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PATENTS ACT 1990
NOTICE OF ENTITLEMENT

We, The Trustees of Columbla Unverslty In the Clty of New York, the applicant/Nominated Person in respect of Application No. 64071/90 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person derives title to the invention from the inventors by assignment.

The Nominated Person is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person is the assignee of the applicants in respect of the application listed in the declaration under Article 8 of the PCT, and because that application was the first application made in a Convention country in respect of the invention.

DATED this TWENTY THIRD day of MARCH 1992



.....
a member of the firm of
DAVIES COLLISON
CAVE for and on behalf
of the applicant(s)

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- (56) Prior Art Documents
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- (57) Claim

1. A method of welding a tissue of a subject comprising:

contacting the tissue to be welded with an exogenous energy absorbing material having an absorption peak at a particular wavelength and which enhances energy absorption by the tissue at said wavelength relative to energy at other wavelengths, and an exogenous tissue soldering agent in sufficient quantities to permit a tissue weld to be formed; and

imparting energy having a characterising wavelength corresponding to the particular wavelength of the energy absorbing material and absorbable by the energy absorbing material to the energy absorbing material in an amount sufficient and under conditions so as to cause heating of the tissue and welding of the tissue.

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24. A tissue soldering composition for use in welding tissue comprising an exogenous energy absorbing material having an absorption peak at a particular wavelength and which enhances energy absorption by the tissue at said wavelength relative to energy at other wavelengths, and an exogenous tissue soldering agent in relative amounts sufficient to effect welding of the tissue when applied to the tissue and when a sufficient amount of energy is imparted to the composition.

PCT

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<p>(21) International Application Number: PCT/US90/05125 (22) International Filing Date: 11 September 1990 (11.09.90) (30) Priority data: 406,297 12 September 1989 (12.09.89) US (71) Applicant: THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US/US]; Broadway and 116th Street, New York, NY 10027 (US). (72) Inventors: OZ, Mehmet, C. ; 4 Horizon Road, Apt. 1007, Fort Lee, NJ 07024 (US). CHUCK, Roy, S. ; 100 Haven Avenue, Apt. 9A, New York, NY 10032 (US).</p>	<p>(74) Agent: WHITE, John, P.; Cooper & Dunham, 30 Rockefeller Plaza, New York, NY 10112 (US). (81) Designated States: AT (European patent), AU, BE (European patent), BR, CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), SU. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> <p style="text-align: center; font-size: 2em; font-weight: bold;">6 4 6 4 0 6</p>	
<p>(54) Title: LASER TISSUE WELDING WITH DYE ENHANCED SOLDERS</p> <p>(57) Abstract</p> <p>A method of welding tissue comprises applying an energy absorbing material such as dye and a soldering agent such as fibrinogen or fibrin glue to tissue to be welded and imparting energy to the energy absorbing material to effect welding of the tissue. Energy can be imparted by directing an energy source such as a laser toward the energy absorbing material, the laser having a dominant wavelength corresponding to a dominant absorption peak wavelength of the dye, so that the dye absorbs energy from the laser, heats the tissue and effects welding of the tissue.</p>		

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Laser Tissue Welding With Dye Enhanced Solders

BACKGROUND OF THE INVENTION

5 The present invention relates to tissue welding with energy absorbing dye and more particularly to tissue welding using a source of energy, such as a laser, with energy absorbing dye and a soldering agent such as fibrinogen applied to the welding site.

10 Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are
15 hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Traditionally, physicians have used suture materials to appose tissue until the natural healing properties of the
20 body allowed scar formation over the wound. Welding of tissue with lasers removes the necessity for the use of sutures, thus allowing primary repair without the creation of excessive scar and foreign body reaction. Successful tissue welding using a laser as an energy source has been
25 reported in several surgical fields (1, 2, 9, 21, 22, 23). Techniques generally rely upon precise primary apposition of the tissue edges and avoidance of thermal injury to this and other surrounding tissue. The possibility of early

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disruption due to the diminished strength of welds immediately after creation has prevented more widespread use of this technology.

5 Laser tissue welding techniques have been employed which can produce an anastomosis which causes less turbulence (1), avoids foreign body reaction and granuloma formation, and is faster healing than a conventional sutured anastomosis (2). However, in the event of weld failure, edges of tissue to be
10 approximated are often thermally damaged and cannot be exposed again to laser energy. In addition, although laser welds usually have sufficient strength to withstand systolic blood pressure, they initially are weaker than their sutured counterparts and are more prone to rupture (3, 4).

15 Fibrinogen in conjunction with additional fibrin glue activators, including thrombin and calcium, has been used to enhance microvascular weld strength (5). Laser energy appears to induce protein glue cross-linking (5) and thus creates stronger welds. However, successful application of
20 the laser for tissue welding especially in conjunction with a biologic bonding material requires a high threshold power, typically above 7.6 watts/cm^2 , and often results in significant injury to surrounding tissue. It would therefore be advantageous to develop a technique of tissue
25 welding which requires lower laser energy output and which reduces the amount of collateral thermal injury, while still achieving a strong if not stronger weld than obtained using suturing techniques.

