes a timer connected with said signal generating means and indicating means connected with said timer for indicating a factor of time of treatment.

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9. An electronic stimulating device for enhancing healing of soft tissue wounds, said device comprising:

first and second pairs of signal generating means each of which provides a stimulating signal suitable for enhancing healing of soft tissue wounds;

first and second pairs of electrodes each of which is connected with a different one of said first and second pairs of signal generating means to receive said stimulating signals therefrom, said electrodes being adapted for positioning contiguous to a soft tissue wound to be healed;

first and second return electrodes adapted to be positioned adjacent to said soft tissue wound to be healed;

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13. An electronic stimulating device for enhancing healing of soft tissue wounds, said device comprising:

first and second pairs of constant current devices providing constant current output pulses, said constant current pluses having a peak current not greater than about 40 ma;

first and second pairs of visual indicating means connected with said first and second pairs of constant current devices, the intensity of said visual indicating means being indicative of the intensities of said constant current output pulses;

first and second pairs of electrodes each pair of which is connected to a different pair of said first and second pairs of constant current devices, said electrodes being adapted to be positioned contiguous to a soft tissue wound to be healed and to receive said constant current output pulses from said first and second pairs of constant current devices;

first and second return electrodes adapted to be positioned adjacent to said soft tissue wound to be healed;

isolation means connected with said return electrodes;

power supply means for supplying a predetermined high voltage to said first and second pairs of constant current devices;

pulse generating means for providing a series of control pulses, said pulse generating means being connected with said constant current devices to control operation of the same whereby each of said constant current devices produces said constant current output pulses for healing of said soft tissue wounds;

intensity control means connected with said pulse generating means, said intensity control means including an on/off switch for powering said device on and off; and

timer means connected with said pulse generating means for enabling treatment by said stimulating pulses over a predetermined timed period, said timer means being connected with said intensity control means whereby operation of said timer means during said timed period can be terminated only by actuation of said on/off switch of said intensity control means from the on position to the off position.

14. The device of claim 13 wherein said device includes pulse limiting means connected with said first and second pairs of constant current devices.

15. The device of claim 13 wherein said timer means is a digital timer.

16. The device of claim 13 wherein said device includes polarity switching means connected with said first and second pairs of constant current devices.

17. The device of claim 16 wherein said device includes pulse rate selection means, and wherein polarity and rate changes of said output pulses effected during operation of said device by switching said polarity switching means and selection at said pulse rate selection means are gradually effected.

18. The device of claim 13 wherein said intensity control means includes switching means for switching the intensity of said applied stimulating pulses and wherein changes in intensity of said applied stimulating pulses are gradually effected.

19. The device of claim 13 wherein said device includes pause/resume switching means connected with said timer means.

20. The device of claim 13 wherein said first and second pairs of constant current devices provide output pulses having pulses and galvanic components.

Description

FIELD OF THE INVENTION

This invention relates to an electronic stimulating device, and, more particularly, to such a device for enhancing healing of soft tissue wounds.

BACKGROUND OF THE INVENTION

Pulse stimulating devices are well known and such devices have heretofore been utilized for pain suppression (see, for example, U.S. Pat. Nos. 4,014,347, 4,632,117 and 4,640,286).

In addition, pulse stimulating devices have been heretofore suggested for use in treating inflammatory conditions, edema, sprains, muscle spasms, and the like (see,

for example, the Vara/Pulse Galvanic Stimulating Unit manufactured and sold by Staodynamics, Inc., Longmont, Colo.).

It has also been heretofore suggested that stimulating units can be utilized for wound healing and extensive experimental work has been accomplished, for example, using the Vara/Pulse Galvanic Stimulating Unit of Staodynamics, Inc..

SUMMARY OF THE INVENTION

This invention provides an electronic stimulating unit for wound healing and, more particularly, for enhancing healing of soft tissue wounds. Stimulating pulses suitable for enhancing wound healing are applied through a plurality of electrodes, which can include two pairs of active electrodes and a pair of return electrodes, with timer means being included to cause treatment to be automatically carried out for a predetermined period of time, and with intensity of applied pulses being visually indicated.

It is therefore an object of this invention to provide an improved electronic stimulating device for enhancing healing of soft tissue wounds.

It is another object of this invention to provide an improved electronic stimulating device for enhancing wound healing utilizing a timer for causing treatment to occur over a predetermined period of time.

It is another object of this invention to provide an improved electronic stimulating device for enhancing wound healing with stimulating pulses being applied through first and second pairs of electrodes and returned through a pair of return electrodes.

It is still another object of this invention to provide an improved electronic stimulating device for enhancing wound healing wherein the intensity of applied pulses is visually indicated.

With these and other objects in view, which will become apparent to one skilled in the art as the description proceeds, this invention resides in the novel construction, combination, and arrangement of parts substantially as hereinafter described, and more particularly defined by the appended claims, it being understood that changes in the precise embodiment of the herein disclosed invention are meant to be included as come within the scope of the claims.

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DESCRIPTION OF THE INVENTION

Electronic stimulating device, or unit, 7 is shown in FIG. 1 as a self-contained portable unit with the entire unit being enclosed within housing 9 and with all operator actuatable controls being accessible at front panel 11. As shown, a carrying case 13 is also preferably provided and includes storage for leads, electrodes, accessories, and

the like.

As also shown in FIG. 1, a combined off/on and intensity control knob 15 is provided at the front panel with operable intensities of 30, 35 or 40 ma being preferably only those identified. Polarity knob 17 is provided at front panel 11 with this knob being preferably positionable to provide either negative or positive polarity of pulses. Pulse rate knob 19 is also provided at front panel 11 and preferably allows only selection of a pulse rate of 64 pulses per second (pps) or 1128 pps.
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United States Patent 6,120,497
Anderson , et al. September 19, 2000
Method and apparatus for treating wrinkles in skin using radiation

Abstract

A method for treating wrinkles in skin involves the use of a beam of pulsed, scanned or gated continuous wave laser or incoherent radiation. The method comprises generating a beam of radiation, directing the beam of radiation to a targeted dermal region between 100 microns and 1.2 millimeters below a wrinkle in the skin, and thermally injuring collagen in the targeted dermal region. The beam of radiation has a wavelength of between 1.3 and 1.8 microns. The method may include cooling an area of the skin above the targeted dermal region while partially denaturing the collagen in the targeted dermal region. The method may also include cooling an area of the skin above the targeted dermal region prior to thermally injuring collagen in the targeted dermal region.
Inventors: Anderson; R. Rox (Lexington, MA); Ross, Jr.; Edward Victor (San Diego, CA); Hsia; James C. (Weston, MA); McMillan; Kathleen (Concord, MA)
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United States of America (Washington, DC)
Appl. No.: 153052
Filed: September 15, 1998
Related U.S. Patent Documents

Application Number	Filing Date	Patent Number	Issue Date
794876	Feb., 1997	5810801	

Current U.S. Class: 606/9 ; 606/2; 606/23
Field of Search: 606/2,9,23
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Primary Examiner: Buiz; Michael
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Parent Case Text

This application is a continuation of application Ser. No. 08/794,876, filed Feb. 5, 1997, now U.S. Pat. No. 5,810,801.

Claims

What is claimed is:

1. A method for treating a wrinkle in human skin, comprising:

generating a beam of radiation having a wavelength of between 1.3 and 1.8 microns and a fluence of between 10 and 150 joulesper square centimeter;

directing the beam of radiation to a targeted dermal region between 100 microns and 1.2 millimeters below a wrinkle in the skin;

cooling an epidermal region of the skin above the targeted dermal region; and

causing thermal injury within the targeted dermal region to elicit a

healing response that produces substantially unwrinkled skin.

2. The method of claim 1 wherein the wherein the cooling step comprises cooling the epidermal region of the skin above the targeted dermal region before the step of causing thermal injury within the targeted dermal region.

3. The method of claim 1 further comprising the step of stretching the skin adjacent the wrinkle before the step of directing the beam of radiation to the targeted dermal region.

4. The method of claim 1 wherein the cooling step comprises cooling an epidermal region of the skin above the targeted dermal region contemporaneously with the step of causing thermal injury within the targeted dermal region.

5. The method of claim 1 wherein the cooling step comprises cooling the epidermal region of the skin above the targeted dermal region before and contemoraneously with the step of causing thermal injury within the targeted dermal region.

6. A method for treating a wrinkle in human skin, comprising:

generating a beam of radiation having a wavelength of between 1.3 and 1.8 microns and a power density of between 5 and 100 watts per square centimeter;

directing the beam of radiation to a targeted dermal region between 100 microns and 1.2 millimeters below a wrinkle in the skin;

cooling an epidermal region of the skin above the targeted dermal region; and

causing thermal injury within the targeted dermal region to elicit a healing response

that produces substantially unwrinkled skin.

7. The method of claim 6 wherein the wherein the cooling step comprises cooling the epidermal region of the skin above the targeted dermal region before the step of causing thermal injury within the targeted dermal region.

8. The method of claim 6 further comprising the step of stretching the skin adjacent the wrinkle before the step of directing the beam of radiation to the targeted dermal region.

9. The method of claim 6 wherein the cooling step comprises cooling an epidermal region of the skin above the targeted dermal region contemporaneously with the step of causing thermal injury within the targeted dermal region.

10. The method of claim 6 wherein the cooling step comprises cooling the epidermal region of the skin above the targeted dermal region before and contemporaneously with the step of causing thermal injury within the targeted dermal region.

Description

FIELD OF THE INVENTION

The invention relates generally to the treatment of wrinkles in human skin using radiation. In particular, the invention relates to a method for treating wrinkles in human skin using a beam of laser or incoherent radiation to cause thermal injury in the dermal region of the skin sufficient to elicit a healing response that produces substantially unwrinkled skin.

BACKGROUND OF THE INVENTION

Undesired wrinkles in skin are commonly seen in dermatologic practice. Wrinkles in skin may be caused by age and by exposure to the sun's ultraviolet rays. Human skin consists mainly of two layers: the top layer of skin known as the epidermis; and the layer beneath the epidermis known as the dermis. The dermis is primarily a cellular and is composed of water, the protein collagen, and glycosaminoglycans. Water constitutes approximately 70 percent of the total weight of the dermis. Collagen constitutes approximately 70 percent of the dry weight of the dermis, and glycosaminoglycans constitute between approximately 0.1 and 0.3 percent of the dry weight of the dermis. Collagen and glycosaminoglycans are constantly produced by fibroblasts, a type of connective tissue cell, and degraded by enzymes. Collagen degradation relies primarily on specific proteinases known as collagenases.

Collagen provides the dermis with the majority of its structural integrity. With aging, the amount of dermal collagen decreases and is replaced by the protein elastin. In addition, the remaining collagen tends to be chaotically oriented as compared to the more organized patterns found in youthful skin. Glycosaminoglycans are very

hydrophilic, and increased amounts of these carbohydrates are associated with the increased skin vigor found in youthful skin. One major difference between the smooth, supple skin of newborns and the drier, thinned skin of older individuals is the far greater relative amount of glycosaminoglycans found in newborn skin. The glycosaminoglycans found in newborns can bind up to 1000 times their weight in water. As the skin ages and the amount of glycosaminoglycans decreases, the skin may become less hydrated and lose some of the suppleness found in youth. Also, the remaining glycosaminoglycans in photo-aged skin are deposited on the haphazardly arranged elastin fibers which have replaced the collagen fibers. The placement of the remaining glycosaminoglycans may partially account for the weather-beaten appearance of photo-aged skin.

Existing procedures for eliminating or reducing the severity of wrinkles include chemical peels, mechanical abrasion and laser ablation. All of these methods remove the top layer of skin. A new top layer forms during healing. Cosmetic improvement is seen when the skin containing wrinkles is replaced by a new layer of horizontally oriented neocollagen in the superficial dermis. However, all of these methods disrupt and completely remove the epidermis. The resulting open wounds require daily care to optimize wound healing. Epidermal destruction and subsequent healing has several undesirable side effects. These undesirable side effects include prolonged hypopigmentation, hyperpigmentation, erythema and edema. Hyperpigmentation occurs frequently in darker skin types as a result of an inflammatory response of the skin. Hyperpigmentation results in the treated area of the subject's skin turning darker than the surrounding untreated skin. Hyperpigmentation can be slow to clear, sometimes taking up to a year to disappear. Hypopigmentation is attributable to damage to the melanin-producing cells in the skin. While generally transient, hypopigmentation can be permanent, and is cosmetically undesirable while it persists. Also, erythema or redness of the skin may be significant for weeks to months after the procedure, requiring the patients to wear conspicuous amounts of make-up.

A known property of collagen fibers, such as those found in the skin. is that the fibers shrink when elevated to a temperature in the range of 60 to 70 degrees Celsius, which is about 30 degrees Celsius above normal body temperature. Temperature elevation ruptures the collagen ultrastructural stabilizing cross-links, and results in immediate contraction in the collagen fibers to about one-third of their original length without changing the structural integrity of the fibers. One known technique shrinks the collagen fibers in the cornea of the eye to change the shape of the cornea and correct refractive disorders. This technique involves the use of laser energy in a wavelength range of about 1.80 to about 2.55 microns. The laser energy is used to irradiate the collagen in the cornea to elevate the collagen to at least 23 degrees Celsius above normal body temperature and thereby achieve collagen shrinkage. U.S. Pat. Nos. 4,976,709, 5,137,530, 5,304,169, 5,374,265, and 5,484,432 to Sand disclose a technique and apparatus for controlled thermal shrinkage of collagen fibers in the cornea.

However, this technique cannot be effectively used to remove wrinkles in skin by

shrinking dermal collagen. The bulk of the shrunken, thermally denatured, collagen fibers do not remain in the skin after treatment with this technique. Unlike the cornea, which is avascular, an aggressive healing response in the skin degrades the denatured collagen in the superficial dermis by collagenases, thereby rapidly eliminating the bulk of the shrunken collagen from the skin.

Additionally, in the 1.80 to 2.55 micron wavelength range, strong absorption of the laser energy by water present in the skin limits the penetration depth of the laser radiation to a small fraction of a millimeter. The depths of thermal injury which can be achieved in skin using the wavelengths in this range are therefore limited to the most superficial layer of the skin. Such superficial injury leads to an inflammatory healing response characterized by prolonged visible edema and erythema, as well as the possibility for long lasting pigmentary disturbances.

SUMMARY OF THE INVENTION

The present invention addresses the foregoing problems and provides a method for inducing remodeling of the skin's extracellular matrix by partially denaturing the dermal collagen deeper in the skin, below the

surface, while avoiding injury to the epidermis and upper layers of the dermis. The invention offers numerous advantages over existing dermatologic procedures and devices. The surface of the skin remains intact, thereby avoiding the need for dressing wounds; pigmentary disturbances are minimized; and any inflammatory response to the injury is mild and less visually evident.

In general, the present invention features a method for treating wrinkles in skin, without removing a layer of skin, using a beam of pulsed, scanned or gated continuous wave (CW) laser or incoherent radiation. The method comprises generating a beam of radiation having a wavelength between 1.3 and 1.8 microns, directing the beam of radiation to a targeted dermal region between 100 microns and 1.2 millimeters below a wrinkle in the skin, and thermally injuring the targeted dermal region to elicit a healing response that produces substantially less wrinkles.

More specifically, causing selective thermal injury to the dermis activates fibroblasts which deposit increased amounts of extracellular matrix constituents (i.e., collagen and glycosaminoglycans). These increases in extracellular matrix constituents are responsible for dermal skin rejuvenation and the reduced appearance of wrinkles.

In one embodiment, the beam of radiation causes partial denaturation of the collagen in the targeted dermal region. The partial denaturation of the collagen accelerates the collagen synthesis process by the fibroblasts and the deposition of new glycosaminogalycans, leading to the elimination or a reduction in the severity of the wrinkle. The method may also include cooling the surface of the skin and epidermal tissue above the targeted dermal region while irradiating the skin. The method may

also include cooling the surface of the skin prior to irradiating the skin.

In a detailed embodiment, the method also includes stretching the skin along the wrinkle before directing the beam of radiation to the targeted dermal region below the wrinkle. Stretching the skin causes thermal injury to the collagen fibers across the wrinkle, while not affecting the fibers along the wrinkle.

The invention also relates to an apparatus for treating wrinkles in skin. The apparatus includes a radiation source and a delivery system which includes a cooling system. The radiation source generates a beam of radiation having a wavelength between 1.3 and 1.8 microns. The delivery system directs the beam of radiation to a targeted dermal region between 100 microns and 1.2 millimeters below a wrinkle in the skin. The cooling system cools the epidermal tissue above the targeted dermal region to minimize injury to the surface of the skin.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention contemplates a system and method for removing wrinkles which includes delivering a beam of laser or incoherent radiation to cause sufficient thermal injury in the dermal region of the skin to elicit a healing response to cause the skin to remodel itself, resulting in more youthful looking (i.e., substantially unwrinkled) skin. In particular, thermal injury may be in the form of partial denaturation of the collagen fibers in the targeted dermal region of skin. In one embodiment, the radiation beam has a set of parameter ranges carefully selected to partially denature collagen in the dermis while protecting the epidermis by surface cooling. As a result, a subject treated using the method of the invention is able to have the appearance of wrinkles lessened without damage to the epidermis.

FIG. 1 is an illustration of a system 10 for practicing the invention. The system 10 includes a radiation source 12 and a delivery system 13. A beam of radiation generated by the radiation source 12 is directed to a target region of a subject's skin including a wrinkle via the delivery system 13. In one embodiment, the radiation source 12 is a laser. The laser may generate a beam of pulsed, scanned or gated CW laser radiation. In another embodiment, the radiation source 12 generates incoherent radiation.

The beam of radiation is directed to a targeted dermal region of skin between 100 microns and 1.2 millimeters below the wrinkle. The parameter ranges for the beam have been specifically selected to cause thermal injury to the dermis while avoiding injury to the epidermis and upper layers of the dermis. In particular, the wavelength of the radiation beam has been chosen to maximize absorption in the targeted region of the dermis, and the fluence or power density, depending on the type of radiation, has been chosen to minimize erythema. The wavelength range chosen has a tissue absorption coefficient preferably in the range of about 1 to 20 cm.sup.-1. Thus, the

beam preferably has a wavelength of between about 1.3 and 1.8 microns in one embodiment. Within this wavelength range, radiation energy applied through the surface of the skin is deposited predominantly in the dermal region of the skin. In one embodiment, the radiation beam has a nominal wavelength of approximately 1.5 microns. Lasers which produce radiation having wavelengths in the range of between about 1.3 and 1.8 microns include the 1.33 micron Nd:YAG laser, the 1.44 micron Nd:YAG laser and the 1.54 micron Er:Glass laser. The radiation beam may be pulsed, scanned or gated continuous wave laser radiation. In embodiments having a laser as the radiation source 12, the laser radiation generated preferably has a fluence of between about 10 and 150 joules.

In another embodiment, the radiation used to thermally injure the dermis is incoherent radiation. In embodiments using incoherent radiation, the incoherent radiation generated by the radiation source 12 preferably has a power density of between about 5 and 100 watts per square centimeter.

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To minimize thermal injury to the epidermis and the upper layers of the dermis, in one embodiment, the delivery system 13 includes a cooling system for cooling the surface of the skin prior to and/or during application of the radiation. In this embodiment, the delivery system 13 is multi-functional and is capable of delivering radiation and cooling the surface of the skin at the same time. FIG. 3 shows one embodiment of a delivery system 13 which includes a cooling system. The handpiece 16 includes a skin contacting portion 20 which is brought into contact with the region of skin 22 receiving the beam of radiation 24. The skin contacting portion 20 cools the epidermal region of skin 22 receiving the beam of radiation. The skin contacting portion 20 includes a sapphire window 26 and a fluid passage 28 which contains a cooling fluid. The cooling fluid may be a fluorocarbon type cooling fluid. The cooling fluid circulates through the fluid passage 28 and past the sapphire window 26 which is in contact with the epidermal region of skin 22 receiving the beam of radiation 24.

In another embodiment, the delivery system 13 and the cooling system are separate systems. The cooling system may comprise a container of a cold fluid. Cooling of the surface of the skin is accomplished by briefly spraying the skin with the cold fluid which extracts heat from the skin on contact. The fluid used can also be a non-toxic substance with high vapor pressure at normal body temperature, such as a freon. These fluids extract heat from the skin by the virtue of evaporative cooling.

FIG. 3 illustrates the treatment of a wrinkle 30 in accordance with the invention. Radiation pulses are produced using the radiation source 12, which may be a pulsed, scanned or gated CW laser or incoherent radiation source. The radiation pulses are directed toward the region 22 of the subject's skin containing the wrinkle 30 by the delivery system 13. The radiation pulses are preferably directed to a targeted dermal

region between 100 microns and 1.2 millimeters below the surface of the skin. In a detailed embodiment, the radiation pulses are focused to a region centered at a depth of about 750 microns. The targeted dermal region including a portion of the wrinkle 30 is then irradiated with radiation pulses exiting from the handpiece 16 until collagen in that region is partially denatured. To accomplish this, the collagen at the selected depth in the targeted dermal region is preferably heated to a temperature in the range of about 50 to 70 degrees Celsius. Partially denaturing collagen in the dermis accelerates the collagen synthesis process by the fibroblasts. The thermal injury caused by the radiation is mild and is only sufficient to elicit a healing response and cause the fibroblasts to produce new collagen. Excessive denaturation of collagen in the dermis causes prolonged edema, erythema, and potentially scarring.

The skin contacting portion 20 preferably cools the area of the skin above the targeted dermal region to temperatures below approximately 50 to 70 degrees Celsius during application of the radiation, so as not to cause collateral thermal damage to the epidermis. The radiation beam, due to its wavelength, does not sufficiently penetrate into depths below the targeted dermal region to cause thermal damage deeper in the skin. In one detailed embodiment, the skin contacting portion 20 cools an area of the skin above the targeted dermal region before the radiation is applied. The relative timing of cooling the surface of the skin to applying radiation depends, in part, on the depth to which thermal injury is to be prevented. Longer periods of cooling prior to the application of radiation allow more time for heat to diffuse out of the skin and cause a thicker layer of skin to be cooled, as compared to the thickness of the layer cooled by a short period of cooling. This thicker layer of cooled tissue sustains less thermal injury when the radiation energy is subsequently applied. Continued cooling of the surface of the skin during the delivery of radiation energy extracts heat from the upper layers of the skin as heat is deposited by the radiation, thereby further protecting the upper layers from thermal injury.

The depth of thermal injury caused by the radiation depends primarily on the penetration depth of the radiation used. The penetration depth can be approximated by taking the reciprocal of the absorption coefficient of the skin at the wavelength of the radiation. The thickness of the tissue overlying the zone of injury which is spared from injury depends primarily on the cooling applied prior to and/or during the delivery of radiation energy. By suitably choosing the radiation wavelength, the timing of the surface cooling, the cooling temperature, the radiation fluence and/or the power density as described above, the depth, the thickness and the degree of thermal injury can be confined to a zone within the dermis. These parameters can be chosen to optimally induce the injury required to elicit remodeling within the dermis, while substantially or completely sparing injury to the overlying epidermis and upper layers of the dermis.

In another detailed embodiment, the region of skin including the wrinkle 30 is stretched along the wrinkle 30 before the beam of radiation is directed to the targeted dermal region below the wrinkle 30. Stretching the skin along the wrinkle before irradiating the skin causes partial denaturation of the collagen fibers across the

wrinkle, while not damaging the fibers along the wrinkle. Partially denaturing the fibers across the wrinkle tightens the skin sufficiently to cause the wrinkle to disappear.

Referring to FIG. 4, in one embodiment, to counteract the effects of scattering, the radiation beam is made highly convergent on the surface of the skin.

Experimental Results

The method of the present invention for treating wrinkles in skin using radiation was applied in a series of in vivo experiments performed on pigs. A pulsed erbium glass laser producing radiation having a wavelength of approximately 1.54 microns was used as the radiation source 12. The laser energy was applied to the pig skin via the skin contacting portion 20 equipped with a cooled sapphire window 26 at the tip, as described above and shown in FIGS. 1-3. The inner surface of the sapphire window 26 was cooled by circulating refrigerated coolant, chilled to approximately minus 25 degrees Celsius through the passage 28. The coolant used was a halocarbon which is transparent to the 1.54 micron laser radiation. The laser beam at the outer surface of the sapphire window 26 was approximately 5 mm in diameter.

The tip of the skin contacting portion 20 was placed in contact with the skin to cool the skin prior to applying the laser radiation. After a set amount of time (hereinafter "the pre-cooling time"), laser energy was applied to the skin. Various combinations of pre-cooling times, laser pulse energies, laser pulse repetition frequencies, time intervals of laser energy delivery, and total number of laser pulses delivered were studied. It was found that by the appropriate choice of these parameters, varying degrees of thermal injury can be achieved at varying depths in the dermis while preserving the viability of the epidermis and upper dermis.

For example, using a pre-cooling time of 5 seconds, a laser energy in the range of between 0.2 and 0.8 joules per pulse at a pulse repetition frequency of 4 Hertz (corresponding to an average laser power in the range between 0.8 to 3.2 watts), and a total of 24 pulses, it was found that varying degrees of thermal injury could be induced in a zone centered at a depth in the range of approximately 0.5 to 1.0 millimeters beneath the surface of the skin, while avoiding injury to the epidermis and upper dermis.

Histology performed on biopsy samples taken at sites treated with the above range of parameters revealed collagen denaturation extending from about 100 microns in the dermis to about 1 mm deep. The epidermis and upper layers of the dermis were preserved as confirmed with nitrotetrazolium blue, a viability stain. In the cases in which only partial collagen denaturation was shown on histology, clinically, the treated areas showed an intact epidermis with mild edema and erythema which resolved completely within two weeks. Histologically, the treated sites showed greatly increased fibroblast activity, new collagen secretion and degradation of denatured collagen. By four weeks post treatment, the treated sites returned to normal, both clinically and

histologically.

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United States Patent 6,428,532
Doukas , et al. August 6, 2002

Selective tissue targeting by difference frequency of two wavelengths

Abstract

A method and apparatus for treating tissue directs first and second wavelengths of energy, e.g., laser or ultrasonic signal energy, into an overlap region of tissue such that the first and second wavelengths mix in situ to form a third wavelength. The third wavelength effectively couples energy into the tissue to treat tissue in said overlap region, producing local heating or cavitation. Wavelengths may be selected such that overlying tissue is unharmed, and the two wavelengths may be applied along different paths and cross in the overlap region. Absorbers or dyes may be used to tailor absorbance of the third wavelength. When using ultrasonic energy, signals of several megahertz may be so mixed to provide a lower frequency treatment signal such as a 200 kHz treatment signal or a subharmonic. Laser signals may be mixed to form a treatment signal in the infrared.

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Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 60/114,271 filed Dec. 30, 1998, the contents of which are incorporated herein by reference.

Claims

What is claimed is:

1. An apparatus for treating tissue using two different wavelengths, comprising a first energy emitter which produces a first wavelength of energy and a second energy emitter which produces a second wavelength of a second energy, such that said first and second wavelengths are focused onto a focal point or region of a tissue, such that said first and second wavelengths produce an overlap region in which the first and second wavelengths mix to form a third wavelength which produces cavitation and/or hyperthermia at said focal point or region within said tissue.

2. The apparatus of claim 1, wherein said first and second energy emitters are lasers.

3. The apparatus of claim 2, wherein said lasers are ND:YAG lasers.

4. The apparatus of claim 2, wherein said piezoelectric transducers are ceramic.

5. The apparatus of claim 1, wherein said first wavelength is about 1.06 .mu.m and said second wavelength is between about 550 nm and 920 nm.

6. The apparatus of claim 1, wherein said energy difference is between about 3 and 6.5 .mu.m.

7. The apparatus of claim 1, wherein said first and second energy emitters are piezoelectric transducers.

8. The apparatus of claim 1, wherein said first wavelength of energy has a frequency of about 3.8 MHZ and said second wavelength of energy has a frequency of about 4.0 MHZ.

9. The apparatus of claim 1, wherein said two different wavelengths of energy are ultrasonic signals having a frequency difference of about 200 kHz.

10. The apparatus of claim 9, wherein said wavelengths mix to form a signal with a subharmonic frequency of about 100 kHz.

11. An apparatus for treating tissue using two different wavelengths, comprising a) a first energy emitter which produces a first wavelength of energy; b) a second energy emitter which produces a second wavelength of a second energy; and c) a control means for said apparatus to carry out treatment, wherein said control means causes transmission and focusing of said first and second wavelengths onto a focal point or region of a tissue, whereby said first and second wavelengths produce an overlap region having an energy difference which produces energy of a third wavelength effective to provide cavitation an/or hyperthermia at said focal point or region within said tissue.

12. The apparatus of claim 11, wherein said control means causes transmission of ultrasonic waves for a duration of between about 1 millisecond and continuous.

13. The apparatus of claim 11, wherein said control means provides transmission of ultrasonic waves by successive pulses.

14. A method for treating tissue using two different wavelengths, comprising the steps of: a) directing a first wavelength of energy into a tissue; and b) directing a second wavelength of a second energy into said tissue, such that said first and second wavelengths are focused onto a focal point or region of said tissue in an overlap region having an energy difference which produces a third wavelength that effectively couples energy to said tissue to provide cavitation and/or hyperthermia at said focal point or region within said tissue, thereby treating said tissue.

15. The method of claim 14, wherein the steps of directing said first and second wavelengths of energy are performed by directing lasers.

16. The method of claim 15, wherein said lasers are ND:YAG lasers.

17. The method of claim 14, wherein said first wavelength is about 1.06 .mu.m and said second wavelength is between about 558 nm and 920 nm.

18. The method of claim 14, wherein said energy difference is between about 3 and 6.5 .mu.m.

19. The method of claim 14, wherein said first and second energy emitters are piezoelectric transducers.

20. The method of claim 19, wherein said piezoelectric transducers are ceramic.

21. The method of claim 14, wherein said first wavelength is about 3.8 MHZ and said second wavelength is about 4.0 MHZ.

22. The method of claim 14, wherein said energy difference is about 200 kHz.

23. The method of claim 22, wherein said energy difference has a subharmonic frequency of about 100 kHz.

24. The method of claim 14, wherein said energy wavelengths are pulsed for a duration of between about 1 millisecond and continuous.

25. A method of treating tissue, such method comprising the step of directing first and second wavelengths of energy into an overlap region of tissue such that the first and second wavelengths mix in situ to form a third wavelength, wherein the third

wavelength effectively couples energy into the tissue to treat tissue in said overlap region.

Description

BACKGROUND OF THE INVENTION

Surgical instruments and surgical methods, methods of chemotherapy, radiation therapy and hyperthermic treatment can be utilized for the medical treatment of various proliferating diseases, e.g., tumors and cancer. Methods of acoustic surgery for similar applications have not presently been widely accepted in these areas of clinical practice.

Laser and improved acoustic techniques are under development for surgical techniques. Lasers are currently used for targeted energy delivery in a variety of medical procedures. The absorption of light is function of wavelength. The 600 nm to about the 1200 nm spectral band has been called a "window" region because there is deep penetration of photons into tissue due to low absorption (1). Measurement of the transmission of coherent photons using optical heterodyne detection has been performed for 2-7 mm thick tissue sections (2). The total extinction coefficient .mu., (790 nm) of skin, liver, and muscle is 0.96-1.75 mm.sup.-1. For targeted delivery the laser energy should be preferentially absorbed in the treatment site. One of the most successful medical uses of lasers occurs when endogenous chromophores are located in the treatment region. Unfortunately selective concentration of endogenous chromophores in the treatment location occurs rarely. Therefore exogenous chromophores that are preferentially delivered into the treatment site are currently under investigation (3).

For acoustic surgery, a mechanically oscillated hollow metal pin, for example, can be used as a therapeutic tool. High-power focused ultrasound acoustic fields are able to destroy human body tissue (see PCT published applications in the name of Fry WO 89/07907 and WO98/07909). Dunn and Fry have also described in "Ultrasonic threshold dosage for the mammalian central nervous system" IEEE transactions, volume BME 18, pages 253-256 how this destruction process involves two effects, more a specifically a thermal effect and a cavitation effect.

In general, the thermal effect predominates when the acoustic power at the point of focus is below the threshold of about 150 W/cm.sup.2 at MHZ. Therefore, thermal effects are due to the acoustic absorption of the tissue which converts the mechanical energy of the acoustic wave into thermal energy.

More specifically, the cavitation effect becomes predominant when the acoustic power at the point of focus exceeds a threshold of 150 W/cm.sup.2. Cavitation is linked to the formation of microscopic bubbles of gas which implode when they reach a critical diameter with a local release of appreciable amounts of energy leading to destruction

of neighboring tissue.

In order to obtain destruction of tissue exclusively by thermal effects, it has been necessary for the acoustic field to be able to reach a threshold of destruction referred to as the "thermal dose." This threshold is a function of temperature reached and of the duration of application. Thus the presently known approaches have been to destroy tissue by application of a moderate temperature increase over a long duration of application or, through application of a significant temperature increase over a short period of application.

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the discovery that by directing two different wavelengths of energy (beams), simultaneously, at a focal point or region within tissue, an overlap region forms having an energy difference which causes selective cavitation and/or hyperthermia in tissue at the site of intersection of the two beams. This localized treatment does not effect tissue surrounding the area where the two beams do not overlap and is, therefore, an effective means to treat tissue, e.g., diseased tissue, without harming the surrounding tissue area about the site of selective cavitation.

The present invention pertains to apparati for treating tissue using two different wavelengths, e.g., energy beams. The apparati include a first energy emitter which produces a first wavelength of energy and a second energy emitter which produces a second wavelength of a second energy. The first and second wavelengths are focused onto a focal point or region of the tissue, so that the first and second wavelengths intersect and produce an overlap region having an energy difference. The energy difference causes cavitation and/or hyperthermia at the focal point or region within the tissue. The two different wavelengths are generated by energy emitters. Suitable energy emitters are lasers, e.g., ND:YAG lasers, or piezoelectric transducers. In a preferred embodiment, the acoustic energy difference between the two energy beams is about 200 kHz with a subharmonic frequency of about 100 kHz.

The present invention also pertains to apparati for treating tissue using two different wavelengths, energy beams, which include a first energy emitter, a second energy emitter and a control means. The first and second energy emitters produce two differing wavelengths. The control means facilitates transmission and focusing of the energy beams onto a focal point or region of the tissue. The energy beams intersect and produce an overlap region which has an energy difference which produces cavitation and/or hyperthermia at the focal point or region within the tissue. The control means causes transmission of cavitation waves, e.g., ultrasound, for a duration of between about 1 milliseconds and continuous. Alternatively, the control means causes hyperthermia when an energy pulse of between about 20 nanoseconds (10-.sup.8 seconds) and 5 femtoseconds (10.sup.-15 seconds). The controls means can also provide transmission of ultrasonic waves, light, coherent light, or energy waves which

result in hyperthermia by successive pulses.

The present invention further pertains to methods for treating tissue using two different wavelengths simultaneously. The methods include directing a first wavelength of energy into a tissue and directing a second wavelength of a second energy into the tissue, such that the first and second wavelengths are focused onto a focal point or region of the tissue. The first and second wavelengths intersect to produce an overlap region which has an energy difference causing cavitation to occur at the focal point or region within the tissue, thereby treating the tissue. This localized treatment does not effect tissue surrounding the area where the two beams do not overlap and is, therefore, an effective means to treat tissue, e.g., diseased tissue, without harming the surrounding tissue area about the site of selective cavitation.
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DETAILED DESCRIPTION OF THE INVENTION

The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

The present invention is based, at least in part, on the discovery that by directing two different wavelengths of energy (beams), simultaneously, at a focal point or region within tissue, an overlap region forms having an energy difference which causes selective cavitation in tissue at the site of intersection of the two beams. This localized treatment does not effect tissue surrounding the area where the two beams do not overlap and is, therefore, an effective means to treat tissue, e.g., diseased tissue, without harming the surrounding tissue area about the site of selective cavitation.

Two energy waves of frequencies w1 and w2 propagating through a medium will generate at the overlap region (a region where the two waves are present at the same time) a wave of frequency (Dw=w1-w2). This is true for any type of wave (e.g., light or sound). The difference frequency is also known as beat frequency. Many of the interactions of the waves depend on the frequency of the wave. Therefore, the overlap region where the beat frequency is generated, can produce interactions that are unique. This is the basis of selectivity and targeting of tissue of the present invention. The two types of waves that are of interest are laser radiation and ultrasound. Because the way that light and sound affect tissue are fundamentally different, the two methods are presented separately where appropriate. In this invention the beat frequency produced by laser radiation causes elevation of temperature at the overlap region because of increased absorption of the beat frequency by the tissue (hyperthermia). On the other hand, the beat frequency ultrasound causes cavitation which destroy tissue by mechanical lysis and/or the generation of free radicals and/or hyperthermia.

For example, the conversion of light in situ at the treatment site to another wavelength

that is strongly absorbed should be considered. The increase in absorption can be dramatic. Water has an absorption that varies from .about.10.sup.-4 (visible light) to .about.10.sup.-4 cm-1 (.about.3 mm), eight orders of magnitude increase in absorbance (4). One advantage of in situ generation is the ability to target specific chromophores (5). There is another advantage to using two or more beams of photons for treatment. By separation of the energy into two different delivery paths, the treatment site (where the beams intersect) will receive the necessary dose while sparing adjacent tissue.

The conversion of light from one wavelength to another is a phenomenon that is familiar to users of commercially available lasers (e.g., output of 532 nm by second harmonic generation with the 1064 nm from a ND:YAG laser). Another example is the generation of intense and tunable coherent vacuum ultraviolet light (70 run to 200 nm) for molecular spectroscopy (6). The number of photons per pulse exceeds 10-.sup.10 -10-.sup.11 when pulsed dye lasers are used to generate the vacuum ultraviolet light. Light with a different wavelength can be produced by sum and/or difference mixing between as many as three different fundamental frequencies via a third-order nonlinear process (7):

Process

where .omega..sub.i and k.sub.i (for i=1, 2, and 3) are the frequencies and wavevectors of the input; .sup..omega. 4 and k.sub.4 is the frequency and wavevector of the output; and the wave-vector mismatch is .DELTA.k.ident.k.sub.4 -k'. As an example, 3000 nm light can be generated by mixing 1200 nm and 750 nm (via the j=2 process).
...
Consider the generation of 3000 nm light with a single pulse of 20 mJ (25 ns) 1200 nm and a single pulse 10 mJ (25 ns) 750 run in water. Using the following constants: third-order nonlinear susceptibility for water is 1.44.times.10.sup.-36 esu (8) .DELTA.k=0; b/L=0.1; f/L=0.5; the dimensionless function F.sub.2 is estimated to be 0.01 (7); density of water at 20.degree. C. is 0.99821 g/cm.sup.3 (9); molar mass of water at 20.degree. C. is 18.01528 g/mol (9); and N.sub.A =6.0221367(37).times.10.sup.23 /mol (9), the total power generated is calculated from Eq. 1 to be 6.times.10.sup.3 W or .about. 162 .mu.J for 25 ns (.about.0.5 % conversion). This calculation is for the case of exact phase matching (i.e., .DELTA.k=0). A more detailed calculation would involve positive contributions to P.sub.4 from generation of photons with .DELTA.k.noteq.0. The increase in tissue temperature can be estimated to be 71.degree. C. from (10) .DELTA.T=Q.DELTA.t/VpC where .alpha.=0, V=628 .mu.m.sup.3, p=1.09 g/cm.sup.3, C=0.8 cal/g.degree. C., and Q.DELTA.t=162 .mu.J.

To determine cell viability as a function of laser parameters, a beam of 1200 nm and a beam of 750 nm light will be focused onto a monolayer of cells. Cell viability will be quantified by the Fluorescein Diacetate/Ethidium Bromide assay. The energy of the two beams will be varied independently. Two other parameters that will be examined is the angle between the beams and focal length.

To determine minimum laser energy needed for photocoagulation, a beam of 1200 nm and a beam of 750 nm light will be focused into skin ex vivo. The energy of the two beams will be varied independently. Biopsies will be obtained for histology. The extend of thermal damage in the tissue will be investigated. This will be repeated for increasing depth of penetration into tissue.

The present invention describes a novel approach to target energy delivery. Light which is delivered via fiber optics can be converted in situ into another wavelength. The ability to generate light with a specific wavelength that is both spatially and temporally localized allows selective targeting of endogenous or exogenous chromophores. Medical instruments that currently incorporate fibers for light delivery may find new uses. The concept of generating light with a different wavelength in situ in a localized region has important implications not only for treatment but also for diagnostic applications.

The two modalities of therapeutic ultrasound or laser treatment are based on hyperthermia, the rise of the local temperature of tissue, and/or cavitation, the formation and collapse of bubbles (11). Bubbles are initiated during the tensile (negative pressure) cycle of energetic waves, such as ultrasound. Once they are formed, they are forced to oscillate in phase with the applied energetic, e.g., ultrasound, pressure, rapidly increasing in size. As the bubble size increases the bubble becomes unstable and implodes. Cavitation is art recognized and is a violent and destructive phenomenon. During the collapse of the bubble, temperatures as high as 5000.degree. C. and pressures of hundreds of atmospheres can be generated. The energy released during cavitation is sufficient to produce light (sonoluminescence), hydrolysis, free radicals, and chemical reactions (12). Cavitation has been shown to cause injury to cells and tissue either by mechanical lysis or by the generation of free radicals, such as H0.sub.2., OH--, HO., and H.sub.2 0., which damage the cell membrane (13). These radical species are identical to those produced in water by ionizing radiation, e.g., Cobalt-60, and are strong oxidizing agents (14).

Cavitation, among the non-thermal effects of energetic beams, e.g., ultrasound, has the greatest potential for therapeutic applications, when controlled. The present invention exploits selective targeting of tissue based on cavitation. Two different energy frequencies are crossed within tissue with the overlap region generating a difference frequency (beat frequency) where the two beams cross. Cavitation, e.g., ultrasonic waves or hyperthermia, e.g., ultrasonic waves and light waves, e.g., laser, occur at the point of intersection of the two beams. The method takes advantage of the fact that the cavitation threshold and/or hyperthermia depends on the frequency of the energetic beam, e.g., laser or ultrasound.

For example, low frequency ultrasound is more effective in producing cavitation than high frequency ultrasound (15). In the present invention, the frequencies of the two ultrasound beams are set above the cavitation threshold. The two beams are made to

overlap spatially and temporally inside the target to produce cavitation. For example, if the frequency of two ultrasound beams are 4.0 and 3.8 MHZ, respectively, 200-kHz ultrasound is generated at the overlap region which is more effective in producing cavitation than either the 4.0-MHZ or the 3.8-MHZ ultrasound. Thus, only at the overlap region of the two ultrasound beams and nowhere else in the tissue, will cavitation be produced. The cavitation will cause destruction of tissue either by mechanical lysis or generation of free radicals. The radical species that are generated have short lifetimes and they remain localized in the target area. Therefore, surrounding areas of the tissue will not be affected. Typical pulse durations are between about 1 millisecond and continuous for ultrasonic treatment and between about 2 nanoseconds and 5 femtoseconds for laser treatment.

Acoustic contrast agents essentially contain stable air bubbles. They enhance the echo in diagnostic applications of ultrasound (16). Bubble-based contrast agents have been shown to enhance cavitation during ultrasound exposure (17). In addition, these contrast agents enhance the production of hydrogen peroxide. Therefore, acoustic contrast agents can be used to increase the efficiency of cavitation and provide an additional degree of selectivity. It is of interest to note that high frequency ultrasound (3.8 MHZ) did not produce any cavitation even in the presence of contrast agents (17). Suitable contrast agent include, for example, Albunex.RTM. (Molecular Biosystems, San Diego and Levovist.RTM.) (Schering AG, Berlin, Germany). Albunex.RTM. is a coated microbubble produced by sonication of an albumin solution. Levovist.RTM. is made of dry particles of galactose which form a microbubble suspension when water is added.

Ultrasound-induced cavitation is responsible for the permeabilization of the cell plasma membrane (18). The membrane permeabilization is transient and the plasma membrane recovers. The permeabilization of the plasma membrane allows large molecules to diffuse into the cytoplasm. Mammalian cells have been successfully transfected with b-galactosidase in vitro (19). In addition, there is evidence that the combination of acoustic contrast agents and ultrasound facilitated transfection in an animal model in vivo (19). Therefore, localized generation of low frequency ultrasound could also be applied to selective sites of an organ or tissue for drug delivery or as a vector for gene therapy.

Although the variety of the tissues to be treated necessitates adaptation of the energy beam parameters, they can be defined quantitatively as follows: Carrier frequency f of the energy (sonic) pulse signal: 3 MHz<f<5 MHZ Duration T of the sonic pulse signal: 1 ms<T<continuous Intensity of sound 20 W/cm.sup.2 to 200 W/cm.sup.2 Recurrence rate r (pulse succession frequency): 100 Hz to continuous

For treating a particular type of tissue, in a predetermined part of the body, the selection of the parameters should be made in the view of clinical experience. For example, the object is to acoustically generate cavitation bubbles in the proliferating cell area to cause mechanically induced significant alterations in the tissue environs and/or alterations in the tissue cells themselves. Such alterations are indicated by a

fall in, an obstruction of, or even an interruption of, the blood supply to a cell, as well as by acoustically induced interference with the existing mode of nutrition of the cell, and/or by destruction of the cell elements (rips in the cell membranes, destruction of elements of the cell contents), but not necessarily by destruction of the structural cohesion of the cell aggregate.

In principle, the carrier frequency, the duration of the energy pulse signal and the amplitude and duration of the negative pressure phase, are the essential quantities which determine the cavitation threshold in a preselected tissue. The higher the carrier frequency, the shorter are the sonic pulse and negative pressure phase durations and the lower is the amplitude of the negative pressure phase, the more unlikely will be the onset of cavitation.

In one aspect the present invention pertains to methods for treating tissue using two different wavelengths simultaneously. The methods include directing a first wavelength of energy into a tissue and directing a second wavelength of a second energy into the tissue, such that the first and second wavelengths are focused onto a focal point or region of the tissue. The first and second wavelengths intersect to produce an overlap region which has an energy difference causing cavitation and/or hyperthermia to occur at the focal point or region within the tissue, thereby treating the tissue. This localized treatment does not effect tissue surrounding the area where the two beams do not overlap and is, therefore, an effective means to treat tissue, e.g., diseased tissue, without harming the surrounding tissue area about the site of selective cavitation.

The terms "treat", "treatment" or "treating" is intended to cover both prophylactic and/or therapeutic applications. The methods of the invention can be used to protect a subject from damage or injury caused by disease, or can therapeutically treat the subject after to the onset of a disease.

The term "subject" is intended to include mammals susceptible to diseases, including one or more disease related symptoms. Examples of such subjects include humans, dogs, cats, pigs, cows, horses, rats, and mice.

The term "disease" is associated with an increase of a pathogen within a subject such that the subject often experiences physiological symptoms which include, but are not limited to, release of toxins, gastritis, inflammation, coma, water retention, weight gain or weight loss and immunodeficiency. The effects often associated with such symptoms include, but are not limited to, fever, nausea, diarrhea, weakness, headache, and even death. Examples of diseases which can be treated by the present invention include undesirable cell proliferation, bacterial infection and cancer.

The language "undesirable cell proliferation" is intended to include abnormal growth of cells which can be detrimental to a mammal's physiological well being. Effects of undesirable cell proliferation can include the release of toxins into the mamnmal,

fever, gastritis, inflammation, nausea, weakness, coma, headache, water retention, weight gain or weight loss, immunodeficiency, death, etc. The undesired cells which proliferate can include cells which are either benign or malignant. Examples of undesirable cell proliferation include bacterial cell proliferation and aberrant cell division and/or proliferation of foreign cells, such as in cancer cells.

The language "aberrant cell division and/or proliferation" is art recognized and is intended to cover those instances where cells are generated in excess of what is considered typical in physiologically similar environment, such as in cancers.

The term "cancer" is art recognized and is intended to include undesirable cell proliferation and/or aberrant cell growth, e.g., proliferation.

The term "tissue" is art recognized and is intended to include extracorporeal materials, such as organs, e.g., mesentery, liver, kidney, heart, lung, brain, tendon, muscle, etc. and corporeal materials, such as blood cells, e.g., red and white blood cells, and extracellular matrix components.

The term "emitter" is intended to include those devices which produce and transmit an energy beam, e.g., one or more wavelengths, in the form of sound waves, e.g., ultrasonic (ultrasound), or light waves, e.g., coherent light (laser). Suitable means to produce ultrasonic waves include piezoelectric transducers driven by an independent frequency generator, such as quartz or ceramic types. Suitable means for producing coherent light is a laser. Examples of suitable lasers include ND:YAG, optical parameter oscillator, continuously tunable oscillator, mode-locked or titanium sapphire.

The phrase "onto a focal point or region" is intended to mean that the focused wavelengths, e.g., energy beams, are concentrated at a position within the tissue where the two beams intersect. This point of intersection can be controlled such that an area from between about 500 microns to about 10 mm (ultrasonic) or 100 microns to 2 mm (light, e.g., laser) can be treated at the point of intersection. To accomplish this, multiple pairs of energy beams which produce overlap regions can be focused at the locus or, one set of mismatched energy beams can be positioned about the tissue to be treated or, alternatively, one or more pairs of emitters can be moved translationally or rotationally in the X, Y, Z planes. It is to be understood that the energy beams can be attenuated such that the depth of penetration into the tissue can be controlled such that such penetration is superficial or penetrates into the tissue to a predetermined depth.

The phrase "overlap region" refers to the area represented by the intersection of two energy beams.

The phrase "energy difference" is intended to mean the difference in energy between two wavelengths. In one embodiment, two ultrasonic beams can be made to overlap

where a first beam is between about 2 MHZ and about 5 MHZ, preferably about 4.0 MHZ and a second beam is between about 2 MHZ and about 5 MHZ, preferably about 3.8 MHZ. In a preferred embodiment, the range for the two beams is between about 2 MHZ and about 3 MHZ. The difference in energy is between about 50 kHz and about 300 kHz, preferably about 200 kHz with a subharmonic frequency of between about one half of the beat frequency, preferably 100 kHz and is generated at the overlap region of the two beams. Alternatively, two laser beams can be made to overlap where a first beam is between about 300 nm and about 2100 nm, preferably 1200 nm and the second beam is between about 300 nm and about 2100 nm, preferably 750 nm. The difference is between about 70 nm and about 8000 nm.

The present invention also pertains to apparati for treating tissue using two different wavelengths, e.g., energy beams, e.g., acoustic waves or coherent light (laser) waves. The apparati include a first energy emitter which produces a first wavelength of energy and a second energy emitter which produces a second wavelength of a second energy. The first and second wavelengths are focused onto a focal point or region of the tissue, so that the first and second wavelengths intersect and produce an overlap region having an energy difference. The energy difference causes cavitation and/or hyperthermia at the focal point or region within the tissue. The two different wavelengths are generated by energy emitters. Suitable energy emitters are lasers, e.g., ND:YAG lasers, or piezoelectric transducers.

The present invention further pertains to apparati for treating tissue using two different wavelengths, energy beams, which include a first energy emitter, a second energy emitter and a control means. The first and second energy emitters produce a two wavelengths of different energies. The control means facilitates transmission and focusing of the energy beams onto a focal point or region of the tissue. The energy beams intersect and produce an overlap region which has an energy difference which produces cavitation and/or hyperthermia at the focal point or region within the tissue. The control means causes transmission of ultrasonic waves for a duration of between about 1 microseconds and continuous. The controls means can also provide transmission of ultrasonic waves by successive pulses.

...

In a preferred embodiment, the frequency of cavitation ultrasonic waves is lower than the frequency of thermal ultrasonic waves.

...
It can thus be seen that the therapy apparatus can be used with, or applied to, all types of therapy using lasers or ultrasound, of all benign of malignant tumors, whether such tumors be internal or external. Applications include the treatment of benign or malignant tumors of the liver, of the prostate, of the kidney, of the breast, of the skin, of the brain and for the treatment of varicose states and of the esophagus.
...

Methodology 1. The experimental arrangement consists of two piezoelectric transducers which are driven by two independent frequency generators. The frequencies of the two transducers are set to differ by 50-300 kHz. For example, one transducer will operate at 3.8 MHZ, while the second transducer will be set at 4.0 MHZ. Both ultrasound frequencies are too high to produce cavitation even in the presence of contrast agents (17). The transducers will be inserted into a container filled with water. The two ultrasound beams will be made to overlap at their focal points. The presence of cavitation will be detected by measuring the subharmonic frequency, one half of the ultrasound frequency inducing cavitation. The driving ultrasound frequency in the proposed experimental arrangement is 200 kHz and the subharmonic frequency will be 100 kHz. A hydrophone will be used to measure the subharmonic signal. The signal will be passed through a narrow band filter to remove all other frequencies present. A spectrum analyzer will be used to measure and analyze the signal. The strength of the subharmonic is related to the level of cavitation generated by the ultrasound. In fact, a strong correlation has been found between cell damage and the emitted subharmonic energy in vitro (20). The strength of the subharmonic signal will be measured as a function of the intensity of the two ultrasound beams, the difference frequency, and the concentration of acoustic contrast agents.

The formation of free radicals will be measured indirectly using the Fricke ferrous sulfate dosimeter (14). This assay has been the standard for chemically measuring ionizing radiation and has also been previously used to measure the free radicals generated by extracorporeal shock wave lithotripters (14). The two beams will be made to cross inside an aqueous solution of ferrous sulfate. The ferrous sulfate will be oxidized to ferric ion by the free radicals. The amount of ferric ion produced will be determined by its absorbance at 224 nm in a spectrophotometer. The Fricke dosimeter will be calibrated against an ionizing radiation source (14). The amount of ferric ion will be determined as a function of the intensity of the two ultrasound beams, the difference frequency, and the concentration of acoustic contrast agents. The amount of ferric ion will also be compared to the strength of the subharmonic frequency. 2. The damage threshold will be determined in an organ (liver) ex vivo. The two transducers will be acoustically coupled to the organ with acoustical gel. The two ultrasound beams will be made to overlap inside the organ. The hydrophone will be inserted into the organ to measure the intensity of the subharmonic frequency. The intensity of the subharmonic measures the generation of cavitation. The threshold intensity will also be measured by acoustic contrast agents that will be injected into the organ prior to the experiments. In addition, biopsies from the overlap regions as well as regions exposed to one beam only will be taken for histological examination. These experiments will determine the cavitation threshold in an organ and the nature and the degree of damage. 3. The two ultrasound beams will be made to overlap inside a tube through which cells can be flown. In this arrangement, the time of cell exposure can be controlled by adjusting the flow rate. Red blood cells (RBC) will be used in these experiments. RBC have been extensively used in studies of molecular loading with ultrasound (8), extracorporeal lithotripters (21), and laser-generated stress waves (22). Cavitation will permeabilize the plasma membrane of RBC and allow hemoglobin to

diffuse out of the cell. The amount of hemoglobin released will be measured by measuring the absorbance of the collected medium at 420 nm (absorption band of hemoglobin). In addition, the number of cells will be measured before and after the experiment to determine the degree of cell lysis. The hemoglobin efflux will be determined as a function of the ultrasound intensity, time of exposure, and concentration of contrast agents. Control experiments will also be performed in which RBC will be exposed only to one ultrasound beam under otherwise identical conditions.

The two ultrasound beams will be made to overlap in a tube through which cells are flown. The flow rate will determine how long the cells are exposed to the difference frequency. RBC and lymphocytes will be used in these experiments. These cells have been selected because we have experience with preparation and handling. In addition, we have a good understanding of how they respond to mechanical forces. RBC exposed to ultrasound will permeabilize and allow hemoglobin to diffuse out of the cell. The amount of hemoglobin released will be measured by measuring the absorbance of the medium. The hemoglobin efflux will be determined as a function of the ultrasound intensity and flow rate. In addition to the efflux the influx of molecules present in the extracellular medium can be determined. Both RBC and lymphocytes will be used in the experiments. The cells will be flown in the tube and exposed to the difference frequency. Probe molecules (labeled dextran of 4, 12 and 70 kDa) will be added to the extracellular medium. The uptake of the molecular probes will be determined by flow cytometry. The parameters that will be studied are ultrasound intensity, flow rate, and concentration of the molecular probes. These measurements will be compared to identical measurements where the cells are exposed only to a single ultrasound beam. The effect of the contrast agents in loading will also be investigated by adding the contrast agents into the extracellular medium and measure the amount of molecular probes that are loaded into the cells.

The influx of molecules present in the extracellular medium can also be measured to determine the loading efficiency of the beat frequency. RBC will be flown inside the tube and exposed to the difference frequency. Probe molecules (fluorescence-labeled dextran 4.sub.1, 12 or 70 kDa molecular weight) will be added to the extracellular medium. The uptake of the molecular probes will be determined by flow cytometry. The parameters that will be studied are ultrasound intensity, time of exposure, concentration, and size of the molecular probes. These measurements will be compared to identical measurements in which the cells will be exposed only to a single ultrasound beam. The effect of contrast agents in enhancing molecular loading will also be investigated by adding contrast agents into the extracellular medium and measuring the number of molecules that are loaded into the cells. The effect of the cavitation on cell viability will be investigated with a tritiated thymidine assay. The incorporation of thymidine will be measured as a function of intensity, flow rate, and concentration of the contrast agent.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in

the scope of the following claims.

* * * * *

United States Patent 6,107,466
Hasan , et al. August 22, 2000
Acceleration of wound healing by photodynamic therapy

Abstract

Disclosed is a method for accelerating wound healing in a mammal. The method includes identifying an unhealed wound site or partially-healed wound site in a mammal; administering a photosensitizer to the mammal; waiting for a time period wherein the photosensitizer reaches an effective tissue concentration at the wound site; and photoactivating the photosensitizer at the wound site. The dose of photodynamic therapy is selected to stimulate the production of one or more growth factor by cells at the wound site, without causing tissue destruction.
Inventors: Hasan; Tayyaba (Arlington, MA); Hamblin; Michael R. (Revere, MA); Trauner; Kenneth (Sacramento, CA)
Assignee: The General Hospital Corporation (Boston, MA)
Appl. No.: 160528
Filed: September 24, 1998

Current U.S. Class: 530/351 ; 530/399; 604/20; 607/88; 607/89
Field of Search: 530/351,399 604/20 607/88,89
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Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of copending application Ser. No. 08/741,816, filed Oct. 31, 1996, which claims benefit from provisional application Ser. No. 60/026,315, filed Sep. 19, 1996, now abandoned.

Claims

We claim:

1. A method for accelerating the healing of a wound in a mammal, comprising:

(a) identifying an unhealed wound site or partially-healed wound site in a mammal;

(b) administering an effective amount of a photosensitizer to the mammal;

(c) waiting for a time period wherein the photosensitizer reaches an effective tissue concentration at the wound site;

(d) photoactivating the photosensitizer at the wound site by delivering specifically to the wound site light of an effective wavelength and intensity, for an effective duration, thereby producing an effective dose of photodynamic therapy;

the dose of photodynamic therapy being sufficient to stimulate production of a growth factor by a cell at the wound site, without causing tissue destruction;

thereby accelerating healing of the wound in the mammal.

2. The method of claim 1, wherein the growth factor is selected from the group consisting of PDGF, TGF-.beta., .alpha.-FGF, .beta.-FGF, TGF-.alpha., EGF, IGF, VEGF, KGF, and HGF.

3. The method of claim 1, wherein the cell at the wound site is selected from the group consisting of a fibroblast cell, a myofibroblast cell, a macrophage cell, an endothelial cell, and an epithelial cell.

4. The method of claim 1, wherein the photosensitizer is a macromolecular conjugate.

5. The method of claim 1, wherein the photosensitizer is selected from the group consisting of: a porphyrin, a chlorin, a bacteriochlorin, a purpurin, a phthalocyanine, a naphthalocyanine, a texaphyrin, and a non-tetrapyrrole photosensitizer, and conjugates thereof.

6. The method of claim 1, wherein the photosensitizer localizes to a particular cell type or to an organelle of a cell at the wound site.

7. The method of claim 1, wherein the photosensitizer is targeted to a particular cell

type or to an organelle at the wound site by conjugation to a targeting moiety.

8. The method of claim 5, wherein the targeting moiety is selected from the group consisting of a polypeptide and a microparticle.

9. The method of claim 1, wherein the administration of the photosensitizer is systemic.

10. The method of claim 9, wherein the administration is between about 0.1 mg/kg and about 50 mg/kg.

11. The method of claim 10, wherein the administration is between about 0.5 mg/kg and about 10 mg/kg.

12. The method of claim 9, wherein the administration is parenteral.

13. The method of claim 1, wherein the administration of the photosensitizer is local.

14. The method of claim 13, wherein the administration is topical.

15. The method of claim 1, wherein the photoactivating step comprises delivering light by means of optical fibers.

16. The method of claim 1, wherein the photoactivating step comprises delivering light by means of transillumination.

17. The method of claim 1, wherein the photoactivating step comprises delivering laser light.

Description

FIELD OF THE INVENTION

This invention relates to cell biology, medicine, wound healing and photodynamic therapy.

BACKGROUND OF THE INVENTION

Delayed or impaired wound healing can result from various conditions. These include diabetes (Rosenberg (1990) Nurs. Clin. North Am. 25:247-261), peripheral vascular disease (Kamler et al. (1992) Adv. Exp. Med. Biol. 316:419-424), advanced age (Reed (1998) Geriatrics 53:88-94), malnutrition (Young (1988) Heart Lung 17:60-67), immune suppression and corticosteroid use (Uthoff et al. (1995) Ann. Thorac. Surg. 59:277-282), psychological stress (Kiecolt-Glaser et al. (1995) Lancet 346:1194-1196), and cancer radiotherapy and chemotherapy (Mason et al. (1992) Clin. Oncol. 4:32-35). There is presently no standard therapy for delayed or impaired

wound healing other than supportive care.

The initiation and control of concerted processes responsible for wound healing are governed by polypeptide molecules known as growth factors (Bennett et al. (1993) Review, Am. J. Surg. 165:728-737). These polypeptides can be categorized by sequence homology or by function.

Based on sequence homology, growth factor family groups include epidermal growth factor family (EGF) (Brown et al. (1988) Ann. Surg. 208:788-794), platelet derived growth factor family (PDGF) (Hill et al. (1991) Comp. Biochem. Physiol A. 100:365-370), insulin-like growth factor family (IGF) (Suh et al. (1992) Endocrinology 131:2399-2403), transforming growth factor-beta family (TGF-3) (Cox (1995) Cell Biol. Int. 19:357-371), fibroblast growth factor family (FGF) (Kusstatscher et al. (1995) J.

Pharmacol. Exp. Ther. 275:456-461), vascular endothelial growth factor family (VEGF) (Frank et al. (1995) J. Biol. Chem. 270:12607-12613).

Growth factors can be grouped functionally, according to the role they play in initiating and controlling the various phases of wound healing. A first group consists of chemotactic growth factors, i.e., those that attract inflammatory cells such as monocyte/ macrophage and fibroblasts to the cell site. A second group consists of growth factors that act as mitogens to stimulate cellular proliferation. A third group consists of that growth factors that stimulate angiogenesis. A fourth group consists of growth factors that affect the production and degradation of the extracellular matrix. A fifth group of growth factors consists of those that influence the synthesis of cytokines and growth factors of neighboring cells.

Growth factors constitute a subclass of cytokines. Growth factors are distinguished from other cytokine subclasses by their ability to act as mitogens, chemoattractants and proliferation inducers on cells of epithelial, endothelial, and mesenchymal origins. In particular, the pleiotropic growth factor TGF-.beta. is important in orchestrating the wound healing response (Beck et al. (1993) J. Clin. Invest. 92:2841-2849).

Exogenously applied growth factors (particularly BFGF, PDGF, EGF, and TGF-B) have been used to stimulate wound healing (Puolakkainen et al. (1995) J. Surg. Res. 58:321-329; Greenhalgh et al. (1996) J. Trauma 41:159-167). A difficulty in using this approach has been in formulating the growth factors in such a way as to ensure their sustained slow release in a biologically active form (Davies et al. (1997) J. Biomater. Appl. 12:31-56; Nicoll et al. (1997) Biomaterials 18:853-859; Arm et al. (1996) Biomaterials 17:703-709; and Fukunaga et al. (1994) J. Pharm. Pharmacol. 46:168-171).

SUMMARY OF THE INVENTION

It has been discovered that low doses of photodynamic therapy (PDT) upregulate the

expression and/or secretion of growth factors involved in wound healing. One effect is an increase in wound breaking strength in vivo.

Based on this discovery, the invention features a method for accelerating the healing of a wound in a mammal. The method includes: (a) identifying an unhealed wound site or partially-healed wound site in a mammal; (b) administering a therapeutically effective amount of a photosensitizer to the mammal; (c) waiting for a time period wherein the photosensitizer reaches an effective tissue concentration at the wound site; (d) photoactivating the photosensitizer at the wound site by delivering specifically to the wound site light of an effective wavelength and intensity, for an effective duration, thereby delivering a therapeutically effective dose of PDT. The therapeutically effective dose of PDT accelerates wound healing by stimulating the biosynthesis and/or secretion of one or more growth factors by cells at the wound site, without causing tissue destruction.

The cells producing the growth factor in response to the PDT can be fibroblast cells, myofibroblast cells, macrophage cells, endothelial cells, epithelial cells, or other cell types at the wound site. Examples of growth factors whose production may be stimulated by the PDT include PDGF, TGF-.beta., .alpha.-FGF, .beta.-FGF, TGF-.alpha., EGF, IGF, VEGF, KGF, and HGF.

Various types of molecules can be used as the photosensitizer, e.g., porphyrins, chlorins, bacteriochlorins, purpurins, phthalocyanines, naphthalocyanines, texaphyrins, and non-tetrapyrroles. Specific examples of photosensitizers are photofrin, benzoporphyrin derivative, tin etiopurpurin, sulphonated chloroaluminium phthalocyanine and methylene blue. The photosensitizer can be conjugated to another molecule or can be unconjugated. The photosensitizer can be in the form of a macromolecular conjugate. A preferred macromolecular conjugate is polylysine chlorin-e6 conjugate.

In some embodiments of the invention, the photosensitizer localizes to a particular cell type or to an organelle in a cell at the wound site. The photosensitizer can be targeted to a particular cell type or to an organelle. Targeting can be achieved by conjugation of the photosensitizer to a targeting moiety, e.g., a polypeptide or microparticle.

Administration of the photosensitizer can be local or systemic. Systemic administration can be oral or parenteral. For systemic administration, dosage can be between about 0.1 mg/kg and about 50 mg/kg. Preferably, the dosage is between about 0.5 mg/kg and about 10 mg/kg. Local administration can be topical or by injection, at or near the wound site.

Photoactivating light can be delivered to the wound site from a conventional light source or from a laser. Delivery can be direct, by transillumination, or by optical fiber.

As used herein, "photoactivation" means a light-induced chemical reaction of a

photosensitizer which produces a biological effect.

As used herein, "photosensitizer" means a chemical compound that produces a biological effect upon photoactivation or a biological precursor of a compound that produces a biological effect upon photoactivation.

As used herein, "without tissue destruction" means without formation of non-viable tissue as a result of necrosis or apoptosis leading to eschar formation and/or sloughing.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present document, including definitions, will control. Unless otherwise indicated, materials, methods, and examples described herein are illustrative only and not intended to be limiting.

Various features and advantages of the invention will be apparent from the following detailed description and from the claims.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a diagram illustrating steps in the preparation of a cationic and anionic polylysine chlorin-e6 conjugate.

DETAILED DESCRIPTION

Wounds

The present invention involves identifying an unhealed wound or a partially-healed wound in a mammal, e.g., a human. The wound can be a non-penetrating wound, or a penetrating wound. Examples of wounds that can be treated by the method of this invention include abrasions, lacerations, surgical incisions, and burns.

The invention is particularly useful where normal healing processes are impaired, retarded or suppressed. This can occur due to conditions such as diabetes, peripheral vascular disease, immune suppression, corticosteroid use, cancer radiotherapy, and cancer chemotherapy.

Photosensitizer

The photosensitizer is a chemical compound that produces a biological effect upon

photoactivation, or a biological precursor of a compound that produces a biological effect upon photoactivation. The photosensitizer must have a sufficiently low toxicity to permit administration to the patient with a medically acceptable level of safety. Preferably, the photosensitizer is essentially nontoxic upon photoactivation.

Various photosensitizers are known and can be used in the practice of this invention. Preferably, the photosensitzer used is not a photosensitizer that inactivates growth factor activity, e.g., CASPc (a phthalocyanine). Photosensitizers typically have chemical structures that include multiple conjugated rings that allow for light absorption and photoactivation. They differ in the properties of light absorption and fluorescence, biodistribution, temporal uptake, clearance, and mechanisms of photoactivatable cytotoxicity. Classes of photosensitizer include hematoporphyrins (Batlle (1993) J. Photochem. Photobiol. Biol. 20:5-22; Kessel (1988) Cancer Let. 39:193-198), uroporphyrins, phthalocyanines (Kreimer-Birnbaum, (1989) Seminars in Hematology 26:157-173), purpurins (Morgan et al. (1990) Photochem. Photobiol. 51:589-592; Kessel, (1989) Photochem. Photobiol. 50:169-174), acridine dyes, bacteriochlorophylls (Beems et al. (1987) Photochem. Photobiol. 46:639-643; Kessel et al. (1989) Photochem. Photobiol. 49:157-160), and bacteriochlorins (Gurinovich et al. (1992) J. Photochem. Photobiol. Biol. 13:51-57). Specific examples of suitable photosensitizer are listed below.

Photosensitizer

1. Photofrin.RTM.

2. Synthetic diporphyrins and dichlorins

3. Hydroporphyrins, e.g., chlorins and bacteriochlorins of the tetra(hydroxyphenyl) porphyrin series

4. phthalocyanines

5. O-substituted tetraphenyl porphyrins (picket fence porphyrins)

6. 3,1-meso tetrakis (o-propionamido phenyl) porphyrin

7. Verdins

8. Purpurins, e.g., tin and zinc derivatives of octaethylpurpurin (NT2), and etiopurpurin (ET2)

9. Chlorins, e.g., chlorin-e6, and mono-l-aspartyl derivative of chlorin-e6

10. Benzoporphyrin derivatives (BPD), e.g., benzoporphyrin monoacid derivatives, tetracyanoethylene adducts of benzoporphyrin, dimethyl acetylenedicarboxylate

adducts of benzoporphyrin, Diels-Adler adducts, and monoacid ring "a" derivative of benzoporphyrin

11. Low density lipoprotein mediated localization parameters similar to those observed with hematoporphyrin derivative (HPD)

12. sulfonated aluminum phthalocyanine (Pc) sulfonated AlPc disulfonated (AlPcS.sub.2) tetrasulfonated derivative sulfonated aluminum naphthalocyanines chloroaluminum sulfonated phthalocyanine (CASP)

13. zinc naphthalocyanines

14. anthracenediones

15. anthrapyrazoles

16. aminoanthraquinone

17. phenoxazine dyes

18. phenothiazine derivatives

19. chalcogenapyrylium dyes cationic selena and tellurapyrylium derivatives

20. ring-substituted cationic PC

21. pheophorbide .alpha.

22. hematoporphyrin (HP)

23. protoporphyrin

24. 5-amino levulinic acid

The photosensitizer can be any of various types of compounds, including porphyrins, chlorins, bacteriochlorins, purpurins, phthalocyanines, naphthalocyanines, texaphyrines, and non-tetrapyrrole photosensitizer. Specific examples of photosensitizers are Photofrin, benzoporphyrin derivative, tin etiopurpurin, sulfonated chloroaluminum phthalocyanine, methylene blue, and chlorin-e6.

Photosensitizer Conjugation, Formulation, and Administration

The photosensitizer can be modified to form a macromolecular conjugate. An exemplary macromolecular conjugate is poly-l-lysine chlorin-e6 conjugate. A macromolecular conjugate can be used to facilitate or promote localization to an

intracellular organelle of a cell.

A single photosensitizer compound can be used alone in the practice of this invention. Alternatively, two or more photosensitizer can be used in combination, provided that light of an effective wavelength for each photosensitizer in the combination is used in the photoactivation step.

An alternative to administration of the photosensitizer compound itself, is administration of a photosensitizer precursor molecule. This approach is illustrated by the use of 5-aminolevulinic acid, which causes endogenous production of the photosensitizer protoporphyrin IX (Morgan et al. (1989) J. Med. Chem. 32:904-908.

The photosensitizer can be chosen, or chemically modified, to optimize its usefulness in specific treatment situations. For example, the photosensitizer can be chemically modified to reduce its interaction with articular cartilage. This could be done by eliminating a positive charge to reduce association with negatively charged proteoglycans of articular cartilage.

For targeting to a particular organ, tissue, cell type, or organelle the photosensitizer can be chemically conjugated to a targeting moiety. In some embodiments, the photosensitizer is chemically conjugated to a targeting moiety that binds to a cell surface receptor, e.g., a macrophage receptor or an endothelium surface receptor.

Macrophages can be targeted through their phagocytic activity. Such targeting involves conjugating the photosensitizer to a microparticle. A suitable microparticle is a 1 .mu.m polystyrene microsphere (Polysciences, Inc.). Photosensitizer-microparticle conjugates are taken up selectively by macrophages, through their characteristic phagocytic activity. Conjugation of a photosensitizer to a microparticle can be by methods known in the art. See, e.g., Bachor et al. (1991) Proc. Natl. Acad. Sci. USA 88:1580-1584.

The photosensitizer can be formulated to optimize its usefulness for particular applications. For example, it can be formulated in a salve or gel for topical application. It can be formulated for parenteral administration or oral administration. Appropriate formulation can be carried out by one of ordinary skill in the art, without undue experimentation.

Administration of the photosensitizer can be local or systemic. The administration can be by any suitable route, including topical, intravenous, intraarticular, subcutaneous, intramuscular, intraventricular, intracapsular, intraspinal, intraperitoneal, topical, intranasal, oral, buccal, rectal or vaginal. The preferred route of administration will depend on the size and nature of the wound, the location of the wound, and the photosensitizer used.

Parenteral formulations may be in the form of liquid solutions or suspensions; for oral

administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods for making formulations are known in the art. Guidance concerning such methods can be found in standard references such as "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain as excipients sterile water or saline, Cremophor EL, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes, biocompatible, biodegradable lactide polymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the present factors. Other potentially useful parenteral delivery systems for the factors include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, liposomes, and antibody conjugates. Formulations for inhalation may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

Where the size, nature, and location of the wound renders local administration of the photosensitizer feasible, local administration is preferred over systemic administration. Advantages of local administration include reducing number of side effects, reducing the likelihood of a particular side effect, or both. In addition, local administration generally yields an effective concentration of photosensitizer at the wound site more rapidly, and permits greater control over photosensitizer concentration at the wound site.

Photoactivation

Following administration of the photosensitizer, it is necessary to wait

for the photosensitizer to reach an effective tissue concentration at the wound site, before photoactivation. Duration of the waiting step varies, depending on factors such as route of photosensitizer administration, wound location, and speed of photosensitizer movement in the body. Determining a useful range of waiting step duration is within ordinary skill in the art.

Following the waiting step, the photosensitizer is activated by photoactivating light applied to the wound site. This is accomplished by applying light of a suitable wavelength and intensity, for an effective length of time, specifically to the wound site. The suitable wavelength, or range of wavelengths, will depend on the particular photosensitizer(s) used. Wavelength specificity for photoactivation depends on the molecular structure of the photosensitizer. Photoactivation occurs with sub-ablative light doses. Determination of suitable wavelength, light intensity, and duration of

illumination is within ordinary skill in the art.

The light for photoactivation can be produced and delivered to the wound site by any suitable means. For superficial wounds or open surgical wounds, suitable light sources include broadband conventional light sources, broad arrays of LEDs, and defocussed laser beams.

For non-superficial wound sites, including those in intracavitary settings, the photoactivating light can be delivered by optical fiber devices. For example, the light can be delivered by optical fibers threaded through small gauge hypodermic needles. Optical fibers also can be passed through arthroscopes. In addition, light can be transmitted by percutaneous instrumentation using optical fibers or cannulated waveguides.

Photoactivation at non-superficial wound sites also can be by transillumination. Some photosensitizers can be activated by near infrared light, which penetrates more deeply into biological tissue than other wavelengths. Thus, near infrared light is advantageous for transillumination. Transillumination can be performed using a variety of devices. The devices can utilize laser or non-laser sources, i.e. lightboxes or convergent light beams.

PDT dosage depends on various factors, including the amount of the photosensitizer administered, the wavelength of the photoactivating light, the intensity of the photoactivating light and the duration of illumination by the photoactivating light. Thus, the dose of PDT can be adjusted to a therapeutically effective dose by adjusting one or more of these factors. Such adjustment is within ordinary skill in the art.

The invention is further illustrated by the following experimental examples. The examples are provided for illustration only, and are not to be construed as limiting the scope or content of the invention in any way.

EXPERIMENTAL EXAMPLES

Poly-l-lysine chlorin-e6 Conjugates

A pair of poly-l-lysine chlorin-e6 (ce6) conjugates possessing opposite charges were made as follows. The ester of ce6 (porphyrin Products, Logan, Utah) was prepared by reacting 1.5 equivalents of dicyclohexylcarbodiimide and 1.5 equivalents of N-hydroxysuccinimide with 1 equivalent of ce6 in dry dimethyl sulfoxide (DMSO). Polylysine hydrobromide (50 mg) (Sigma, St. Louis, Mo.) (average M.Wt. 11000, DP=100) was dissolved in dry DMSO (50 ml) containing N-ethylmorpholine (1 ml), and subsequently added to dry DMSO (1 ml) containing ce6-N-hydroxysuccinimide ester (25 mg). The solution was kept in the dark at room temperature for 24 hours and the resultant ce6 conjugate in the solution had a cationic charge (pl-ce6). The preparation of the anionic ce6 conjugate (pl-ce6-succ) further involved treating the cationic ce6

solution with an excess of succinic anhydride (100 mg dissolved in 0.5 ml dry DMSO) (FIG. 1). The cationic ce6 conjugate-containing solution and the anionic ce6 conjugate-containing solution were dialyzed in DMSO-resistant dialysis membrane with a 3500 MW cutoff (Spectrum Medical Industries, Los Angeles, Calif.) for 24 hours against three changes of 10 mM phosphate buffer (pH7).

The degree of ce6 substitution on the polylysine chains was estimated by measuring the absorbance at 400 nm and calculating the amount of ce6 present using e400nm=150000. The amount of polylysine was assumed to be the original quantity weighed out. After exhaustive dialysis it was assumed that the remaining ce6 was covalently bound to the polylysine. On each polylysine chain it was estimated that there were 5 ce6 molecules.

Increased Production of TGF-.beta. in Keratinocytes

Balb/c murine keratinocytes (Balb/mk) were grown to 60% confluency in P100 dishes in medium containing 10% FBS. The cells were then incubated with different concentrations (O.mu.M, 0.5.mu.M and 2.mu.M) of pl-ce6-succ (d.p. 100, substitution ratio <5%) for 4h. Following incubation of cells with pl-ce6-succ, a fluence (1 Jcm.sup.-2, 5Jcm.sup.-2, and 20 Jcm.sup.-2) of red light from a light emitting diode array centered on 670 nm was delivered to the cells. After 24 hours, cells were lysed and equal amounts of cell protein (25 .mu.g per lane) from each sample was then loaded on a 15% SDS PAGE gel. Following protein separation by electrophoresis, the gel was electroblotted to a nylon membrane and subsequently probed with anti-TGF-.beta. antibody. The bound murine anti TGF-.beta. was then detected by a second antibody conjugated to alkaline peroxidase. Cells that were not treated with pl-ce6-succ were used as a control.

Western blot analysis revealed that cells incubated with 0.5 .mu.M pl-ce6-succ and subsequently exposed to a fluence of 5 Jcm.sup.-2 showed an increase in TGF-.beta. production. No further increase in TGF-.beta. production was observed in cells exposed to a fluence of 20 Jcm.sup.-2.

The largest increase in TGF-.beta. production was observed in cells incubated with 2 .mu.M pl-ce6-succ equivalent and exposed to a fluence of 5 Jcm.sup.-2. Substantial phytotoxicity to the cells was observed when cells were exposed to a fluence of 20 Jcm.sup.-2, accounting for the reduced TGF-.beta. levels observed.

Increased Production of VEGF in Keratinocytes

Balb/mk, OVCAR-5 and PAM 212 cells were grown to 60% confluency in P100 dishes in medium containing 10% FBS. The cells were then incubated with varying concentrations (0 .mu.M, 0.5.mu.M, 2 .mu.M) of pl-ce6-succ (d.p. 100, substitution ratio <5%) for 4h, and following incubation, a fluence (0 Jcm.sup.-2, 5 Jcm.sup.-2, 20 Jcm.sup.-2) of red light from a light emitting diode array centered on 670 nm was

delivered to the cells (see Table 1). After 24 hours, cells were lysed and equal amounts of cell protein (25 .mu.g per lane) from each sample were then loaded on a 15% SDS PAGE gel. Following protein separation by electrophoresis, the gel was electroblotted to a nylon membrane and subsequently probed with anti VEGF antibody. The bound anti-VEGF antibody was then detected by a second antibody conjugated to alkaline peroxidase.

PAM 212 cells showed a moderate increase in VEGF production in cells exposed to 0.5 .mu.M ce6 and 5 Jcm.sup.-2 ; 0.5 .mu.M ce6 and 20 Jcm.sup.-2 ; and 2 .mu.M ce6 and 5 Jcm.sup.-2. A significant increase in VEGF production was observed in cells exposed to 2 .mu.M pl-ce6-succ and 20 Jcm.sup.-2.

Balb/mk cells showed a minor increase in VEGF production when treated with 0.5 .mu.M ce6 and 5 Jcm.sup.-2 ; and 0.5 .mu.M ce6 and 20 Jcm.sup.-2.

OVCAR-5 cells showed no increase in VEGF production when treated with 0.5 .mu.M ce6 and 5 Jcm.sup.-2 ; 0.5 .mu.M ce6 and 20 Jcm.sup.-2 ; 2 .mu.M ce6 and 5 Jcm.sup.-2 ; and 2 .mu.M ce6 and 20 Jcm.sup.-2. However, a decrease in VEGF production was observed in cells exposed to 2 .mu.M ce6 and 20 Jcm.sup.-2.

The two cell types that showed an increase in production of VEGF were keratinocyte cell lines. Keratinocytes have been reported to be a good source of VEGF for wound healing.

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TGF-.beta. secretion

A bioassay, as described by Nunes et al. ((1995) J. Immunol. Methods 186:267-274), was used to investigate whether cells increased their secretion of TGF-.beta. into the extracellular medium following administration of an effective dose of PDT. The bioassay determined TGF-.beta. activity by measuring the ability of TGF-.beta. to inhibit proliferation of epithelial cells. The cell line Mv1Lu, derived from lung epithelial cells of the mink was used because this cell line was known to be particularly susceptible to this effect. The ability of TGF-.beta. to inhibit proliferation was quantified by measuring tritiated thymidine (3H-thymidine) uptake by the Mv1Lu cells.

To determine whether PDT caused target cells to actually synthesize more TGF-.beta., or to release existing intracellular stores of TGF-.beta., both Mv1Lu cell lysates and conditioned medium were tested for TGF-.beta. bioactivity.

Various cell lines, i.e., J774, OVCAR-5, NB Rat, EA HY926 and PAM 212, were grown in P35 dishes to 80% confluency. The cells were then incubated with 2 .mu.M pl-ce6-succ and pl-ce6 for 3 hours. Following incubation, the cells were washed with PBS and then illuminated with red light from the diode array for a fluence of 1 cm.sup.-2 or 2 cm.sup.-2 delivered at 12 mWcm.sup.-2. The cells were then returned to the incubator for 24 hours whereupon the medium was aspirated, acidified, neutralized, and applied

to Mv1Lu cells. The inhibition of 3H-thymidine uptake was compared to that produced by authentic TGF-.beta. standards.

The cells, which were returned to the incubator for 24 hours, were then lysed by acidification, neutralized and added to Mv1Lu cells. The inhibition of 3H-thymidine uptake was compared to that produced by authentic TGF-.beta. standards.

Table 2 below gives the percentage inhibition of 3H-thymidine uptake produced by the PDT conditioned medium. The acid/alkaline treated control cell conditioned media was used as the 100% value. Data presented represent at least duplicate results.

A significant increase in TGF-.beta. secretion was observed in all cell lines that underwent PDT (Table 2). This indicated that PDT upregulated the secretion of TGF-.beta. from cells.

The cell lysates did not produce any significant growth inhibition or stimulation under these conditions.

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Intracellular Expression and Localization of TGF-.beta. and VEGF

Immunochemical analysis was used to investigate whether PDT had an effect on the expression and localization of TGF-.beta. and VEGF.

Cells (J774, OVCAR-5, NB rat keratinocyte, EA hy 926, and Pam 212) were grown on glass cover slips, incubated with conjugate and treated with light as described above. After 24 hours the cells were fixed with methanol, and reacted with rabbit monoclonal antibodies against TGF-.beta. or VEGF. Then a second alkaline peroxidase conjugated goat anti-rabbit monoclonal antibody was applied. The staining was developed using the appropriate reagents and conventional techniques.

The intensity of the staining in both the plasma membrane and cytoplasm was assessed on a scale of 0-3, and the frequency with which of negative cells arose was determined. Cells were either untreated (control) or treated with pl-ce6 and pl-ce6-succ with and without the application of 2 Jcm.sup.-2 light.

Table 3 summarizes data on TGF-.beta. expression and localization in five cell types, in response to non-ablative PDT. The application of photoactivating light to the photosensitizer-treated cells affected both the expression and localization of TGF-.beta. (Table 3). In some combinations, e.g., in the NB rat keratinocyte cells, the treatment of cells with light and the photosensitizer caused the number of negative cells to increase sharply (presumably because TGF-.beta. was secreted into the medium). In other cells (OVCAR-5 or Ea.hy926 cell lines) there was an increase in the membrane or cytoplasmic staining.

...
Table 4 summarizes data on VEGF expression and localization in five cell types, in response to non-ablative PDT. The effect of photoactivating light on the expression level and localization of VEGF (Table 4) was smaller than that found for TGF-.beta. (Table 3).
...
Wound Breaking Strength

Several reports have shown that the wound healing response in healthy experimental animals was so robust that it could not be accelerated (Beck et al. supra; Broadley et al. (1989) Biotechnol. Ther. 1:55-68). Therefore, in this experiment, the wound healing was suppressed so that an effect of PDT on wound healing could be evaluated.

Copenhagen rats were given a single dose of 6-alpha-methylprednisolone (40 mg/kg body weight, i.v. in tail vein), and 48 h later they were shaved and two longitudinal full-thickness incisions 3 cm long and down to the panniculus carnosus were made on the dorsum either side of the spine. Immediately following the wounding the incisions were closed with Michel clips. After 24 hours the conjugate pl-ce6-succ was injected into the tissue surrounding the wound. The dose was 0.2 mg pl-ce6-succ equivalent/kg body weight, and was administered in six 50 .mu.l aliquots of phosphate buffered saline around the wound. Four hours later the entire wound and surrounding tissue was exposed to red light from a diode array centered on 670 nm. 2Jcm.sup.-2 was delivered at a power density of 12 mW cm.sup.-2. After five days the Michel clips were removed, and after a further two days the rats were sacrificed and the wounds excised. The wound breaking strength was measured on the fresh wound strips using a Chantillon TCD 200 tensiometer (Commercial Scale Co. Inc., Agawam, Mass.).

The wound breaking strength in control mice, i.e., mice that did not receive PDT (n=13), was 55.7 g.+-.9.9 g. At least a three-fold increase in the wound strength, was observed in mice (n=13) that underwent PDT (187 g.+-.26.8 g). These data indicated acceleration of wound healing in mice by the PDT.

** * * *

United States Patent 5,942,534
Trauner , et al. August 24, 1999
Photodynamic therapy for the treatment of osteoarthritis

Abstract

A method of treating a patient who has osteoarthritic disease by administering a therapeutic composition containing a photoactivatable compound, or a precursor thereof, and administering light of a photoactivating wavelength that activates the compound.

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Government Interests

This invention was made in part with government support under grant number DEFG02-91-ER61228 awarded by the Department of Energy. The United States government has certain rights in the invention.
Parent Case Text

This application claims priority from U.S. Provisional application Ser. No. 60/028,198, filed on Oct. 10, 1996, which is incorporated herein by reference in its entirety.
Claims

What is claimed is:

1. A method of treating a patient who has osteoarthritic disease, said method comprising

(a) administering to the patient a therapeutic composition comprising a photoactivatable compound, or a precursor thereof; and

(b) administering light of a photoactivating wavelength which activates the photoactivatable compound,

wherein the amount of the therapeutic composition and the amount of light administered are sufficient to reduce the level of osteoarthritic disease in the patient.

2. The method of claim 1, wherein the photoactivatable compound is a hematoporphyrin derivative.

3. The method of claim 1, wherein the photoactivatable compound is a benzoporphyrin derivative.

4. The method of claim 1, wherein the photoactivatable compound is a product of aminolevulinic acid (ALA).

5. The method of claim 1, wherein the therapeutic composition is delivered

systemically.

6. The method of claim 1, wherein the therapeutic composition is delivered locally to the area of the joint.

7. The method of claim 1, wherein the light is administered directly to the joint.

8. The method of claim 7, wherein the light is adiministered using an arthroscopic instrument.

9. The method of claim 7, wherein the light is administered using a fiber optic instrument.

10. The method of claim 1, wherein the light is administered external to the joint, transilluminating the periarticular structure of the joint.

11. The method of claim 1, wherein the light is provided by a laser light source.

12. The method of claim 1, wherein the light is provided by a non-laser light source.

13. The method of claim 1, wherein the light is derived from a broad band light source.

14. The method of claim 1, wherein the patient is a human.

15. The method of claim 1, wherein said patient is selected from the group consisting of a cat, a dog, a rabbit, a horse, a cow, a sheep, and a goat.

Description

BACKGROUND OF THE INVENTION

The invention relates to photodynamic therapy.

Photodynamic therapy (PDT) was first developed as an experimental treatment for cancer. The treatment was based on the observation that cancer cells could retain photoactivatable compounds and could be selectively killed when these compounds subsequently interacted with absorbed light (see e.g., Bottiroli et al., Photochem. Photobiol. 47:209-214, 1988; Salet et al., Photochem. Photobiol. 53:391-393, 1991; Gross, In Photobiological Techniques, Valenzeno et al. Eds., Plenum Press, New York, 1991; and Jori et al., In Photodynamic Therapy of Neoplastic Disease, Kessel Ed., CRC Press, Boca Raton, Fla., 1989). A photodynamic compound that is widely used is marketed as Photofrin.RTM.. Photofrin.RTM./HPD (hematoporphyrin derivative) was the first FDA approved photosensitizing agent available for PDT trials. Photofrin.RTM. has subsequently been tested extensively for the destruction of multiple tumors in numerous medical disciplines (Dougherty et al., In Photodynamic Therapy of

Neoplastic Disease, Kessel Ed., supra).

The mechanism of action for hematoporphyrin derivatives such as Photofrin.RTM. in the treatment of neoplastic disease is well delineated. Large molecular aggregates of the porphyrins accumulate around tumor neovasculature. This accumulation is caused by poor lymphatic drainage from the neoplastic tissues. Once sequestered in the tissue, the molecular aggregates dissociate, and the hydrophobic components of the porphyrin cause it to partition into cell membranes, primarily into the cellular and mitochondrial membranes.

Initiation of photodynamic activity is caused by excitation of the photodynamic compound by light that falls within its absorption band. The wavelength specificity depends on the molecular structure of the photodynamic compound; a greater degree of conjugation within a molecule leads to greater absorbance at longer wavelengths. Activation of photodynamic compounds occurs with subablative light fluences. Toxicity is achieved by O.sub.2 radical toxicity. The singlet O.sub.2 reacts with, for example, double bonds to produce reactive species, for example, organoperoxides. These, in turn, initiate free radical chain reactions which degrade and disorganize membranes, uncouple oxidative phosphorylation, and lead to cellular disruption (Jori et al., supra; Weishaupt et al., Cancer Res 36:2326-2329, 1976). Nucleic acids and proteins are also damaged by photooxidation (Henderson et al., In Porphyrin Localization and Treatment of Tumors, Doiron et al. Eds., Liss, N.Y., 1984).

Studies demonstrating destruction of synovium without significant side effects indicate that photochemical synovectomy is an effective treatment for rheumatoid arthritis (U.S. Pat. No. 5,368,841).

SUMMARY OF THE INVENTION

The invention features a method of treating a patient who has an osteoarthritic joint by administering a photoactivatable compound, or a precursor thereof, and administering light of a wave-length that activates the compound. The method of the invention may be used to treat a human patient or another mammal, such as a dog, cat, rabbit, horse, cow, sheep or non-human primate.

Another embodiment of the invention is the use of a photoactivatable compound, or a precusor thereof, for the manufacture of a medicament for treating a patient who has an osteoarthritic joint. This treatment involves administering the medicament containing the photoactivatable compound, or a precursor thereof, and administering light of a wave-length that activates the compound.

Photoactivatable compounds can be administered to a patient according to established guidelines, so that the concentration of the compound in the target tissue (i.e., in the joint) will be greater than the concentration of the compound in the surrounding tissue. The ratio of the compound in the affected tissue to the compound

in the surrounding tissue is preferably 2:1 or greater. Furthermore, the compound may be administered so that an adequate level of the compound will be maintained in the target tissue. In general, this objective, i.e., an adequate level of a differentially localized compound, can be achieved using standard techniques known to skilled pharmacologists in which the clearance time course for the compound is considered.

Compounds may be administered either systemically or locally to the area of the joint. Systemically or locally administered compounds that are useful in the invention include those that are preferentially taken up by the target tissue or those that are retained substantially longer by the target tissues than by the surrounding tissues of a patient. Furthermore, photoactivatable compounds may be administered alone, or in mixtures containing two or more such compounds. If compounds are combined, light of an effective wavelength for each compound in the mixture must be used to photoactivate the compounds.

Generally, the photoactivatable compound used must have a sufficiently low toxicity to permit administration to a patient with a medically acceptable level of safety. Various photoactivatable compounds are known and can be used in the practice of the invention. These compounds typically have chemical structures that include multiple conjugated rings that allow for light absorption and photoactivation. They differ in the properties of light absorption and fluorescence, biodistribution, temporal uptake, and clearance. Classes of photoactivatable compounds include hematoporphyrins (Kessel, Cancer Lett. 39:193-198, 1988), uroporphyrins, phthalocyanines (Kreimer-Birnbaum, Sem. in Hematol. 26:157-173, 1989), purpurins (Morgan et al., Photochem. Photobiol. 51:589-592, 1990; Kessel, Photochem. Photobiol. 50:169-174, 1989), acridine dyes, bacteriochlorophylls (Beems et al., Photochem. Photobiol. 46:639-643, 1987; Kessel et al., Photochem. Photobiol. 49:157-160, 1989), and bacteriochlorins (Gurinovich et al., J. Photochem. Photobiol. B-Biol. 13:51-57, 1992). Specific photoactivatable compounds which may be used to treat osteoarthritis are summarized, in part, in Table 1. Any photoactivating compound which displays no systemic toxicity and which is useful for photodynamic therapy of neoplasias may generally be useful in the methods of the invention. Preferably, Photofrin.RTM. (which is semi-purified hematoporphyrin derivative), benzoporphyrin derivatives, or aminolevulinic acid is administered in accordance with the invention.

TABLE 1

Compounds for Photodynamic Therapy of Osteoarthritis

1. Photofrin.RTM.

2. Synthetic diporphyrins and dichlorins

3. Hydroporphyrins such as chlorins and bacteriochlorins of the tetra(hydroxyphenyl) porphyrin series

4. phthalocyanines (PC)

with or without metal substituents,

e.g., chloroaluminum phthalocyanine (CASP)

with or without varying substituents

5. O-substituted tetraphenyl porphyrins

(picket fence porphyrins)

6. 3,1-meso tetrakis (o-propionamido phenyl) porphyrin

7. Verdins

8. Purpurins

tin and zinc derivatives of octaethylpurpurin (NT2)

etiopurpurin (ET2)

9. Chlorins

chlorin e6

mono-l-aspartyl derivative of chlorin e6

di-l-aspartyl derivative of chlorin e6

10. Benzoporphyrin derivatives (BPD)

benzoporphyrin monoacid derivatives

tetracyanoethylene adducts of benzoporphyrin

dimethyl acetylenedicarboxylate adducts of benzoporphyrin

Diels-Adler adducts

monoacid ring "a" derivative of benzoporphyrin

11. sulfonated aluminum PC

sulfonated AlPc

disulfonated (AlPcS.sub.2)

tetrasulfonated derivative

sulfonated aluminum naphthalocyanines

12. naphthalocyanines

with or without metal substituents

with or without varying substituents

13. anthracenediones

14. anthrapyrazoles

15. aminoanthraquinone

16. phenoxazine dyes

17. phenothiazine derivatives

18. chalcogenapyrylium dyes

cationic selena and tellurapyrylium derivatives

19. ring-substituted cationic PC

20. pheophorbide derivative

21. hematoporphyrin (HP)

22. other naturally occurring porphyrins

23. 5-aminolevulinic acid and other endogenous metabolic precursors

24. benzonaphthoporphyrazines

25. cationic imminium salts

26. tetracyclines

In addition to free photactivatable compounds, photoactivatable compounds may be delivered in various formulations, including liposomal, peptide/polymer-bound, or detergent-containing formulations.

An alternative to administration of the photoactivatable compound itself, is administration of a precursor of that compound. This approach is illustrated by the use of 5-aminolevulinic acid, which causes endogenous production of the photoactivatable compound protoporphyrin IX (Morgan et al., J. Med. Chem. 32:904-908, 1989).

Light of the appropriate wavelength for a given compound may be administered by a variety of methods known to one skilled in the art. These methods may involve laser, nonlaser, or broad band light and may result in either extracorporeal or intraarticular generation of the light of the appropriate wavelengths. Light used in the invention may be administered using any device which generates the appropriate wave form including, but not limited to, fiber optic instruments, arthroscopic instruments, or instruments which provide transillumination, as is known to one of ordinary skill in the art.

The therapeutic method described herein can provide effective treatment for osteoarthritic joints and the inflammation that may accompany any mechanical injury of a joint. As described herein, photoactivatable chemicals are administered, and the local joint region is then exposed to light via optical fibers threaded through small gauge hypodermic needles. Alternatively, the light source may be provided extracorporeally by transillumination. Thus, photodynamic therapy offers an effective, novel, and minimally invasive treatment which may benefit a large number of patients; 60-80% of the population develop some degree of osteoarthritis during their lifetime.

The invention also features an in vitro method for screening for a photoactivatable compound useful in PDT of osteoarthritis. The method involves contacting chondrocytes with the test photoactivatable compound, administering light of an appropriate wave length and determining whether this treatment has decreased the number of viable chondrocytes. Compounds that decrease the number of viable chondrocytes after treatment with light can be useful for PDT of osteoarthritis.

The term "osteoarthritic disease" as used herein is meant to encompass primary osteoarthritis, which may be of unknown etiology, and secondary osteoarthritis, which may occur as the result of a degenerative arthrosis. A patient that has osteoarthritis and, accordingly, an "osteoarthritic joint," may or may not have apparent focal damage, such as lesions, on the articular surfaces of an affected joint. It is expected that most patients availing themselves of the method of treatment described herein will be symptomatic, but the treatment may be also be applied as a prophylactic measure.

As used herein, "precursor" means a compound that is metabolically converted to a photoactivatable compound after administration to a patient.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. Unless otherwise indicated, these materials and methods are illustrative only and are not intended to be limiting. All publications, patent applications, patents and other references mentioned herein are illustratiive only and not intended to be limiting.

Other features and advantages of the invention, e.g., treatment of human osteoarthritis, will be apparent from the following description, from the drawings and from the claims.

...

DETAILED DESCRIPTION

Photoactivatable chemicals localize selectively to the synovium or joint fluid and can be used to treat osteoarthritic disease. The anti-inflammatory effects of PDT and its ability to modulate enzymatic activity in chondrocytes can diminish the pathology seen in osteoarthritis. Decreased production, release, or activation of, for example, metalloproteinase enzymes, may allow for prolonged preservation of articular surfaces in oteoarthritis. Photofrin.RTM. is one of many examples of a photoactivatable therapeutic agent which may be used in the method of the invention. Its relevant characteristics, which include localization to the synovial tissue and particular clearance characteristics, are typical of many other photoactivatable compounds.

Osteoarthritis is a disease characterized by mechanically or biologically induced breakdown of articular cartilage. The degeneration of the cartilage due to biologic or mechanical effects changes load transmission through the joints and produces painful symptoms. Pain may also be due to inflammation of the joint lining tissue (synovium) which reacts to the free floating particles of cartilage (meniscal or articular). The degenerative cartilage does two things: (a) it produces enzymes which digest the extracellular matrix; and (b) it produces inflammatory mediators which spread throughout the joint and lead to inflammation of the synovium. The synovial inflammation in osteoarthritis is a response to irritating cartilage particles and is not due to an autoimmune response. PDT of osteoarthritis may slow the production of degradative enzymes, destroy inflammatory mediators in joint fluid and modulate inflammation in synovial tissue.

Numerous possibilities exist for delivery of both photosensitizing agents and light energy to the joints. Determining the most appropriate parameters for any photodynamic compound to be used for the treatment of osteoarthritis can be done using the experimental techniques provided herein.

I. Delivery of Photoactivatable Compounds

Therapeutic photoactivatable compounds may be either injected into the joints, or administered systemically according to the methods of the invention. The choice of localized versus systemic administration is determined, in part, by the number of joints to be treated during a given therapeutic regime. If a small number of joints require treatment, the therapeutic compounds may be administered locally. Conversely, if many joints require treatment, the therapeutic compounds may be administered systemically.

The therapeutic compounds to be administered for use in photodynamic therapy can be formulated for pharmaceutical or veterinary use by combination with an acceptable diluent, carrier, or excipient and/or in unit dosage form. In using therapeutic compounds in the methods of the invention, conventional pharmaceutical or veterinary practice may be employed to provide suitable formulations or compositions.

Thus, the formulations of the invention can be administered parenterally by, for example, intravenous, intraarticular, subcutaneous, intramuscular, intraventricular, intracapsular, intraspinal, intraperitoneal, topical, intranasal, or intrapulmonary administration. Patients may also be treated by oral, buccal, rectal, or vaginal administration.

Parenteral formulations may be in the form of liquid solutions or suspensions; oral formulations may be in the form of tablets, liquids, powders or capsules; and intranasal formulations may be in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations can to be found in, for example, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., latest edition. Formulations for parenteral administration may, for example, contain as excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymers or polyoxyethylene-polyoxypropylene copolymers in the form of microspheres may be used to control the in vivo release of the present compounds. Other potentially useful parenteral delivery systems for the compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, liposomes, and antibody conjugates including, for example, liposomes into which joint tissue-specific antibodies have been incorporated. Formulations for inhalation may contain an excipient, for example, lactose; or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and/or deoxycholate; or may be oily solutions for administration in the form of nasal drops; or a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

The present factors can be used as the sole active agents or can be used in

combination with other active ingredients.

The concentration of the present factors in the formulations of the invention will vary depending upon a number of issues, including the dosage to be administered, and the route of administration.

In general terms, the compounds of the invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. General dose ranges for systemic administration are from about 0.01 mg/kg to about 20 mg/kg of body weight; a preferred dose range is from about 0.2 mg/kg to 2 mg/kg of body weight. When administered directly to the joint, the compounds may given at 0.01 to 10 mg per joint. The preferred dosage to be administered is likely to depend upon the type and extent of progression of the joint condition being addressed, the overall health of the patient, the patient's size, body surface area, age and sex, the formulation, and the route of administration.

II. Delivery of Photoactivating Light

Newer photosensitizing compounds, which do not cause systemic skin photosensitivity, allow for activation in the near infrared and longer wavelengths of the visible light spectrum. This allows for joint transillumination, which may be performed using a variety of devices involving laser or non-laser sources, i.e., lightboxes or convergent light beams. Alternatively, optical fibers may be passed either through arthroscopes, which will allow direct visual targeting and activation of the compounds, or directly through hypodermic needles which, preferably, have a small gauge. Light may also be passed via percutaneous instrumentation using optical fibers or cannulated waveguides. Activation may also be performed by open arthrotomy.

III. Method of Screening Therapeutic Agents for Use in Photodynamic Therapy for Osteoarthritis

A. Models of Osteoarthritic Joints

The following protocols may be used to generate a model of an osteoarthritic joint in a mammal. The mammal may be, for example but not restricted to, a rat, mouse, guinea pig, rabbit, dog, cat, or non-human primate. Mammals prepared in the manner described below can be used to screen various photoactivatable compounds for their application to the treatment of mechanically injured joints.

i. Section of the Medial Collateral and Both Cruciate Ligaments Combined with Resection of the Medial Meniscus

The following procedure is performed with New Zealand white rabbits, but other species of rabbits and any mammals with analogous joint structures may also be used.

Adult New Zealand white rabbits ranging in weight from three to six kilograms and judged to be mature by roentgenographic demonstration of epiphyseal closure may be used. Each animal is anesthetized (for example with diabutal supplemented with Xylocaine.TM.), and the right knee joint is entered through a median parapatellar incision. The medial collateral ligament, both cruciate ligaments, and the flexor digitorum longus tendon (the muscle has an intraarticular origin in the rabbit) are divided, and the medial meniscus is excised. The capsule is then loosely approximated and the skin is closed, for example, with a continuous nylon suture (Ehrlich et al., J. Bone and Joint Surg.--American Vol. 57:392-396, 1975).

ii. Section of the Fibular Collateral and Sesamoid Ligament and Removal of the Anterior Horn of the Lateral Meniscus

Osteoarthritis may also be induced, for example in adult Dutch Belted rabbits, as described by O'Byrne et al. (Agents and Actions 39:C157-159, 1993) by sectioning the fibular collateral and sesamoid ligaments and removing the anterior horn of the lateral meniscus. This procedure has been shown to result in severe focal lesions of the cartilage on opposing surfaces of the tibia and femur (O'Byrne et al., supra).

B. Gross Pathology

Upon awakening, the animals may be permitted full weightbearing activity. Severe degenerative arthritis secondary to instability will develop over the ensuing three to six months, with visible changes apparent after one month. The knee joint may exhibit gross instability characterized by tibiofemoral and patellofemoral subluxation and occasionally by dislocation. In the early months, the articular surface of the femoral condyles, particularly that of the medial condyle, may appear dulled. In later months, fibrous tissue may cover portions of the articular surface of the tibial condyles. If the animals are allowed to survive for one year, the cartilage on the femoral surface may be thinned and exhibit focal erosion. Osteophytes may be seen on both the patella and the patellar surface of the femoral condyles.

C. Histological Analysis of the Osteoarthritic Joint

Histological studies employed to examine an osteoarthritic joint are well known to skilled artisans and include routine hematoxylin and eosin staining, and staining with safranin O, fast green, and iron hematoxylin. Hydroxyproline can be quantified by the method of Woessner; hexosamine can be quantified by the method of Rondle and Morgan; and acid phosphatase can be quantified by the method of Lowry (Lowry, J. Histochem. 1:420-428, 1953; Lowry et al., J. Biol. Chem. 207:19-37, 1954). Metabolic determinations may be performed using liquid scintillation spectrometric assays of incorporated isotopes after in vitro exposure, as described by Mankin et al. (J. Bone and Joint Surg. 51:1591-1600, 1969).

D. Animal Studies of Photodynamic Compounds Using Animals with Osteoarthritic

Joints

In order to determine whether a particular photoactivatable compound is suitable for use in the method of the invention, New Zealand white rabbits weighing 3-4 kg each are divided into 3 groups: a control group consisting of normal, healthy animals (control group 1), a control group consisting of animals that have undergone surgical sectioning of the anterior cruciform ligament but do not receive PDT (control group 2) and an experimental group consisting of animals that have undergone this surgery and do receive PDT. Once instability of the joint is apparent, 24 animals in the experimental group will receive a systemic injection of 2 mg/kg of the compound to be tested via a 25 gauge needle into an ear vein. Additional, localized injections may also be given 48 hours later, or at any other time indicated by drug clearance studies. A comparable number of animals in control group 1 will also receive injections of the therapeutic agent. The animals will be sedated with rompen and ketamine, according to standard protocols, and given light activation treatments. Both knees of all animals in the experimental group and one knee, preferably the right knee, of animals in control group 1 will receive light activation treatments. 400 nm-690 nm wavelength light energy, or any wavelength which is activating for the chosen therapeutic, will be transmitted via a 400 micron optical fiber through a 23 gauge needle into the knee joint cavities. Alternatively, light may be applied extracorporeally. A total light energy of 100 J/cm.sup.2, or that energy range deemed appropriate for a given compound, will be applied to each joint over 20 minutes with an average laser power setting of 3-5 watts, or that wattage and time which is effective for a given compound.

Six animals from the experimental group and from control group 2, and 4 animals from control group 1 will be sacrificed one-, two-, four-, and 10 weeks after the photodynamic compound was injected into the experimental group and control group 1. After the animals have been killed, samples of synovium, articular cartilage, meniscus, and tendon will be harvested and fixed in formalin. Specimens are then embedded in paraffin, sectioned, stained with hematoxylin and eosin, and then examined microscopically for signs of inflammation, scarring, and necrosis.

It will be understood that specific modifications in dosage, timing, light wavelength, and duration may be necessary for each therapeutic compound tested. These general parameters are known to those skilled in the art and are summarized, in part, in the following papers and references cited therein which are incorporated by reference in their entirety: Gomer, J. Photochem. Photobiol. 54:1093-1107, 1991; Maziere et al., J. Photochem. Photobiol. 8:351-360, 1991; Allison et al., Photochem. Photobiol. 54:709-715, 1991; Allison et al., Photochem. Photobiol. 52:501-507, 1990; Poon et al., J. Neurosurg. 76:679-686, 1992; Reddi et al., Br. J. Cancer 61:407-411, 1990; Richter et al., Br. J. Cancer 63:87-93, 1990.

This protocol allows the practitioner to document, with pathology: (1) the ability of a photoactivatable compound to affect, e.g., inflammation at the joint, and (2) the non-deleterious effects of an activated photodynamic compound on articular cartilage, meniscus, and other periarticular tissues.

In order to document (1), samples of synovium from animals terminated at various times after treatment are fixed, embedded in paraffin, stained with a histological stain, and examined microscopically. In order to document (2), the same procedure is followed with samples of articular cartilage, meniscus, tendon, and muscle. Gross observations at the time of harvest should also be noted. Knee inflammation at the time of light application will be examined clinically and recorded. A test compound that ablates or diminishes, for example, inflammation of the joint or production of proteolytic enzymes by chondrocytes, without concomitant deleterious effects on articular cartilage, meniscus and other periarticular tissues, could be a useful compound for PDT of osteoarthritis.

The following examples are meant to illustrate, not limit, the invention.

IV. EXAMPLES

Materials and Methods

Chondrocyte Isolation

Chondrocytes were harvested by established collagenase digestion techniques. Articular cartilage was aseptically dissected from the femoral condyles and patellas of calf knee joints. Condyle surface shavings were finely minced and digested overnight at 37.degree. C. with collagenase 1 mg/ml (Type II; 355 U/mg dry weight (dw)) and hyaluronidase 0.1 mg/ml (1060 USP/NF units/mg dw) (Worthington Biochemicals, Inc., Freehold, N.J.) in Dulbecco's Modified Eagle's Medium (DMEM; Mediatech, Herndon, Va.) supplemented with 20 mM HEPES, 100 U/ml penicillin G, 100 .mu.g/ml ascorbic acid (serum free TCM). The resulting cell suspensions were filtered through a cell sieve and washed twice with serum free TCM. The cells were resuspended in the same medium containing 5% heat inactivated fetal calf serum (BioWhittaker, Walkerville, Md.) (FCS-TCM) for seeding. Cell number was determined using a hemocytometer. Cell viability, which was determined by trypan blue exclusion, was greater than 99% for all preparations. Cells were plated at a density of 1.5.times. 10.sup.6 cells/ml into Falcon 96 well cell culture plates (Becton Dickinson Labware, Franklin Lakes, N.J.) for the cytotoxicity and cell proliferation experiments and into Falcon 6 well cell culture plates for the Photosensitizer uptake studies. Chondrocyte cultures were maintained at 37.degree. C. in a 5% CO.sub.2 incubator for 1 week prior to initiation of the photosensitization studies.

Photosensitization Studies

Photofrin.RTM. (PF) and Benzoporphyrin Derivative (BPD-MA) were obtained from Quadralogic Technologies (Vancouver, Canada). Chlorin e6 (Ce6) and chloroaluminum phthalocyanine (CASP) were obtained from Ciba Geigy (Basel, Switzerland) and Porphyrin Products (Logan, Utah), respectively. All photosensitizers

were added to the chondrocytes in the dark 3 hours prior to light exposure. Photosensitizer stock solutions were prepared immediately prior to each experiment; serial dilutions were prepared using FCS-TCM. All irradiations were done at the appropriate excitation wavelengths for each photosensitizer (BPD-MA 690 nm; Ce6 658 nm; CASP 680 nm; PF 624 nm) using light provided by an argon ion pumped dye laser (Coherent, Palo Alto, Calif.). Following light exposure, control and irradiated cells were maintained for 72 hours prior to the assessment of cellular viability and proliferation rates. Cells were examined daily by light microscopy for morphologic changes.

Screening studies of toxicity determined the range of doses and light response for each photosensitizer. These predetermined ranges were used for all of the studies described. For CASP, no dose response was seen. Dosages greater than 50 .mu.g/ml were considered not clinically relevant and a dose range extending to 50 .mu.g/ml was chosen for these studies.

Cytotoxicity Assays

PDT-induced cytotoxicity was determined 72 hours after irradiation. 3-(4,5-dimethylthiazol-2yl)-2-5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St. Louis, Mo.) was used for the determination of cellular viability (Mosmann, J. Immunol. Methods 65:55-63, 1983). MTT is metabolized via mitochondrial dehydrogenase enzymes to a formazan dye which can be measured spectrophotometrically. Cytotoxicity is expressed as the percentage of formazan produced in cells treated with visible light of different wavelengths and intensities relative to control cells (% control). The data are expressed as the mean .+-.S.E.M. from triplicate experiments.

Cell Proliferation Assays

Cell proliferation was determined by [.sup.3 H]-thymidine incorporation for all chondrocyte cultures 72 hours after drug and/or light exposure. The cell cultures were labeled with 5 .mu.Ci/ml [.sup.3 H]-thymidine (35 Ci/mmol) (ICN, Irvine, Calif.) in FCS-TCM. After incubation for 20 hours, the radioactive medium was removed and the cells were rinsed three times with phosphate buffered saline (PBS) to remove unincorporated label. The cells were then lysed with buffer containing 150 mM Tris pH 8.0, 200 mM sodium chloride, 10% triton X-100 and 1% SDS. [.sup.3 H]-thymidine incorporation was determined by liquid scintillation methods and the data are expressed as mean % control .+-.S.E.M. of samples irradiated without photosensitizer addition.

Cellular Imaging: Direct Immunofluorescence (Confocal Microscopy)

Glass coverslips were coated with 20 .mu.g/ml fibronectin (Collaborative Biomedical Research Corp., Bedford, Mass.) at 4.degree. C. overnight. 1.5-3.times.10.sup.6 chondrocytes were seeded onto the fibronectin-coated coverslips, which were

maintained at 37.degree. C. for 2 hours prior to incubation with 10 .mu.M rhodamine for 15 minutes. The cells were fixed with 2% formalin for 5 minutes and mounted onto histological slides. Rhodamine fluorescence was examined using an epifluorescence illumination microscope under 40.times. and 100.times. magnifications (Axiophot, Zeiss, Oberkochen, Germany). Fluorescent images resulted from rhodamine excitation using a 545 nm band pass filter for excitation and a 625 nm band pass filter for emission.

Light Microscopy

The cells were examined daily by light microscopy. Morphologic changes recorded were cellular differentiation, membrane blebbing and cellular exclusion of trypan blue.

Photosensitizer Uptake Studies

Articular chondrocytes were maintained in primary culture for five days after plating. Photosensitizer stocks were serially diluted in FCS-TCM and added to triplicate wells. The cells were incubated in the dark for 3 hours. The photosensitizer-containing FCS-TCM was then removed and the cells were rinsed with 1 ml PBS. To digest the extracellular matrix, the cells were then immersed in 1 ml of a solution of 1 mg/ml collagenase and 0.1 mg/ml hyaluronidase in serum-free TCM. The resulting single cell suspensions were transferred to microfuge tubes and were centrifuged at 3.3.times.g for 5 minutes. The collagenase solution was removed and the cells were rinsed three times with calcium/magnesium-free PBS. Aliquots were taken for cell counting prior to cell lysis with 1 ml 0.1 N NaOH containing 1% SDS. Cell counting was performed by hemocytometer and Coulter counter. Fluorescent spectra of the cell lysates were obtained using a Spex FluoroMax spectrofluorometer (ISA Instruments, Edison, N.J.) at the appropriate excitation/emission wavelengths for each of the four photosensitizers studied. Fluorescence spectra were corrected for background fluorescence. Relative uptake values were obtained by regression analysis of standard curves of known photosensitizer concentrations. The concentration in the cultures at which each photosensitizer inhibited chondrocyte proliferation (i.e., [.sup.3 H]-thymidine incorporation) by 50% (IC.sub.50) was calculated from plots of relative proliferation (expressed as a percentage of control) versus the concentration of the photosensitizer in the culture. Values of uptake at the IC.sub.50 are shown in fg/cell. Average protein per cell was calculated from the cell counts and protein concentration determined by the Lowry protein assay.

Gelatin Zymography

Aliquots of conditioned medium were mixed with 1 ml of acetone and maintained at -20.degree. C. for 16-20 hours. The aliquots were microcentrifuged at 14,000.times.g for 15 minutes, the supernatant was removed and the precipitated protein was dried in a vacuum concentrator (Savant, Farmingdale, N.Y.) to remove residual acetone. Conditioned medium samples were mixed with sample buffer (0.4 M Tris pH 6.8, 5%

SDS, 20% glycerol, 0.003% bromophenol blue) and applied directly, without boiling or reduction, to 10% acrylamide gels containing 1% gelatin. After removal of SDS from the gel by incubation in 2.5% Triton X-100 for 1 hour at room temperature, the gels were incubated for 16-18 hours at 37.degree. C. in buffer containing 50 mM Tris pH 7.6, 0.2 M NaCl, 5 mM CaCl.sub.2 and 0.02% Brij 35. The gels were stained for 1 hour at room temperature in 30% methanol/10% acetic acid containing 0.5% coomassie blue R-250 and destained in the same solution without dye. The gelatinolytic activity of the proteins was evidenced by clear bands against the blue background of the stained gelatin.

Example 1

Studies of PDT Efficiency Using Several Clinically Relevant Photosensitizers

Photosensitizer Effects on Chondrocyte Morphology

In the bright field and corresponding rhodamine fluorescence images of articular chondrocytes plated onto fibronectin-coated coverslips, the cells exhibited an overall polygonal to spheroid morphology and formed confluent monolayers in primary culture. Cell density was 70-80% confluence. At plating, staining of the cells with 10 .mu.M rhodamine indicated that >99% of the cells were metabolically active. Subcellular distribution of rhodamine as bright, discrete, punctate regions of fluorescence outside of nuclei was suggestive of mitochondrial localization. Dedifferentiation occurred in 5-10% of control cells over the seven day protocol period.

Fluorescence of Photosensitizers in Chondrocytes

Cellular and subcellular fluorescence patterns as determined by confocal microscopy varied among the photosensitizers evaluated. The strongest fluorescence signals were observed with BPD-MA, which demonstrated diffuse cytoplasmic fluorescence for all drug concentrations studied. No nuclear or membrane staining was observed. Fluorescence occurred uniformly in both differentiated and dedifferentiated chondrocytes. For Ce6, cells exhibited elevated levels of cytoplasmic fluorescence comparable in intensity and distribution to BPD-MA. Cells treated with PF demonstrated a similar cytoplasmic distribution but lower levels of fluorescence than BPD-MA. Photosensitizer uptake was observed only in differentiated chondrocytes. Minimal CASP fluorescence was observed at the drug concentrations studied; fluorescence at the highest drug concentration was difficult to visualize above baseline levels of cytoplasmic autofluorescence.

Photosensitizer Effects on Cellular Viability and Proliferation

Irradiation of chondrocyte cultures with light dosages up to 10 J/cm.sup.2 in the absence of photosensitizer at all wavelengths caused no decrease in cellular viability. In the presence of photosensitizer, treatment of chondrocyte cultures with 1, 5 or 10 J/

cm.sup.2 of light elicited general dose-dependent decreases in cellular viability (FIGS. 1A-1D). For BPD-MA (FIG. 1A) and Ce6 (FIG. 1B), saturation of toxicity effects occurred in the light dose range of 5 to 10 J/cm.sup.2 and for PF (FIG. 1C) at 10 J/cm.sup.2. No saturation effects were observed for CASP in the range studied (FIG. 1D). BPD-MA produced the most toxic responses in chondrocyte cultures. At BPD-MA concentrations exceeding 0.1 .mu.g/ml, cellular viability decreased 80% for irradiations of 5 or 10 J/cm.sup.2 light, and decreased by 60% for 1 J/cm.sup.2 light. CASP was minimally toxic to articular chondrocytes. Irradiation of cells with 1, 5 or 10 J/cm.sup.2 light in the presence of 5 .mu.g/ml CASP attenuated cell viability by less than 20%. Cellular exposure to more than 6 .mu.g/ml Ce6 and 5 or 10 J/cm.sup.2 light decreased viability by approximately 50%. For PF concentrations greater than 12.5 .mu.g/ml, irradiation of cell cultures with 1 J/cm.sup.2 light produced less than a 10% decrease in cellular viability while irradiations at the higher fluences decreased cellular viability by nearly 70%.

All photosensitizers elicited a dose-dependent inhibitory response on cellular proliferation rates determined by [.sup.3 H]-thymidine incorporation (FIG. 2). BPD-MA produced the most toxic effects (IC.sub.50 =50.3 ng/ml) relative to Ce6 (IC.sub.50 =4.4 .mu.g/ml) and PF (IC.sub.50 =9.3 .mu.g/ml). CASP was found to be the least toxic. At the highest concentration of CASP investigated, 50 .mu.g/ml, [.sup.3 H]-thymidine incorporation was reduced only 25%. The photosensitizing potentials determined by [.sup.3 H]-thymidine incorporation correlated with the MTT assay results.

Photosensitizer Uptake Studies

Photosensitizer uptake increased linearly across the dose range studied for all photosensitizers following a three hour incubation period. At the IC.sub.50 concentrations, BPD-MA exhibited the lowest relative uptake. Higher uptake was observed for Ce6 which was markedly less than for CASP and PF (Table 2).

TABLE 2 _____ In Vitro Uptake of Photosensitizers by Chondrocytes. IC.sub.50 Uptake Photons Absorbed at Drug IC.sub.50 (.mu.g/ml) (fg/cell) IC.sub.50 (photons/cell) _____ BPD-MA 0.05 1.02 5.86 .times. 10.sup.6 CASP >50 64.77 1.86 .times. 10.sup.6 Ce6 4.4 2.32 1.44 .times. 10.sup.8 PF 9.3 57.61 9.13 .times. 10.sup.6 _____

Zymography Studies

PDT effects on modulation of metalloproteinase (MMP2 and MMP9) activity, as determined by zymography, varied with the photosensitizer used, light dose applied and drug concentration. For BPD-MA and PF, MMP2 production decreased as a function of cell killing. At the IC.sub.50 doses, MMP2 production was reduced by approximately 50% and at a highly toxic dose, production was non-detectable.

For Ce6, MMP2 production and MMP9 production were reduced at PDT parameter levels non-toxic to chondrocytes. At high drug doses, correspondingly greater decreases in MMP production occured. At the IC.sub.50 dose, MMP2 production decreased by >95%. Total protein concentration did not vary with MMP values.

For the CASP parameters tested, no decrease in cellular viability was observed by MTT production and [.sup.3 H]-thymidine incorporation. However, significant reductions in MMP2 levels were recorded by zymography. At drug concentrations greater than 0.2 .mu.g/ml and a light dose of 10 J/cm.sup.2, MMP2 levels decreased by 25%. At a drug concentration of 3.12 .mu.g/ml with a light dose of 10 J/cm.sup.2 (conditions under which no decrease in cell viability was observed), complete reduction in MMP2 levels was observed.

In summary, the findings of decreased MMP production and [.sup.3 H]-thymidine incorporation after photodynamic treatment of the cells indicate the potential to photochemically modulate the disease process in osteoarthritis. In addition to anti-inflammatory effects on inflamed synovium, photochemical treatments may be used to retard the biologic progression of the disease, as mediated by, for example, metalloproteinase enzymes. The method described in this Example can be applied to testing a wide variety of photoactivatable compounds, such as those shown in Table 1, for utility in treatment of osteoarthritis.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

* * * * *

United States Patent 5,913,884
Trauner , et al. June 22, 1999

Inhibition of fibrosis by photodynamic therapy

Abstract

Disclosed is a method for modulating wound healing in a mammal. The method includes the steps of: (a) administering a photosensitizer to a mammal that has an unhealed or partially-healed wound; (b) waiting for the photosensitizer to reach an effective tissue concentration at the wound site; (c) photoactivating the photosensitizer by delivering specifically to the wound site light of a effective wavelength and intensity, for an effective length of time. The modulation of wound healing can include hastening healing by administering a low dose of photodynamic therapy. Alternatively, the modulation can include inhibiting fibrosis by administering a high dose of photodynamic therapy. The photosensitizer can be targeted, for example, to macrophages or myofibroblasts. Targeting can be by conjugation to a targeting moiety

such as a protein, peptide or microparticle.

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Field of Search: 607/88 1/92

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CROSS-REFERENCE TO RELATED APPLICATION

This application claims benefit from U.S. provisional application Ser. No. 60/026,315, filed Sep. 19, 1996.
Claims

We claim:

1. A method for inhibiting fibrosis in the healing of a wound in a mammal, comprising:

(a) administering to a mammal that has an unhealed or partially-healed wound an effective amount of a photosensitizer targeted to macrophages or myofibroblasts by conjugation of a targeting moiety;

(b) waiting for a time period wherein said photosensitizer reaches an effective tissue concentration at the wound site;

(c) photoactivating said photosensitizer at said wound site by delivering specifically to said wound site light of an effective wavelength and intensity, for an effective length of

time, wherein the dose of photodynamic therapy is sufficient to kill from about 10% to about 90% of the targeted cells exposed to photoactivating light,

thereby inhibiting fibrosis in said healing of said wound in said mammal.

2. The method of claim 1, wherein said photosensitizer is selected from the group consisting of: porphyrins, chlorins, bacteriochlorins, purpurins, phthalocyanines, naphthalocyanines, texaphyrins, and non-tetrapyrrole photosensitizers.

3. The method of claim 1, wherein said targeting moiety is selected from the group consisting of a protein, a peptide and a microparticle.

4. The method of claim 1, wherein the administration of said photosensitizer is systemic.

5. The method of claim 4, wherein said administration is at a dosage level between about 0.1 mg/kg and about 50 mg/kg.

6. The method of claim 5, wherein said administration is at a dosage level between about 0.5 mg/kg and about 10 mg/kg.

7. The method of claim 1, wherein the administration said photosensitizer is local.

8. The method of claim 7, wherein said administration is parenteral.

9. The method of claim 7, wherein said administration is topical.

10. The method of claim 1, wherein said photoactivating step comprises delivering light by means of optical fibers.

11. The method of claim 1, wherein said photoactivating step comprises delivering light by means of transillumination.

12. The method of claim 1, wherein said photoactivating step comprises delivering light by means of open arthrotomy.

13. The method of claim 1, wherein said photoactivating step comprises delivering laser light.

14. The method of claim 1, wherein said inhibition is performed prophylactically, prior to surgery, to reduce surgical adhesions.

Description

FIELD OF THE INVENTION

This invention relates to wound healing and photodynamic therapy.

BACKGROUND OF THE INVENTION

Dysfunctional healing CAN involve a slowing of the process, which can lead to indolent and chronic wounds. Such slowing can be due to factors such as hemorrhagic shock, infection, immune suppression, or prolonged psychological distress. Alternatively, dysfunctional healing can involve a hyperproliferative response, which can lead to, e.g., post-surgical adhesions.

Macrophages are central to the complex process of wound healing, which involves removal of dead tissue, formulation of granulation tissue, neovascularization, stimulation of locomotion and proliferation of fibroblasts and keratinocytes, and production of collagen types I and III. Photodynamic therapy can destroy large amounts of tissue with a good healing response and good cosmetic result (Koren et al, Int. J. Radiat. Oncol. Biol. Phys. 28:463-466 (1994)). Photodynamic therapy can be used either to stimulate or suppress cellular responses such as cytokine release and immune function. Whether the photodynamic therapy causes stimulation or suppresion depends on the dosage. Low dose photodynamic therapy stimulates cytokine release and immune function, while high dose photodynamic therapy suppresses those processes (Obochi et al., SPIE Proc. 2675:122-131 (1996); Yamamoto et al., Photochem. Photobiol. 60:160-164 (1994)).

Photodynamic therapy has major effects on macrophages. Low dose photodynamic therapy activates macrophages. This enhances their cytotoxicity against tumor cells (Yamamoto et al., Photobiol. B 13:295-306 (1992)). High dose photodynamic therapy leads to production of TNF alpha, and eventually, macrophage death (Evans et al., J. Natl. Cancer Inst. 82:34-39 (1990)).

Fibrosis is a response to injury in which new extracellular matrix is rapidly laid down producing dense bands of collagen that are the microscopic hallmark of scarring. The extent and duration of fibrosis often far exceeds the apparent need for wound healing, causing hypertrophic scars and contractures that limit function or distort anatomy. In the peritoneum, joints, tendon sheaths, or essentially any body space with an epithelial lining, injury and fibrosis can lead to adhesions which bind tissues together. Scars remain metabolically hyperactive long after injury, both producing and degrading extracellular matrix at a rate many times that of uninjured tissue.

The initiation and control of many concerted processes responsible for wound healing are governed by molecules which direct cell activity such as cytokines, growth factors, and adhesion molecules. In particular, the extracellular matrix growth factors TGF-.beta., platelet derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) appear to initiate and/or sustain fibrosis. Specifically TGF-.beta. appears to be the dominant cytokine governing the aggressiveness of the scarring response.

TGF.beta. has been implicated in hepatic fibrosis, pulmonary fibrosis, scleroderma, and keloids. It stimulates collagen and fibronectin formation, suppresses collagenase and induces production of collagenase inhibitors. Increased TGF-.beta. levels, increasing scarring, and more rapid healing responses are associated with disorientation and thinning of type I collagen fibers with abnormal production of proteoglycans and glycosaminoglycans in wound extracellular matrix.

Photodynamic therapy has been used to treat cancer. See, e.g., Dougherty et al., In Photodynamic Therapy of Neoplastic Disease, (Kessel, ed.), CRC Press, Boca Raton, Fla. (1989). Photodynamic therapy has also been used for destruction of the synovium in the treatment of rheumatoid arthritis (U.S. Pat. No. 5,368,841).

SUMMARY OF THE INVENTION

The invention features a method for modulating the healing of a wound in a mammal. The method includes the steps of: (a) administering an effective amount of a photosensitizer to a mammal that has an unhealed or partially-healed wound; (b) waiting for a time period wherein the photosensitizer reaches an effective tissue concentration at the wound site; (c) photoactivating the photosensitizer at the wound site by delivering specifically to the wound site light of a effective wavelength and intensity, for an effective length of time. The modulation of wound healing can include hastening healing by administering a low dose of photodynamic therapy. Alternatively, the modulation can include inhibiting fibrosis by administering a high dose of photodynamic therapy.

Photosensitizers include members of the following classes of compounds: porphyrins, chlorins, bacteriochlorins, purpurins, phthalocyanines, naphthalocyanines, texaphyrins, and non-tetrapyrrole photosensitizers. Specific examples include Photofrin, benzoporphyrin derivative, tin etiopurpurin, sulfonated chloroaluminum phthalocyanine and methylene blue. The photosensitizer can be targeted, for example, to macrophages or myofibroblasts, by conjugation to a targeting moiety such as a protein, peptide, or microparticle. Administration of the photosensitizer can be local or systemic. For systemic administration, the preferred dosage is between about 0.1 mg/kg and about 50 mg/kg. More preferably, it is at a dosage level between about 0.5 mg/kg and about 10 mg/kg. In other embodiments of the invention, the administration of the photosensitizer is local. Local administration can be perilesional or topical.

Light for photoactivation of the photosensitizer can be delivered to the wound site using various light sources and various means of delivery. For superficial wounds or open surgical wounds, suitable light sources include broadband conventional light sources, broad arrays of LEDs, and defocussed laser beams. For deeper wound sites, including those in intracavitary settings, useful means of light delivery include optical fiber devices and transillumination.

The invention can be used prophylactically, i.e., before surgery, to modulate the healing of surgical wounds. Such modulation can be used to reduce surgical adhesions, e.g., intrapleural adhesions, intraperitoneal adhesions, tendon sheath adhesions, and intraarticular adhesions.

As used herein, "low dose" photodynamic therapy means a dose sufficient to kill from 0% to about 10% of all cells exposed to the photoactiviting light if the photosensitizer is untargeted, or from 0% to about 10% of the targeted cells exposed to the photoactiviting light, if the photosensitizer is targeted. As used herein, "high dose" photodynamic therapy means a dose sufficient to kill from about 10% to about 90% of all cells exposed to the photoactivating light if the photosensitizer is untargeted, or from about 10% to about 90% of the targeted cells exposed to the photoactivating light, if the photosensitizer is targeted.

As used herein, "high dose" photodynamic therapy means a dose sufficient to kill from about 30% to about 100% of all cells exposed to the photoactivating light if the photosensitizer is untargeted, or from about 30% to about 100% of the targeted cells exposed to the photoactivating light, if the photosensitizer is targeted. The dose of photodynamic therapy is calculated as the product of photosensitizer dose and photoactivating light dose. Thus, photodynamic therapy dose can be adjusted by adjusting the photosensitizer dose, photoactivating light dose, or both.

As used herein, "photoactivation" means a light-induced chemical reaction of a photosensitizer which produces a biological effect.

As used herein, "photosensitizer" means a chemical compound that produces a biological effect upon photoactivation, or a biological precursor of a compound that produces a biological effect upon photoactivation.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present document, including definitions, will control. Unless otherwise indicated, materials, methods, and examples described herein are illustrative only and not intended to be limiting.

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DETAILED DESCRIPTION

The present invention involves applying photodynamic therapy ("PDT") to an unhealed or partially healed wound. In general, photodynamic therapy involves administration of

a photosensitizer to a patient, followed by photoactivation of the photosensitizer, to produce a cytotoxic effect. In the present invention, photoactivating light is delivered specifically to an unhealed or partially-healed wound, where the biological effect modulates wound healing.

The modulation of wound healing can be achieved according to this invention by modulating macrophage function, myofibroblast function, endothelial cell function, or any combination thereof, through photodynamic therapy at a wound site. Preferaby, the photodynamic therapy includes targeting a photosensitizer to macrophages and myofibroblasts. Targeting can be accomplished, for example, by conjugating the photosensitizer to a targeting moiety that binds to a receptor on the macrophage or myofibroblast surface, e.g., an LDL receptor or a "scavenger" receptor. Alternatively, macrophage targeting can be accomplished by exploiting the phagocytosis that characterizes macrophages and myofibroblasts. The photosensitizer can be conjugated to a microparticle, e.g., a 1 .mu.m polystyrene microsphere (Polysciences, Inc.). Such photosensitizer-microparticle conjugates are taken up selectively by macrophages and myofibroblasts, due to phagocytotic activity of those cell types. A photosensitizer-microparticle conjugate can be produced by known methods, e.g., those described in Bachor et al., Proc. Natl. Acad. Sci. USA 88:1580-1584 (1991).

Fibrosis is a complex process involving different cell types such as fibroblasts, myofibroblasts, and macrophages. Fibrosis also involves interactions between cells by means of biologically active molecules such as growth factors, cytokines, and cell adhesion molecules. Therefore, fibrosis can be advantageously inhibited according to the present invention in various ways. For example, in one embodiment of the invention inhibition of fibrosis results from localized killing of cells involved in fibrosis, e.g., macrophages. Such localized killing can be rendered selective for a particular cell type through the use of a targeted photosensitizer. Preferably, the inhibition of fibrosis results from localized inactivation of extracellular growth factors or cytokines. More preferably, it results from inactivation of TGF-.beta. or bFGF. Inhibition of fibrosis according to this invention can involve one or more of the following: decreased collagen production, modified collagen cross-linking, changes in remodeling of the extracellular matrix, and breakdown of collagen.

Inhibiting fibrosis according to the present invention is a useful adjunct to surgical practice in general. Post operative scarring and fibrosis in the healing of surgical incisions is a common complication from surgery. In hand surgery, excessive scarring of skin can cause joint contracture. Any joint subjected to surgery or injury is at risk of arthrofibrosis. Tendon repair surgery can result in adhesions surrounding the tendon repair. In reconstructive orthopaedic surgery, stiffness and decreased range of motion often follow surgery and compromise clinical outcome. Arthrofibrosis is a frequent complication of procedures such as anterior cruciate ligament reconstruction and total hip replacement. Intraabdominal adhesions are a frequent complication following abdominal surgery. This invention is useful in all such surgical situations. The present invention can also be used to inhibit fibrosis and excessive scarring in the healing of wounds other than surgical incisions.

Photosensitizer

The photosensitizer is a chemical compound that produces a biological effect upon photoactivation, or a biological precursor of a compound that produces a biological effect upon photoactivation. The photosensitizer must have a sufficiently low toxicity to permit administration to the patient with a medically acceptable level of safety. Preferably, the photosensitizer is essentially nontoxic, except for the desired cytotoxic effect produced locally, upon photoactivation.

Various photosensitizers are known and can be used in the practice of this invention. Photosensitizers typically have chemical structures that include mutiple conjugated rings that allow for light absorption and photoactivation. They differ in the properties of light absorption and fluorescence, biodistribution, temporal uptake, clearance, and mechanisms of photoactivatable cytotoxicity. Classes of photosensitizers include hematoporphyrins (Batlle, J. Photochem. Photobiol. B-Biol. 20:5-22 (1993); Kessel, Cancer Let. 39:193-198 (1988)), uroporphyrins, phthalocyanines (Kreimer-Birnbaum, Seminars in Hematology 26:157-173 (1989)), purpurins (Morgan et al., Photochem. Photobiol. 51:589-592 (1990); Kessel, Photochem. Photobiol. 50:169-174 (1989)), acridine dyes, bacteriochlorophylls (Beems et al., Photochem. Photobiol. 46:639-643 (1987); Kessel et al., Photochem. Photobiol. 49:157-160 (1989)), and bacteriochlorins (Gurinovich et al., J. Photochem. Photobiol. B-Biol. 13:51-57 (1992)). Specific examples of suitable photosensitizers are listed in Table 1.

TABLE 1

Photosensitizers

1. Photofrin.RTM.

2. Synthetic diporphyrins and dichlorins

3. Hydroporphyrins, e.g., chlorins and bacteriochlorins of the tetra(hydroxyphenyl) porphyrin series

4. phthalocyanines

5. O-substituted tetraphenyl porphyrins (picket fence porphyrins)

6. 3,1-meso tetrakis (o-propionamido phenyl) porphyrin

7. Verdins

8. Purpurins, e.g., tin and zinc derivatives of octaethylpurpurin (NT2), and etiopurpurin (ET2)

9. Chlorins, e.g., chlorin e6, and mono-l-aspartyl derivative of chlorin e6

10. Benzoporphyrin derivatives (BPD), e.g., benzoporphyrin monoacid derivatives, tetracyanoethylene adducts of benzoporphyrin, dimethyl acetylenedicarboxylate adducts of benzoporphyrin, Diels-Adler adducts, and monoacid ring "a" derivative of benzoporphyrin

11. Low density lipoprotein mediated localization parameters similar to those observed with hematoporphyrin derivative (HPD)

12. sulfonated aluminum phthalocyanine (Pc)

sulfonated AlPc

disulfonated (AlPcS.sub.2)

tetrasulfonated derivative

sulfonated aluminum naphthalocyanines

chloroaluminum sulfonated phthalocyanine (CASP)

13. zinc naphthalocyanines

14. anthracenediones

15. anthrapyrazoles

16. aminoanthraquinone

17. phenoxazine dyes

18. phenothiazine derivatives

19. chalcogenapyrylium dyes cationic selena and tellurapyrylium derivatives

20. ring-substituted cationic PC

21. pheophorbide .alpha.

22. hematoporphyrin (HP)

23. protoporphyrin

24. 5-amino levulinic acid

Photosensitizers include members of the following classes of compounds: porphyrins, chlorins, bacteriochlorins, purpurins, phthalocyanines, naphthalocyanines, texaphyrines, and non-tetrapyrrole photosensitizers. Specific examples are Photofrin, benzoporphyrin derivative, tin etiopurpurin, sulfonated chloroaluminum phthalocyanine and methylene blue. BPD is a second generation porphyrin photosensitizer that diffuses rapidly from microvasculature and disseminates throughout a joint. In addition, BPD has a low affinity for chondrocytes and articular cartilage following systemic or intra-articular injection. CASPc, a phthalocyanine inactivates growth factors TGF-.beta. and bFGF.

A single photosensitizer compound can be used alone in the practice of this invention. Alternatively, two or more photosensitizers can be used in combination, provided that light of a effective wavelength for each photosensitizer in the combination is used in the photoactivation step.

An alternative to administration of the photosensitizer compound itself, is administration of a photosensitizer precursor molecule. This approach is illustrated by the use of 5-aminolevulinic acid, which causes endogenous production of the photosensitizer protoporphyrin IX (Morgan et al., J. Med. Chem. 32:904-908 (1989).

The mechanism of the biological effect produced upon photoactivation need not be of a particular type, as long as the desired inhibition of fibrosis is achieved. The mechanism of the cytotoxic effect will depend upon the particular photosensitizer used. Typically, the cytotoxic effect results from the generation of a short-lived, highly reactive, diffusible species such as singlet oxygen or free radicals. Singlet oxygen or free radicals can then react with double bonds and initiate free radical chain reactions which can inactivate biologically active molecules such as growth factors or cytokines. Such a reaction process can also degrade or disorganize biological membranes of cells that participate directly or indirectly in fibrosis.

The photosensitizer can be chosen, or chemically modified, to optimize its usefulness in specific treatment situations. For example, the photosensitizer can be chemically modified to reduce its interaction with articular cartilage, when used to inhibit arthrofibrosis. This could be done by eliminating a positive charge to reduce association with negatively charged proteoglycans of articular cartilage. For targeting to a particular organ or tissue, the photosensitizer can be chemically conjugated to a targeting moiety such as a monoclonal antibody.

The photosensitizer can be formulated to optimize its usefulness for particular applications. For example, it can be formulated in a salve or gel for topical application. It can be formulated for parenteral administration or oral administration. Appropriate formulation can be carried out by one of ordinary skill in the art, without undue

experimentation.

Administration of the photosensitizer can be local or systemic. The administration can be by any suitable route, including topical, intravenous, intraarticular, subcutaneous, intramuscular, intraventricular, intracapsular, intraspinal, intraperitoneal, topical, intranasal, oral, buccal, rectal or vaginal. The preferred route of administration will depend on the size and nature of the wound, and on the location of the wound.

Parenteral formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are to be found in, for example, "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain as excipients sterile water or saline, Cremophor EL, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes biocompatible, biodegradable lactide polymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the present factors. Other potentially useful parenteral delivery systems for the factors include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, liposomes, and antibody conjugates. Formulations for inhalation may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

Where the size, nature, and location of the wound being treated renders local administration of the photosensitizer feasible, local administration is preferred over systemic administration. Local adminstration generally reduces the likelihood of unwanted side effects. In addition, it typically permits greater control over photosensitizer concentration at the wound site.

Photoactivation

In preferred embodiments of the invention, the method includes a waiting step between the step of administering the photosensitizer and the step of photoactivating the photosensitizer. The waiting step is designed to allow the photosensitizer to reach an optimal tissue concentration at the wound site, prior to photoactivation. The length of the waiting step will depend on factors such as the route by which the photosensitizer is administered, the location of the wound, and the speed with which the photosensitizer moves in the body. Typically, the waiting step will be longer when the photosensitizer is administered systemically.

The wound site is exposed to light of an effective wavelength and intensity, for an effective length of time. What wavelength, or range of wavelengths, is effective will depend on the photosensitizer(s) used. Wavelength specificity for photoactivation depends on the molecular structure of the photosensitizer. Photoactivation occurs with sub-ablative light doses.

Preferably, delivery of the light used to photoactivate the photosensitizer is limited to the wound site and the area immediately surrounding the wound site. This minimizes undesirable cytotoxic side effects.

The light for photoactivation can be produced and delivered to the wound site by any suitable means. For example, the light can be delivered via optical fibers threaded through small gauge hypodermic needles. Alternatively, the light can be provided extracorporeally by transillumination. Some photosensitizing compounds can be activated by near infrared and longer wavelength visible light. This allows deeper tissue penetration of the photoactivating light, thereby facilitating transillumination. Optical fibers can be passed via arthroscopes with direct visual targeting and activation of the compounds. Optical fibers can also be passed directly via small gauge hypodermic needles. Light can also be transmitted via percutaneous instrumentation using optical fibers or cannulated waveguides. Transillumination can be performed using a variety of devices. The devices can utilize laser or non-laser sources, i.e. lightboxes or convergent light beams. Photoactivation can also be performed by open arthrotomy.

EXPERIMENTAL INFORMATION

Selective Uptake of LDL-HP by Macrophages

Experiments have been carried out to demonstrate selective uptake of low density lipoprotein-haematoporphyrin conjugates (LDL-HP) by J774 macrophages, as compared to 3T3 fibroblasts. As a negative control, uptake of unconjugated HP by the macrophages was also measured. The macrophage uptake of LDL-HP was approximately 9-fold greater than macrophage uptake of unconjugated HP (FIG. 1). Macrophage uptake of LDL-HP was approximately 13-fold greater than fibroblast uptake of LDL-HP (FIG. 1).

In the LDL-HP uptake experiments, LDL-HP was prepared essentially as described in Hamblin et al, J. Photochem. Photobiol. B 26:45-56 (1994)). Heamatoporphyrin N-hydroxysuccinamide ester (HP-NHS) was prepared by mixing 20 umol HP with 40 um NHS and 40 umol DCC in 2 ml dry dimethyl sulfoxide, and leaving the mixture in the dark at room temperature overnight. Conjugation to low density lipoprotein was performed by dissolving 10 mg of protein in 2 ml of 0.1 M HP-NHS solution. The solution was mixed thoroughly and allowed to stand overnight at room temperature. All subsequent operations with conjugates were carried out in subdued light. The crude conjugation reaction product was applied to a column of Sephadex G50 and eluted

with 5 mM NaHCO.sub.3 (pH 8). The brown band of protein conjugate rapidly separated from the red band of free porphyrin. The conjugates were stored at -20.degree. C., in solutions containing 10-20% glycerol.

The cell lines were grown in 1:1 Dulbecco's modified Eagle medium (DMEM)-Hams F12 medium supplemented with glutamine, antibiotics, and 10% fetal calf serum. Experimental cultures were grown to semi-confluence in 24-well tissue culture plates containing 1 ml medium per well. The medium was replaced and conjugates were added in phosphate-buffered saline (PBS). Free photosensitizer was added in a small volume (5 .mu.l) of dimethyl sulfoxide.

At the end of incubation, the medium was removed, the cells were washed with PBS (3.times.1 ml) and incubated with trypsin-EDTA for 10 minutes (3T3 fibroblasts) or one hour (J774 macrophages). The cells were centrifuged, and the pellet (usually fluorescent under long-wave UV) was dissolved in 1 ml of a mixture of 1% sodium dodecylsulfate (SDS) and 0.1 M NaOH. The fluorescence of the cell extract was measured (emission, 632 nm; excitation 412 nm) and the protein content was determined by the Lowry method. Results were expressed as fluorescence units per mg of cell protein. One fluorescence unit per milligram of protein is approximately equal to 1.7 pmol porphyrin per milligram of cell protein, i.e., about 10.sup.6 molecules porphyrin per cell.

The following experimental protocols provide additional guidance enabling one of ordinary skill in the art to practice the present invention.

Macrophage Targeted Photodynamic Regulation of Wound Healing

In this protocol, 64 hairless rats receive multiple surgical incisions to their backs. Incisions are applied at 2 time points prior to, and 1 time point after, application of photodynamic therapy. The photodynamic therapy entail systemic or local photosensitizer administration followed by regional light therapy.

For each treatment, either for surgical incisions, PDT treatment, or biopsy, animals are sedated with general anesthesia. Anesthesia is induced using an intramuscular injection of either Ketamine (80 mg/kg), or Xylazine (12 mg/kg). Anaesthesia is determined approximately 15 minutes post-injection by the absence of spontaneous movement, with maintenance of spontaneous respiration. The assessment of deep anaesthesia is determined every 15 minutes by gentle touching of the edge of the sclera with a cotton swab to look for the blinking response. Reinjection of the anaesthetic is done only if necessary. During the experiment, care is taken to keeping the animals warm, such as by placing them on a warm table. Postoperatively, pain is monitored by observation of behavior. Pain control is provided with buprenorphine 0.03 mg/kg SQ, q 12 hrs.

At 3 days prior to, one hour prior to, and one hour after photodynamic treatment, a

series of full thickness incisions are made with a #10 scalpel blade on the back of each animal. Incisions are 25 mm in length, and spaced 1 inch apart. At time 0, rats 1-24 receive systemic injection of varying concentrations (0.5 mg/kg to 10 mg/kg) of CASP via a 30 gauge needle into the tail vein. Wounds are irradiated with 25, 50 or 100J laser energy at a wavelength of 675 nm at 5, 180 minutes, and 24 hours post injection. Animals 25-48 are treated with topical application of methylene blue 5 minutes before, and one hour before, photo activation. Using similar laser treatment parameters, 660 nm light is applied to the incision sites. Animals 49-56 are treated with systemic injections of BPD-MA (0.5 mg/kg to 10 mg/kg) and 692 nm light. Animals 57-64 are treated with systemic injections of SnEt.sub.2 (0.5 mg/kg to 5 mg/kg) and 700 nm light. Incisions sites not exposed with light serve as controls. Dark toxicity controls are performed for each photosensitizer.

At 1 day, one week, 2, 4, 8 and 10 weeks post treatment, incisions are harvested as 5 mm.times.25 mm specimens and serially sectioned into 1 mm thick specimens. Biopsy sites are closed with interrupted 3-0 nylon suture. Wounds at each time point prior to biopsy are photographed and degree of scarring evaluated by visual analogue and categorical scales. For each time point, specimens are fixed, sectioned and stained with H+E for histologic evaluation. Immunofluorescence staining of histologic sections are performed for growth factors and their receptors, to determine the depth of effect below the skin surface. Instron testing on three 1 mm thick strips are performed to characterize tensile strength of the wound repair. TGF-.beta., bFGF, and PDGF tissue concentrations are quantified by RIA on specimens weighed immediately post harvest. mRNA levels, reflecting growth factor production, are assessed by Northern blot techniques or RNase protection assays, if greater sensitivity is required. Type I collagen content is measured by hydroxyproline assay. Collagen synthesis is assayed by measurement of tritiated hydroxyproline incorporation. GAG content is evaluated by carbazole biochemical assay. All animals are euthanized at 10 weeks post treatment by CO.sub.2 inhalation.

Photodynamic Inactivation of Extracellular Growth Factors in Wound Healing

Three targeting moieties and one photosensitizer are used. Conjugates are prepared using LDL (ligand for modified LDL receptor), maleylated BSA (ligand for scavenger receptor), polystyrene nanospheres (100-200 nm dia.)(ligand for macrophage phagocytosis) and the photosensitizer, ce6. Conjugates are characterized by SDS polyacrylamide gel electrophoresis, reverse phase HPLC, fluorescence and absorption spectrophotometry.

Quantitative binding studies and cellular fluorescence imaging are carried out using a two photon fluorescence confocal microscopy system. Four cell lines are used for in vitro studies: a normal fibroblast cell line designated HSF, BALB/MK keratinocytes, a microvascular endothelial line designated Ea.hy.926, and a macrophage cell line designated J774.A1. The four cell lines are examined for in vitro uptake of LDL-ce6, Mal-BSA-ce6, and nanospherece6 conjugates. Variables tested are concentration of conjugate, incubation time, and comparison of uptake at 37.degree. and 40.degree.

C., to measure internalization.

Concentrations of conjugate and incubation time will be chosen for a light dose-cell survival response curve. Doses of PDT are administered to give survivals of 95%, 75%, 50%, and 25%. At various time points after PDT (1, 6, and 24 hours), cells are harvested. Total RNA is extracted and assayed for the presence of mRNA coding for TGF.beta., VEGF, and IGF by Northern hybridization. Controls receiving conjugate alone or light alone are also tested for comparison. An immunofluorometric assay for VEGF in culture supernatant, which is capable of detecting 10 femtomoles of VEGF is used (Detmar et al., J. Invest. Dermatol. 105:44-50 (1995)). The culture supernatant from PDT-treated macrophages is tested to see if it is capable of inducing the expression of VEGF by fibroblasts and keratinocytes, and if it has any mitogenic effect on their growth rate. Similarly, the culture supernatant of treated fibroblasts and keratinocytes is tested to see if it has a mitogenic effect on microvascular endothelial cells.

Three wound models are used for in vivo testing. One model is a rabbit ear excisional model, which produces an avascular ulcer which can only heal from the edges. The second model is an FEL induced wound model on the rat dorsum. Intravenous and perilesional injection is compared for doses ranging from 50 .mu.g to 1 mg/kg of ce6 equivalent. Various times are allowed for macrophage accumulation of conjugate (1, 6, and 24 hours). In vivo fluorescence imaging is carried out by the two photon confocal fluorescence system. The lesions are irradiated with varying fluences of 666 nm light with appropriate controls. The rate of formation of granulation tissue, and re-epithelization is measured. Punch biopsies (2 mm) for total RNA extraction and analysis of TGF.beta., IGF, and VEGF mRNA by Northern hybridization are taken at early and late time points. At 3, 7, and 14 days post treatment, animals are sacrificed and the wounds removed. Strips of the wounds are removed and their strength is measured with a tensometer. Cross sections of the wounds are removed and analyzed histologically. They are stained by immunoperoxidase techniques to quantitate the number of macrophages, endothelial cells, and myofibroblasts.

For studies on photodynamic therapy for abdominal adhesions, a third model, the rat model described by Langer et al. (J. Surg. Res. 59: 344-348 (1995)), is employed. In that model, rats develop intra-abdominal adhesions that can be graded from grade 1 (thin easily separable) to grade 3 (extensive dense tissue masses) (Elkins et al., Fertil. Steril. 41: 926-928 (1984). Intra-abdominal photodynamic therapy is performed using methods described by Molpus et al. (Cancer Res. 56:1075-1082 (1996)) for treatment of experimental ovarian cancer. Photosensitizers or conjugates are injected intraperitoneally, followed by administration of red light. The red light is administered through an optical fiber that penetrates the abdominal wall and into a peritoneal cavity, which has been filled with intralipid as a light diffuser. To test whether intra-abdominal PDT can positively affect the degree of adhesion formation, four variables are investigated: (1) light dose, (2) conjugate dose, (3) time between cecal injury and the treatment, and (4) time between i.p injection of conjugate and the delivery of i.p. light. These parameters are varied systematically. Rats are sacrificed four weeks after

treatment, and their adhesions are graded in a blind experimental design. Samples of the adhesion tissue are removed for histological staining.

Other embodiments are within the following claims.

APPENDIX TWO
REFERENCE OF MENTIONED WAVELENGTHS

UV
UV-B at 280 to 315
282 for Vitamin D - does not penetrate glass
282 or others in uv range to kill pathogens (200-400)
265 peak germicidal

blue
420
450 beta carotene
465
470 bactericidal, periodontal, acne

yellow
525 570
550 phycoerythrin (absorb at 566, emit at 575)

orange-red

600-720 average wavelength of cell tissue
620 phycocyanin
630 to 1000 red to NIR wound healing, ischemic recovery, possible retinal/optic nerve repair, repair mitochondrial dysfunction, trigger cytochrome c oxidase reversing reduction, antioxidant.
633 anti-wrinkle...skin care/stimulate dermal cells and fibroblasts
634 phagocytosis ehhanced
635 DNA and nerve repair
612
660 hemoglobin absorption, wound healing 1 inch penetration
680
infrared

728 730

far infrared

810 spider veins
830
880 wound healing 2-3 inch penetration
1540 remove benign tumors, resurface skin
1.06 blue leg veins

Orders of magnitude (length)
From Wikipedia, the free encyclopedia

To help compare different orders of magnitude, the following list describes various lengths between 1.6×10−35 m and 1.3×1026 m.
List of orders of magnitude for length Factor (m) Multiple Value Item
10−35 1.6×10−35 m Planck length; size of a string; lengths smaller than this do not make any physical sense, according to current theories of physics
. . .
10−24 1 yoctometre (ym)
10−21 1 zeptometre (zm)
10−18 1 attometre (am) size of a quark, electron radius
sensitivity of the LIGO detector for gravitational waves
10−15 1 femtometre (fm) size of a proton
classical electron
10−14 10 fm scale of the atomic nucleus
range of the weak nuclear force
10−13 100 fm
10−12 1 picometre (pm) distance between atomic nuclei in a white dwarf
wavelength of gamma rays
2.4 pm Compton wavelength of electron
5 pm wavelength of shortest X-rays
10−11 10 pm 25 pm radius of hydrogen atom
31 pm radius of helium atom
53 pm Bohr radius
10−10 100 pm wavelength of X-rays
100 pm 1 Ångström
100 pm (0.1 nm) covalent radius of sulfur atom
126 pm (0.126 nm) covalent radius of ruthenium atom
135 pm (0.135 nm) covalent radius of technetium atom
153 pm (0.153 nm) covalent radius of silver atom
154 pm (0.154 nm) length of a typical covalent bond (C–C).
155 pm (0.155 nm) covalent radius of zirconium atom
175 pm (0.175 nm) covalent radius of thulium atom
225 pm (0.225 nm) covalent radius of caesium atom
500 pm (0.50 nm) width of protein α helix
10−9 1 nanometre (nm) 1 nm diameter of Carbon nanotube
2 nm diameter of DNA helix

3.4 nm length of a DNA turn (10 bp)
3 × 8 nm size of an albumin protein molecule
6 - 10 nm thickness of cell membrane
10–8 10 nm 10 nm typical diameter of nanowire
10 nm thickness of cell wall in gram-negative bacteria
20 nm thickness of bacterial flagellum
20 - 80 nm thickness of cell wall in gram-positive bacteria
40 nm extreme ultraviolet wavelength
65 nm Feature size of Intel chips (as of 2006)
90 nm human immunodeficiency virus (HIV) (generally, viruses range in size from 20 nm to 450 nm)
10–7 100 nm size of chromosomes
100 nm 90% of particles in wood smoke are smaller than this
120 nm typical penetrating particle size for a ULPA (Ultra Low Penetration Air) filter (removes up to 99.999% at 0.12 micrometers) and a SULPA (Super ULPA) filter (removes up to 99.9999% at 0.12 micrometers)
280 nm near ultraviolet wavelength
300 nm most-penetrating particle size for a HEPA (High Efficiency Particulate Air) filter (N100 removes up to 99.97% at 0.3 micrometers, N95 removes up to 95% at 0.3 micrometers)

Distances shorter than 100 nm

* 100 nm — greatest particle size that can fit through a surgical mask]
* 120 nm — greatest particle size that can fit through a ULPA filter
* 125 nm — standard depth of pits on compact discs (width: 500 nm, length: 850 nm to 3.5 μm)
* 180 nm — typical length of the rabies virus
* 200 nm — typical size of a Mycoplasma bacterium, among the smallest bacteria
* 280 nm — near ultraviolet wavelength
* 300 nm — greatest particle size that can fit through a HEPA filter
* 380-420 nm — wavelength of violet light (see color and optical spectrum)
* 420-440 nm — wavelength of indigo light
* 440-500 nm — wavelength of blue light
* 500-520 nm — wavelength of cyan light
* 520-565 nm — wavelength of green light
* 565-590 nm — wavelength of yellow light
* 590-625 nm — wavelength of orange light
* 625-740 nm — wavelength of red light

10–6 1 micrometre (μm) 1 μm also called 1 micron
1-3 μm particle size that a surgical mask removes at 80-95% efficiency
1–10 μm diameter of typical bacterium
1.55 μm wavelength of light used in optical fibre
6–8 μm diameter of a human red blood cell
6 μm anthrax spore

7 μm width of strand of spider web [1]
7 μm diameter of the nucleus of typical eukariotic cell
10−5 10 μm 10 μm typical size of a fog, mist or cloud water droplet
10 μm width of cotton fibre
10.6 μm wavelength of light emitted by a carbon dioxide laser
12 μm width of Acrylic fibre
13 μm width of nylon fibre
14 μm width of polyester fibre
15 μm width of silk fibre
17 μm dust mite excreta [1]
20 μm width of wool fibre
25.4 μm 1/1000 inch, commonly referred to as 1 mil
50 μm typical length of Euglena gracilis, a flagellate protist
80 μm average width of human hair (ranges from 18 to 180 μm)
10−4 100 μm 125 μm dust mite
200 μm typical length of Paramecium caudatum, a ciliate protist
300 μm diameter of Thiomargarita namibiensis, the largest bacterium ever discovered
500 μm MEMS micro-engine
500 μm diameter of a human ovum
500 μm typical length of Amoeba proteus, an amoeboid protist
10−3 1 millimetre (mm) 2.54 mm distance between pins in old DIP (dual-inline-package) electronic components
5 mm length of average red ant
7.62 mm common military ammunition size
10−2 1 centimetre (cm) 1.5 cm length of a large mosquito
2.54 cm 1 inch
3.1 cm 1 attoparsec (10−18 parsecs)
4.267 cm diameter of a golf ball
10−1 1 decimetre (dm) 10 cm wavelength of the highest UHF radio frequency, 3 GHz
10 cm diameter of the cervix upon entering the second stage of labour
10.16 cm 1 hand used in measuring height of horses (4 inches)
12 cm wavelength of the 2.45 GHz ISM radio band
15 cm height of a Lilliputian from Gulliver's Travels
22 cm diameter of a typical soccer ball
30.48 cm 1 foot
50-65 cm a pizote's tail
66 cm length of the longest pine cones (produced by the sugar pine)
89 cm average adult height of a Hobbit
90 cm average length of a rapier, a fencing sword
91 cm 1 yard
100 1 metre 100 wavelength of the lowest UHF and highest VHF radio frequency, 300 MHz
1.435m Standard gauge of railway track
1.7 m (5 feet 7 inches) average height of a human being

2.45 m highest jump by a human being (Javier Sotomayor)
2.72 m tallest known human being (Robert Wadlow)
2.77 - 3.44 m wavelength of the broadcast radio FM band 87–108 MHz
3.048 m (10 feet) height of the basket in basketball
5.5 m height of tallest animal, the giraffe
8.95 m longest jump by a human being (Mike Powell)
101 1 decametre (dam) 10 m wavelength of the lowest VHF and highest shortwave radio frequency, 30 MHz
18.44 m (60 feet 6 inches) distance between the pitcher's rubber and home plate on a baseball field
20 m length of a cricket pitch
21 m height of High Force waterfall in England.
23 m height of the obelisk of the Place de la Concorde, Paris.
25 m wavelength of the broadcast radio shortwave band at 12 MHz
27.43 m (90 feet) distance between bases on a baseball field
30 m length of a blue whale, the largest animal
31 m wavelength of the broadcast radio shortwave band at 9.7 MHz
40 m average depth beneath the seabed of the Channel tunnel
49 m width of an American football field (53 1/3 yards)
49 m wavelength of the broadcast radio shortwave band at 6.1 MHz
52 m height of Niagara Falls
55 m height of the Leaning Tower of Pisa
62 m Height of Pyramid of Djoser
70 m width of a typical soccer field
70 m length of the Bayeux Tapestry
91.44 m length of an American football field (100 yards, measured between the goal lines)
102 1 hectometre (hm) 100 m wavelength of the lowest shortwave radio frequency and highest medium wave radio frequency, 3 MHz
105 m length of a typical soccer field
109.73 m total length of an American football field (120 yards, including the end zones)
112.34 m height of the world's tallest tree, a Coast redwood
137 m height of the Great Pyramid of Giza
147 m original height of the Great Pyramid of Giza
187 m shortest wavelength of the broadcast radio AM band, 1600 kHz
193 m the approximate length of New Jersey State Highway 59
244 m height of the City Gate building in Ramat-Gan, Israel
300 m height of the Eiffel Tower
340 m distance sound travels in air in one second; see speed of sound
400–500 m approximate heights of the world's tallest skyscrapers of the past 70 years.
458 m length of the Knock Nevis, the world's largest supertanker
541 m (1,776 ft) height of the planned Freedom Tower at the World Trade Center site
553.33 m height of the CN Tower, the world's tallest free-standing land structure

555 m longest wavelength of the broadcast radio AM band, 540 kHz
647 m height of the Warsaw radio mast, formerly the tallest man-made structure, collapsed in 1991
979 m height of the Salto Angel, the world's highest free-falling waterfall (Venezuela)
103 1 kilometre (km) 1 km wavelength of the lowest medium wave radio frequency, 300 kHz
1609 m 1 international mile
1852 m 1 nautical mile
8848 m height of the highest mountain on earth, Mount Everest
104 10 km 10.911 km depth of deepest part of the ocean, Mariana Trench
25 km height of the highest known mountain of the solar system, Olympus Mons on Mars
31.1 km highest parachute jump (Joseph Kittinger)
33 km narrowest width of the English Channel at the Strait of Dover
34.668 km highest manned balloon flight (Malcolm D. Ross and Victor E. Prather)
42.195 km length of the Marathon, the longest mainstream long-distance road running event
53.9 km length of the Seikan Tunnel, as of February 2006, the longest in the world
105 100 km 111 km one degree of latitude on Earth
560 km distance of Bordeaux-Paris, formerly the longest one-day professional cycling race
804.672 km (~500 miles) distance of the Indy 500 automobile race
975 km greatest diameter of the largest solar system asteroid, 1 Ceres
106 1,000 km = 1 megametre (Mm) 3,480 km diameter of the Moon
5,200 km typical distance covered by the winner of the 24 hours of Le Mans automobile endurance race
6,400 km length of the Great Wall of China
6,600 km approximate length of the two longest rivers, the Nile and the Amazon
7,821 km length of the Trans-Canada Highway
107 10,000 km 12,756 km equatorial diameter of the Earth
40,075 km length of the Earth's equator
108 100,000 km 142,984 km diameter of Jupiter
384,000 km = 384 Mm Moon's orbital distance from Earth
109 1 million km = 1 gigametre (Gm) 1,390,000 km = 1.39 Gm diameter of the Sun
3,600,000 km = 3.6 Gm greatest mileage ever recorded by a car (A 1966 Volvo P-1800S, still driving)
1010 10 million km
1011 100 million km 150 million km = 150 Gm 1 astronomical unit (AU); mean distance between Earth and Sun.
1012 1000 million km = 1 terametre (Tm) 1.4 ×109 km orbital distance of Saturn from Sun
5.9 ×109 km = 5.9 Tm orbital distance of Pluto from Sun
1013 10 Tm 14.56×109 km = 14.56 Tm distance of the Voyager 1 spacecraft from sun (as of November 2005), the farthest man-made object so far
1014 100 Tm

1015 1 petametre (Pm) 9.46×1012 km = 9.46 Pm= 1 light year distance travelled by light in one year; at its current speed, Voyager 1 would need 17,500 years o travel this distance
1016 10 Pm 3.2616 light years
(3.08568×1016 m = 30.8568 Pm) 1 parsec
4.22 light years = 39.9 Pm distance to nearest star (Proxima Centauri)
1017 100 Pm
1018 1 exametre (Em)
1019 10 Em
1020 100 Em 10,000 light years
1021 1 zettametre (Zm) 100,000 light years diameter of galactic disk of Milky Way Galaxy
52 kiloparsecs = 1.6×1021 m = 1.6 Zm distance to the Large Magellanic Cloud (a dwarf galaxy orbiting the Milky Way)
54 kiloparsecs = 1.66×1021 m = 1.66 Zm distance to the Small Magellanic Cloud (another dwarf galaxy orbiting the Milky Way)
1022 10 Zm 22.3 Zm = 2.36 million light years= 725 kiloparsecs = 22.3 Zm distance to Andromeda Galaxy
50 Zm (1.6 Mpc) diameter of Local Group of galaxies
1023 100 Zm 300–600 Zm = 10–20 megaparsecs distance to Virgo cluster of galaxies
1024 1 yottametre (Ym) 200 million light years= 2 Ym = 60 megaparsecs diameter of the Local Supercluster
500 million light years= 5 Ym = 150 megaparsecs
1025 10 Ym
1026 100 Ym 10×109 light years= 1026 m = 100 Ym estimated distance to certain quasars
13.7×109 light years= 1.3×1026 m = 130 Ym distance the cosmic background radiation has travelled since the Big Bang

APPENDIX THREE

PRODUCT RESOURCES

My Favorites:

with respect to Dr. Whelan and colleagues mailto:hwhelan@mcw.edu

parrys blog http://ledgrowlights.blogspot.com/

The patented LED grow bars http://www.solaroasis.com/
and the distributor http://www.led-grow-master.com/

http://www.ledgrowlights.com/

http://www.elixa.com great site..friendly with wide array of products -My personal choice as a first stop place for a variety of electronic healing options!

Canadian Bioresonance/homotoxicology http://www.vibranthealth.com

http://www.intota.com expert consulting/witness

http://www.quantumdev.com nasa-mcw products warp10

http://www.medsurgeadvances.com/index.php

LED center http://led.linear1.org/ great all around LED info

lumiere http://www.uvasun.com/index.php skin therapy with lights/rude/not for public

http://www.calpaclasers.com/laser_pointers.html%20 some lasers are cheap and in an appropriate range
http://www.wholesaleforeveryone.com/page/WFE/CTGY/Lasers?utm_id=ID206&OVRAW=laser%20pointers&OVKEY=laser%20pointer&OVMTC=standard
DIY laser pointer http://www.lasermate.com/DIY.HTM

http://www.theledlight.com uv maglite, for forensics and security

http://www.lighttherapyproducts.com very good info/formulas and an unique light visor

http://www.intl-light.com uv

http://www.hxmzh.com%20 underwater leds

http://www.allergybuyersclub.com/%20 uv the air
http://www.care2.com/
http://www.servicelighting.com/

LED effects is not about LED effects in general but also about how light can affect our lives http://www.my-tronic.com/#

http://www.eled.com/ loose lights
http://www.besthongkong.com/
http://www.mouser.com
http://www.hebeiltd.com.cn/

led's 101 http://www.theledlight.com/LED101.html
troubled times-topic leds http://www.zetatalk.com/energy/tengy19a.htm both for how to..

http://www.theskyfactory.com/ extreme lighting
http://http://www.sunlight-led.com/ENjianjie.htm
www.servicelighting.com/%20 just lights

From LED market place:

CompanyName: RVSI / NER

EmailAddress: merva@nerlite.com
Website: http://www.nerlite.com
Description: Manufacturers of a complete line of LED based illuminators for machine vision applications.

CompanyName: Applied Image Group / Optics
Address: 4400 S. Santa Rita Avenue
City: Tucson
StateOrProvince: AZ
PostalCode: 85714
Country: United States
PhoneNumber: 520-806-3800
EmailAddress: bkuo@appliedimage.com
Website: http://www.appliedimage.com/
Description: Custom molder of precision plastic optics.

CompanyName: ARI International Corp.
Address: 2015 S. Arlington Heights Rd.
City: Arlington Heights
StateOrProvince: IL
PostalCode: 60005
Country: United States
PhoneNumber: 847-364-1000
FaxNumber: 347-364-1270
EmailAddress: daly@ari-corp.com
Website: http://www.ari-corp.com/
Description: ARI International distributes parts manufactured by Asahi Rubber Inc. of Japan

CompanyName: Thorlabs Inc.
Website: http://www.thorlabs.com
Description: Manufacture Optics, Diodes, and a full line of photonics and laser equipment. Optomechanics, Optoelectronics, Laser Diodes, Fiber Optics, Optics, ASE Sources

CompanyName: Advanced illumination, Inc.
:
EmailAddress: dalbert@advill.com
Website: http://www.advill.com
Description: High performance LED lighting for machine vision and other applications. Patented Evenlight process targets each LED for extremely consistent structured light output.

CompanyName: Infos, Inc.
Address: 2349 State Route 8
City: Cold Brook
StateOrProvince: NY
PostalCode: 13324
Country: United States
PhoneNumber: (315) 826-5131
FaxNumber: (315) 826-7099
EmailAddress: infos@infos-inc.com
Website: http://www.borg.com/~infos
Description: The mission of INFOS, INC. is to identify products required by the fiber optic industry, that can be designed, manufactured, and sold at a substantially lower price then equivalent products offered by competitors.

CompanyName: Metaphase Technologies, Inc.
Address: 70 Stacy Haines Road
City: Medford
StateOrProvince: NJ
PostalCode: 08055
Country: United States
PhoneNumber: (609) 953-2444
FaxNumber: (609) 953-2442
EmailAddress: info@metaphase-tech.com
Website: http://www.metaphase-tech.com/
Description: Manufacturer of lighting for machine vision systems.

CompanyName: Silonex, Inc.
Address: 5200 St. Patrick Street
City: Montreal
StateOrProvince: Quebec
PostalCode: H4E 4N9
Country: Canada

PhoneNumber: (514) 768-8000
FaxNumber: (514) 768-8889
EmailAddress: silonex.sales@silonex.com
Website: http://www.silonex.com/
Description: Manufacturer of optoelectronic devices and packaging solutions.

CompanyName: Lightspan, LLC
Address: 14 Kendrick Road, Unit #2
City: Wareham
StateOrProvince: MA
PostalCode: 02571
Country: United States
PhoneNumber: 1-508-295-9110
FaxNumber: 1-508-295-9248
EmailAddress: info@light-span.com
Website: http://www.light-span.com
Description: Lightspan manufactures optically clear polymer materials for packaging of high brightness blue, UV, and white LEDs.

CompanyName: Opto Technology Inc.
Address: 160 E. Marquardt Drive
City: Wheeling
StateOrProvince: IL
PostalCode: 60090
Country: USA
PhoneNumber: 847-537-4277
EmailAddress: sales@optotech.com
Website: www.optotech.com
Description: Opto Technology Inc., is an LED based solutions provider specializing in the design and integration of high brightness and high power standard and custom LED sources with optics, thermal management and supporting electronics for the general lighting industry, marine, aerospace and bio-medical market places.

APPENDIX FOUR
FDA
http://www.fda.gov

Important Information for Consumers on Internet Sales of Laser Products

The Food and Drug Administration (FDA) is aware that some laser products being sold on the internet may not meet federal safety requirements and should not be available for purchase by the general public. In some cases, these products are overpowered and may be unsafe if not used responsibly. In other cases, these products are intended for use only by licensed medical professionals, trained operators, or other approved users.

FDA's Concerns

FDA is concerned about recent reports of laser products directed at aircraft—a potentially hazardous situation. The agency is particularly concerned about the increased availability of overpowered green laser pointers. Overpowered green laser pointers are those that may have been modified to emit more radiation than the manufacturer's original product.

What FDA Can Do

FDA's authority is over the manufacturers of laser products. These products must meet a federal standard for the amount of radiation they can emit and must be properly labeled. FDA is working to identify manufacturers of overpowered green laser pointers and other illegal laser products and will take action to prevent unsafe products from being sold in the United States. If illegal products are imported into the U.S., they may be refused entry, returned to the seller, or destroyed.

Buyer Beware

FDA recommends that consumers be cautious when buying laser products over the internet. Consumers may unknowingly purchase an illegal laser product or may lose their money if the illegal product is refused entry into the U.S. or destroyed.

Consumers should be aware that:

1. Medical lasers may only be sold to licensed medical practitioners.
2. Class IIIb and class IV laser light show projectors, identified as such on the label, may only be sold by or to individuals or firms with current, approved laser light show variances from FDA. Laser products that are advertised as uncertified components may only be sold to other manufacturers and may not be sold to the public for general use.
3. Laser products should have certification and identification labels (shown below) stating the product complies with the federal laser standard.

Complies with 21 CFR 1040.10 and 1040.11

Manufactured or distributed by
Date of Manufacture
Complies with 21 CFR Chaper 1, Subchapter J

4. Products should have a warning label (shown below) advising the user to avoid exposure to the laser radiation.

"Danger: Laser radiation. Avoid direct eye exposure. Maximum output < 5 mW. Laser Diode 630 - 680 nm. Class 3 a laser product.

Consumers who can not verify the above, or do not understand what it means, probably should not sell or purchase the products.

Contact FDA

If you have any questions about a laser product you are considering offering for sale or purchasing on the internet, contact FDA's Center for Devices and Radiological Health at 240-276-0326.

You can also report web sites that you suspect are illegally selling laser products online at http://www.fda.gov/oc/buyonline/buyonlineform.htm.

For More Information

FDA provides general information about buying regulated products online at http://www.fda.gov/oc/buyonline/default.htm.

FDA warns against purchasing potentially illegal or recalled pharmaceuticals or medical devices on the eBay web site at http://cgi3.ebay.com/ws/eBayISAPI.dll?ViewUserPage&userid=usfda

Does FDA have a mandatory limit on the power emitted by laser pointers?

Yes. Laser products promoted for pointing and demonstration purposes are limited to hazard Class IIIa by FDA regulation.

21 CFR 1040.11(b) and 1040.11(c), limit surveying, leveling, and alignment, and demonstration laser products to Class IIIa. This means that pointers are limited to 5 milliwatts output power in the visible wavelength range from 400 to 710 nanometers. There are also limits for any invisible wavelengths and for short pulses. Pointers may not exceed the accessible emission limits of CDRH Class IIIa or IEC1 Class 3R.

Are Class IIIa laser pointers dangerous?

Class IIIa or IEC Class 3R lasers can be dangerous. Class IIIa lasers can cause temporary visual effects such as flash blinding, which could distract or startle the person exposed. The risk of injury is very small when Class IIIa pointers are used responsibly because natural body motion of a person holding the pointer or motion of a person who might be exposed makes it difficult to expose the eyes for a long period of time. People also have a natural aversion to bright lights and are likely to close their eyes and turn their heads if exposed.

What are class IIIb lasers and are they dangerous?

Lasers that emit between 5mW and 500mW output power are in Class IIIb or IEC Class 3B. Class IIIb lasers cannot legally be promoted as laser pointers or demonstration laser products. Product labels and user instructions must describe the hazard classification of the product and its output characteristics.

With any laser product, the potential for injury depends both on the product itself and how the product is used. Higher powered Class IIIb or IEC Class 3B lasers are dangerous and can cause either temporary visual effects or an eye injury.

What is the problem with more powerful Class IIIb lasers being promoted and sold as pointers?

Class IIIb hand-held lasers are too dangerous for use as pointers or amusement articles. Furthermore, promotion of Class IIIb or IEC Class 3B products for pointing or amusement violates FDA requirements and United States law. Manufacturers of such products may be required to repair, replace, or refund the purchase price of violative products distributed in the U.S. These products are also subject to detention and seizure by the U.S. Customs and Border Protection when imported.

Irresponsible use of more powerful laser pointers poses a significant risk of injury to the people exposed. Persons who misuse or irresponsibly use lasers are open to personal liability and prosecution.

What are the FDA requirements for Class IIIa and IIIb laser systems?

The FDA standard 21 CFR 1040.10 and 1040.11) requires a warning label on Class

IIIa and IIIb products. Class IIIb products must also have a key switch and connector for remote interlock. The products are also required to have identifying and certifying labels and instructions for safe use.

For more information, see the Radiation Products page on our web site.

Do other organizations have requirements or standards for lasers?

Yes. Several states have registration requirements and annual registration fees for operators of Class IIIb lasers. Check with the Department of Health in your state for additional information.

The American National Standards Institute (ANSI) publishes standards for the Safe Use of Lasers2:

* Z136.1 American National Standard for the Safe Use of Lasers: 2000
* Z136.5 American National Standard for the Safe Use of Lasers in Educational Institutions: 2000
* Z136.6 American National Standard for the Safe Use of Lasers Outdoors: 2000

The ANSI standards contain procedures for avoiding exposure to laser light, designation of a Laser Safety Officer, training of operators, and the posting of warning signs in laser operating areas for Class IIIb lasers. People who operate Class IIIb lasers should be familiar with these standards to ensure the laser is used safely. In cases where a laser pointer is misused and results in an injury or alleged injury, authorities may refer to these standards as criteria for appropriate use and safety precautions.

Can battery-operated, portable laser systems be sold in the U.S?

Yes, battery-operated, portable laser systems can be sold in the U.S., providing that they fully comply with the standard, are certified and reported, and are not Class IIIb lasers sold or promoted for pointing or amusement purposes.

People who operate Class IIIb portable laser systems should be familiar with the above ANSI standards for safe use of lasers. This ANSI series of standards includes specific information for the safe use of such laser products in their applications of use.

1. IEC – International Electrotechnical Commission Standard 60825-1, Ed. 1.2: 2001-08

2. ANSI Z136 standards are available from the Laser Institute of America (http://www.laserinstitute.org)

APPENDIX FIVE
12V/24 VOLT KITS, CIRCUITS AND RESOURCES

http://www.melexis.com voltage regulators

12/24 volt systems
Google Answers http://answers.google.com/answers/

AUTOMATED BUSINESS POWER http://www.ABP.com supply 24v from 12v
http://www.bepmarine.com

http://www.sailgb.com/ nice unit

http://www.analyticsystems.com/home.htm :
The VTC120i Series of Isolated Voltage Converters are variable duty cycle switching power supplies with precision linear regulator outputs. They can be configured to run from a 12V, 24V or 48VDC battery system to provide input/output voltages as shown below.

Applications include running 24V PLC controls from a 12 Volt battery system, or any other application requiring 24 VDC when only 12 VDC is available or to provide ground isolation between two 12 VDC or 24 VDC power systems, or between a 12 VDC and 24 VDC power system.

http://www.geotechenv.com also has a variable 12v controller

12V DC Variable Speed Pump Controller

energyalternatives have the flexcharge timer:

"The Flexcharge digital timer is a 7-day, 8-event digital clock based programmable load controller. Multiple load ON and OFF times can be programmed and each day can be programmed with it's own unique timing pattern. Eight ON an eight OFF events can be programmed independently.

For example: use one ON event to have a light come on at 7pm every day then use seven OFF events to turn the light off at a different time each day. The replaceable internal battery maintains the clock and programmed memory in the event of a system power failure (for up to 3 months). Consumes less then 3mA in standby mode. Internal DPST(double-pole-single-throw) relay switch can turn one load on at the same time that it turns another load off. Timer switch can handle up to 8 amps of inductive load or up to 16 amps of resistive load, at 6 to 36 VDC or 120VAC. Timer requires 12VDC or AC to operate."

http://www.analyticsystems.com/home.htm

http://www.q-tran.com/products.html

qx-qt-universal.pdf

and from http://www.backwoodshome.com :
Powering medical equipment
during a utility blackout
By Michael Hackleman

"The first step in finding the overall load on a system is to build a load chart. There's a line for each load—light, appliance, tool, refrigerator, etc.—and columns for various entries ...
and a good article if you are considering larger emergency needs such as things that have motors!

Feb 2003 vmars newsletter (http://www.vmars.org.uk/) has Simon Dabbs, G4GFN's article on operating 24v from 12v source.

kits at http://www.action-electronics.com/kits.htm
http://web.telia.com/~u85930032/ or http://hem.passagen.se/sm0vpo / Harry Lythall - SM0VPO
  thanks harry!

also, places like National Semiconductor (http://www.national.com/) have product information sheets such as LMC555 timer which include simple circuits (lmc555.pdf) like:

comments :
mailto:dr.karlagottschalk@gmail.com

may the true light that heals be within
bright monday 2006

Journal of Clinical Laser Medicine & Surgery 21,67-74, 2003.

Effect of NASA Light-Emitting Diode (LED) Irradiation on Molecular changes for Wound Healing in Diabetic Mice

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4Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin

5Department of Plastic Surgery, University of Texas Medical Branch, Galveston, Texas

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Objective: The purpose of this study was to assess the changes in gene expression of near-infrared light therapy in a model of impaired wound healing. **Background:** Light-Emitting Diodes (LED), originally developed for NASA plant growth experiments in space, show promise for delivering light deep into tissues of the body to promote wound healing and human tissue growth. In this paper we present the effects of LED treatment on wounds in a genetically diabetic mouse model. **Methods:** Polyvinyl acetal (PVA) sponges were subcutaneously implanted in the dorsum of BKS.Cg-*m* +/+ *Leprdb* mice. LED treatments were given once daily, and at the sacrifice day, the sponges, incision line and skin over the sponges were harvested and used for RNA extraction. The RNA was subsequently analyzed by cDNA array. **Results:** Our studies have revealed certain tissue regenerating genes that were significantly upregulated upon LED treatment when compared to the untreated sample. Integrins, laminin, Gap junction proteins, Kinesin superfamily motor proteins are some of the genes involved during regeneration process. These are some of the genes that were identified upon gene array experiments with RNA isolated from sponges from the wound site in mouse with LED treatment. **Conclusions:** We believe that the use of NASA Light-Emitting Diodes (LED) for light therapy will greatly enhance the natural wound healing process, and more quickly return the patient to a pre-injury/illness level of activity. This work is supported and managed through the Defense Advanced Research Projects Agency (DARPA) and NASA Marshall Space Flight Center – SBIR Program.

INTRODUCTION

The need to care for a population with chronic wounds is a growing challenge that requires innovative approaches. One approach that specifically addresses the identified pathophysiological processes involved in wound healing is light therapy. We believe that the use of NASA Light-Emitting Diodes (LED) for light therapy will greatly enhance the natural wound healing process. This will save valuable time and resources for both patients and health care facilities. Furthermore, improved wound healing will reduce the risk of infection for the patient, decrease the amount of costly dressings required, and more quickly return the patient to a pre-injury/illness level of activity.

Laser light has been widely acclaimed to speed wound healing of ischemic, hypoxic, and infected wounds (Conlan, 1996)1. Lasers provide low energy stimulation of tissues that results in

increased cellular activity during wound healing (Beauvoit, 1994, 1995)2,3. These activities include collagen production and angiogenesis (Abergel, 1987)4.

Wound healing has three phases: first a substrate is laid down, then cells proliferate, and finally there is remodeling of tissue. The data published so far suggests that laser biostimulation produces its primary effect during the cell proliferation phase of the wound healing process. It has been demonstrated that mitochondria are receptive to monochromatic near-infrared light and that laser light likely increases respiratory metabolism of certain cells (Beauvoit, 1994, 1995; Cooper, 1997)2,3,5. Processes such as fibroblast proliferation, attachment and synthesis of collagen and procollagen, growth factor production [including keratinocyte growth factor (KGF), transforming growth factor (TGF) and platelet-derived growth factor (PDGF)], macrophage stimulation, lymphocyte stimulation (Mester , 1998)6 and greater rate of extracellular matrix production have been reported with laser light treatment (Mester, 1973; Lubart, 1992; Miller, 1993; Yu, 1994; Whelan, 1999, 2000, 2001; Sommer, 2001).7,8,9,10,11,12,13,14 Animal studies on the enhanced wound healing effect of laser light of low power density have been performed in toads, mice, rats, guinea pigs, and swine (Bibikova 1995, Al-Watban, 1997)15,16. Human studies with laser light have demonstrated greater amounts of epithelialization for wound closure and stimulation of skin graft healing (Miller, 1993; Conlan, 1996)9,1. An excellent review of recent human experience with near-infrared light therapy for wound healing was published by Conlan, et al in 1996.

Lasers, however, have some inherent characteristics which make there use in a clinical setting problematic, including limitations in wavelength capabilities and beam width. The combined wavelengths of the light for optimal wound healing cannot be efficiently produced, the size of wounds which may be treated is limited (due to laser production of a beam of light; a fact inconsistent with treating large areas), heat production from the laser light itself can actually damage tissue, and the pin-point beam of laser light can damage the eye. NASA developed LEDs offer an effective alternative to lasers. These diodes can be configured to produce multiple wavelengths, can be arranged in large, flat arrays (allowing treatment of large wounds), and produce no heat. It is also of importance to note that LED light therapy has been deemed a nonsignificant risk by the FDA; thus FDA approval for the use of LEDs in humans for light therapy has been obtained.

NASA LEDs stimulate the basic energy processes in the mitochondria (energy compartments) of each cell, particularly when near-infrared light is used to activate the wavelength sensitive constituents inside (chromophores, cytochrome systems). Optimal light wavelengths [proven in prior studies of laser and LED light (Karu, 1989; Lubart, 1992; Beauvoit, 1994, 1995; Whelan, 1999, 2000, 2001; Sommer 2001)17,8,2,3,11,12,13,14] to speed wound healing include 680nm, 730nm, and 880 nm. These wavelengths can be produced accurately be NASA LEDs, which have a bandwidth of 25nm. The depth of near-infrared light penetration into human tissue has been measured spectroscopically (Chance, 1988; Beauviot, 1994, 1995)18,2,3. Spectra taken from the wrist flexor muscles in the forearm and muscles in the calf of the leg demonstrate that most of the photons at wavelengths between 630-800 nm travel approximately 23 cm through the skin surface (light input) and muscle, exiting at the photon detector. Data collection and cataloging to elucidate the absorption coefficients of the various human tissues is currently underway by this principle investigator.

We have used the cDNA array technology to discover the expression of various genes that are induced upon LED treatment and followed through the entire process of healing trying to identify some of the early mid and late events at the molecular level. Knowledge gained from determining cellular and molecular mechanisms will direct our improvements in non-invasive therapeutic technologies.

MATERIALS AND METHODS

Eighty genetically diabetic mice (BKS.Cg-*m* +/+ *Leprdb*) from Jackson Laboratory (Bar Harbor, ME) were divided into two groups of 40 mice each. The groups included a control and a group treated with a 670nm LED. The LED treatments were given daily at a fluence of 4J/cm2 for 14 days.

Animal care and surgery were performed in accordance with an approved protocol by the Animal Resource Center at the Medical College of Wisconsin. The animals were anesthetized through

inhalation of Isoflurane. The dorsum of the anesthetized animal was shaved and disinfected with Betadine. Using aseptic technique, two 0.5cm incisions were made on either side of the spine just inferior to the scapulae. Two individual, subcutaneous pockets were dissected caudally from the incisions. Two PVA sponges (Merocel, Mystic, CT) that measured 3x3x5mm were inserted into the pockets (one sponge per pocket). The incisions were closed with 6-0 Ethilon (Ethicon, Somerville, NJ). The animals were caged individually and given food and water ad libitum.

Once per day, the animals were placed in an open-top plexiglass restrainer, and the 670nm LED was placed over the animals. The LED treatments were given at a power of 28mW/cm2 for 2 minutes 24 seconds to achieve a dose of 4J/cm2.

Each of the two groups were subdivided into four groups of ten animals each. On days 2, 4, 7, and 14 of the treatment period, animals were sacrificed and samples were collected for cDNA microarray analysis. The incision line was excised, the sponges were removed, and the skin overlying the sponges was excised. These samples were immediately placed in Trizol reagent (Invitrogen, Carlsbad, CA). The samples from each group were pooled, homogenized using a polytron, and the RNA was precipitated using the manufacturer's instructions. The precipitated RNA was stored on ethanol at -80[illegible]C. The samples were shipped on dry ice for microarray analysis.

RNA isolation and microarray:

a) RNA obtained using the Trizol method (Das et al, 1998)19 was further processed for DNAse digestion and purification.

b) For RNA samples obtained during the study, we used mouse cDNA arrays containing ~1200 genes. For each cDNA array RNA was labeled with 33P and exposed to a Kodak screen that was scanned using BIORAD Multiflour Scanner FX. The scanned images were then analyzed using ATLAS Image 2.0 software. The data obtained was then analyzed for gene clustering using Cluster and TreeView programs (Eisen, 1998)20. The unsupervised clustering algorithm was applied to microarray analysis of those samples. More comprehensive analysis of the differential gene

expression was carried out using GeneSpring from Silicon Genetics.

RESULTS

Our initial studies with skin wound healing model in mouse have revealed certain patterns of gene expression induced by LED. Tissue regenerating genes were significantly upregulated upon LED treatment when compared to the untreated sample. Integrins, nidogen, laminin, actin, kinesin motor proteins are some of the genes that have been reported to be involved during regeneration process. These are some of the genes that were identified upon gene array experiments with RNA isolated from sponges from the wound site in mouse with and without LED treatment.

WOUND HEALING

Fig. 1. Type 2 Diabetic Mice with excisional skin wounds treated with combined LED wavelengths, 4J/cm2, 50mW/cm2. The square root of wound area is used in the dependent variable in the analysis. This transformation was needed to correct for non-constant error in the General Linear Model. SqrtArea could be interpreted as being proportional to the radius of a circular wound. The interaction effect Day*Treat is significant (p-value = 0.0095).

A wound healing impaired type 2 diabetic mouse model has been studied. As previously reported, genetically diabetic mice treated with low level laser irradiation demonstrated significantly enhanced wound closure grossly, and improved wound epithelialization, cellular content, granulation tissue formation, collagen deposition, and extensive neovascularization on histological evaluation (Yu, 1997)21. In our study, type 2 diabetic mice with excisional skin wounds were treated with LEDs at individual wavelengths of 680nm, 730nm, and 880 nm at 4J/cm2 and 50mW/cm2. LED treatment produced increased healing rates, compared to surgical controls as seen in Figure 1.

A repeated measures analysis was conducted using a General Linear Model with *SqrtArea* as the

dependent variable and *Treat* as the independent variable. The interaction effect Day*Treat is significant (p-value = 0.0095), indicating that there is a significant difference between treatments on some days. This test is of primary interest in this situation, because it shows that the treatments are effective for some part of the treatment period. This analysis was carried out using the SAS statistical software package, published by The SAS Institute, Inc.

Gene expression studies: Gene changes induced by LED can be categorized into two major groups, gene that were upregulated (Fig. 2A) in both time periods and genes that were downregulated in both time periods (Fig. 2B). LED stimulated genes coding for improved tissue regeneration and basement membrane repair.

2d 14d

Fig. 2A. Cluster analysis of gene upregulated by LED when compared to the untreated control in the tissue obtained from the sponge section of the wound of diabetic mouse model. Red color indicates upregulation of genes brighter color indicates higher fold changes.

Basement membrane and tissue regenerating genes were significantly upregulated in LED vs. untreated control.

Downregulated genes were clustered using the hierarchical cluster and genes that were downregulated in both time periods were selected (Fig. 2B).

2d 14d

Figure 2B. Cluster analysis of downregulated genes in both time points after LED treatment. Green color indicated downregulation of genes, brighter color indicates higher fold change compared to the dull green color.

The basement membrane consists of a supramolecular network of collagen type IV, laminin (LN), nidogen, and associated proteoglycans. Increased expression of basement membrane components during sequential phases of wound angiogenesis and healing was repeatedly observed upon LED treatment.

Few selected genes out of approximately 306 genes that were significantly altered have been compared for their expression levels at the two time points studied after LED treatment (Fig. 3). These preliminary results are based on gene array experiments however they wound need to be confirmed by other quantitative techniques.

Fig. 3. Expression pattern of few selected genes induced by LED at both time points in the sponge site of the wound.

Laminin, nidogen, myosin were among the many genes that are part of the basement membrane were upregulated at both time points by LED. Gene from the kinesin superfamily proteins were also altered that are involved in regeneration. Kinesin superfamily motor proteins are involved during regeneration.

Semaphorins/collapsins, a family of genes with a semaphorin domain conserved from insects through to mammals, have been shown to be involved in axon guidance during neuronal development in addition to the axon repellent function of semaphorin D. Semaphorins are involved in axon guidance during neuronal development in addition to the axon repellent function of

semaphorin H.

Galectin-7 is a beta-galactoside binding protein of the lectin family, specifically expressed in stratified epithelia and notably in epidermis. Its production coincides with the degree of stratification of the epithelia. It can be considered as a marker of all subtypes of keratinocytes. This gene was upregulated at day 2 and continued to be elevated after14 d of LED treatment (Fig. 3).

Fibroblast growth factor 7 and 12 were also upregulated by 2d upon LED treatment in the sponge site of diabetic mice. These are growth factors known to be involved in the regeneration process (Fig. 3). Genes for TGF-beta 1 and thrombospondin 1 (TSP-1) were however upregulated by 14d of LED treatment.

Calcium regulated genes, Calpactins, were also altered by LED treatment. Calpactins are a family of related Ca++-regulated cytoskeletal proteins. The light chain is a member of the S100 family known to be associated with cell differentiation, malignant transformation, and S-phase of cell cycle.

Fig 4. Selected genes that were downregulated at both time periods in the sponge site of the wound upon LED treatment.

Genes such as receptor for cytokines, cytokines such as interleukin-1, IL-10, macrophage inflammatory protein-2 and proapoptosis associated genes are a few that were downregulated at both time points studied (Fig. 4).

Expression levels of each of these genes have been observed only by several gene array experiments. However each of these selected genes will need to be confirmed by other quantitative techniques such as RT-PCR or Real time PCR.

DISCUSSION

The biochemical mechanism by which LED enhances the process of wound healing is not known. The current theory is that the infrared light is absorbed by some photoreceptors, which then trigger

a cascade of reactions in a cell. The major biological photoacceptors in the near-infrared range have been determined to be hemoglobin, myoglobin, and cytochrome oxidase. LED treatment effectively energized the cells by stimulating their cytochrome oxidase (Whelan et al, 2000; 2001)12,13 and triggered a cascade of cellular and molecular events that have significant biological benefits.

Using the gene array technology we observed a variety of gene families such as basement membrane components to be upregulated by LED when compared to the untreated controls. Expression of basement membrane components occurs during sequential phases of wound healing and angiogenesis. Nidogen is one such protein along with gap junction proteins, actin that were upregulated by LED treatment. Laminin and nidogen transcripts are greatest during the early proliferative-migratory phase of angiogenesis but decrease significantly in later phases, when vessel maturation and tube formation predominate. There are reports that suggest that wound-induced epithelial cell migration is a finely tuned process that is dependent upon the regulated function and localization of specific laminins and their integrin receptors (Lotz et al, 1997)22.

Integrin alpha 7 beta 1 is a specific cellular receptor for the basement membrane protein laminin-1, as well as for the laminin isoforms -2 and -4. The alpha 7 subunit is expressed mainly in skeletal and cardiac muscle and has been suggested to be involved in differentiation and migration processes during myogenesis. Both integrins and laminins were among the many upregulated genes upon LED treatment when compared to the untreated controls. Principal stages of epidermal wound healing in human skin implies a linkage between BM assembly, integrin distribution and the compartment of proliferation competent cells, which in turn determines the onset of differentiation. Thus, apart from the balance of diffusible growth regulators, there is positional control of keratinocytes, largely accomplished by integrin-matrix interactions, which seems to be prerequisite to establishment and maintenance of tissue homeostasis (Breitkreutz et al, 1997)23.

Homeobox genes are another family of genes, which were altered by LED treatment. Hox 7 and Hox 8 genes are known to play a role for the msh-like family of genes in mesodermal and muscle differentiation and patterning and may act as a key factor up-regulating a variety of proangiogenic stimuli (Izpisua-Belmonte and Duboule, 1992)24. The formation of new blood vessels from pre-existing blood vessels is thought to be critical for wound repair.

We have identified semaphorins/collapsins to be markedly increased upon exposure to LED that may in turn decrease pain. Mouse semaphorin H functions as a chemorepellent to guide or block sensory peripheral nerve ingrowth, most likely via neuropilin as a receptor (Miyazaki et al, 1999)25. With the increase of semaphoring D at the site of the wound, nerve growth would likely be directed to occur around, rather than through the wound area. Numerous studies have shown that pain slows the healing process probably due to CNS-directed recruitment of inflammatory cells to the site of injury and their subsequent release of cytokines/eicosanoids and other mediators.

A cluster of calcium binding proteins was altered upon LED treatment. Calpactins are a family of related Ca++-regulated cytoskeletal proteins that were upregulated upon LED treatment. The calpactin I complex is composed of two heavy chain (39K) and two light chain (11K) subunits. The heavy chain is a member of a protein family that includes lipocortins, endonexin and chromobindins while the light chain is a member of the S100 family (7 distinct members are known). Many new members of the S-100 genes are known to be associated with cell differentiation, malignant transformation, and cell cycle. The messenger RNA levels of Calpactins have been reported to increase parallel to the S phase population of cells. Calpactins I and II are proteins that bind Ca2+, phospholipids, actin and spectrin; they are also major substrates of oncogene and growth-factor-receptor tyrosine kinases.

Transforming growth factor-beta (TGF-beta), a potent regulator of wound healing and scar formation, is thought to have a key role in the response to injury (Sinha et al, 2002)26. TSP-1 promotes angiogenesis in the rat aorta model. TSP-1 has a predominant role in the activation of latent TGF-beta in malignant glioma cells (Sasaki et al, 2001)27. TSP-1 is known to up-regulate the plasminogen activator system through a mechanism involving the activation of TGF-beta 1 (Yevdokimova, 2001)28. Both TGF beta-1 and TSP-1 were upregulated by 14d of LED treatment in the current study suggesting they play an important role in the wound healing process.

A large number of proapoptotic genes along with cytokines and their receptors were downregulated by LED. Activator of apoptosis harakiri (HRK), programmed cell death 1 protein precursor (PDCD-1; PD-1) and RIP were among the many genes involved in apoptosis that were inhibited by LED. Receptor-interacting protein (RIP), a Ser/Thr kinase component of the tumor

necrosis factor (TNF) receptor-1 signaling complex, mediates activation of the nuclear factor kappaB (NF-kappaB) pathway (Sun et al, 2002)29. RIP2 has a C-terminal death domain, and RIP2, which has a C-terminal caspase activation and recruitment domain (Holler et al, 2000)30. These cell death-associated genes were downregulated upon LED treatment in the mouse model, which suggest there is, increased proliferation induced by LED.

CONCLUSION

Using gene discovery techniques one can begin to understand the biochemical mechanisms that are triggered by LED and may be playing a role in ultimately enhancing the healing process. LED effects the expression of genes involved in wound healing and possibly pain modulation thus enhancing the healing process. This work will directly lead to improvements in manipulating basic mechanisms to enhance rapid LED-healing of acute combat trauma.

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