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SUMMARY OF THE INVENTION

In accordance with the present invention, a method of welding a tissue of a subject is provided comprising

5 contacting the tissue to be welded with an energy absorbing material and a tissue soldering agent in sufficient quantities to permit a tissue weld to be formed, and imparting energy absorbable by the energy absorbing material to the energy absorbing material in an amount sufficient and

10 under conditions so as to cause heating of the tissue and welding of the tissue. Preferably, the imparting of energy comprises imparting electromagnetic energy such as laser energy to the energy absorbing material. Also preferably, the contacting of the tissue comprises contacting the tissue

15 with a mixture of energy absorbing material such as dye and a tissue soldering agent such as fibrinogen. The laser energy may be from a laser consisting of a phased array of gallium-aluminum-arsenide semiconductor diodes, or an argon ion laser, for example. When the laser is the phased array

20 of diodes, the energy absorbing material preferably comprises indocyanine green (ICG) dye, whereas when the laser is an argon ion laser, the dye is preferably fluorescein isothiocyanate (FITC). The soldering agent may comprise human or animal fibrinogen, but can be any

25 polypeptide. The soldering agent may be a serum protein, albumin, or fibronectin.

The present invention also provides a tissue soldering composition for use in welding tissue comprising an energy

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absorbing material and a soldering agent in relative amounts sufficient to effect welding of tissue when applied to tissue and when a sufficient amount of energy is imparted to
5 the composition.

By providing an exogenous dye at the welding site, a lower threshold power can be used, and consequently a smaller laser. Also, using a smaller laser will result in less tissue damage. Moreover, the use of a dye enhances
10 selective delivery of energy to target tissue, thus reducing the amount of collateral thermal injury to exposed tissue in general. Mixing a soldering agent such as fibrinogen with the exogenous dye prior to application to a welding site further enhances the weld strength of the resultant weld.
15 Moreover, use of soldering agents such as fibrinogen requires neither precise apposition of tissue edges nor excessive heating of host tissue, thus allowing greater room for error during the weld. If initial attempts with the soldering agent fail, reapplication of the soldering agent
20 allows repeated trials without destroying the host tissue. Bonds created with soldering agents are structurally stronger than primary laser welds. The fact that the addition of the laser energy enhancing dye to the soldering agent, such as fibrinogen, allow selective heating of the
25 solder without injury to underlying host tissue (6) is especially important for tissue which does not contain endogenous pigments. Use of exogenous compounds can photosensitize the solders on the tissue to allow enhanced

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and selective laser energy uptake. As in photodynamic therapy or diagnostic fluorescence imaging (10,11,12) a laser and dye combination can be chosen such that the laser's output frequency matches an absorption peak of the dye closely, thereby providing efficient and target-specific laser energy delivery.

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DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, a method of bonding or welding a tissue in a subject is provided comprising contacting the tissue to be welded with an energy absorbing material and a tissue soldering agent in sufficient quantities to permit a tissue weld to be formed, and imparting energy absorbable by the energy absorbing material to the energy absorbing material in an amount sufficient and under conditions so as to cause heating of the tissue and welding of the tissue. Preferably, energy is imparted by directing an electromagnetic energy source such as a laser to the energy absorbing material. The contacting of the tissue comprises preferably contacting the tissue with a mixture of energy absorbing material and a tissue soldering agent. The energy absorbing material is preferably a dye. The dye preferably has a dominant absorption peak at a particular wavelength and the laser preferably has a dominant wavelength corresponding to that dominant absorption peak of the dye. The energy source also preferably emits energy having a frequency component in the visible spectrum to thereby aid in directing the energy source toward the tissue to be welded. One preferred laser/dye combination is wherein the laser comprises a phased array of gallium-aluminum-arsenide semiconductor diodes having a dominant wavelength of about 808 nm and wherein the dye comprises indocyanine green (ICG). Another

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preferred laser/dye combination is wherein the laser is an argon ion laser having a dominant wavelength of about 488 nm and wherein the dye is fluorescein isothiocyanate (FITC).

5 The soldering agent may be human or animal fibrinogen, but may be any polypeptide. The soldering agent may comprise a fibrin glue comprising fibrinogen combined with additional fibrin glue activators such as thrombin, with or without calcium, as set forth in U.S. Patent No. 4,627,879,

10 incorporated by reference herein. The tissue soldering agent may comprise a cryoprecipitated suspension comprising fibrinogen and Factor XIII and a fibrin glue activator comprising thrombin. The soldering agent may be for example serum protein, albumin or fibronectin. The laser may be a

15 continuous laser or a discontinuous pulse or chopped laser. In accordance with another aspect of the invention, a tissue soldering composition is provided for use in welding tissue comprising an energy absorbing material and a soldering agent in relative amounts sufficient to effect welding of

20 tissue when applied to tissue and when a sufficient amount of energy is imparted to the composition. The composition may be dye and fibrinogen. The dye may be indocyanine green (ICG) or fluorescein isothiocyanate (FITC), for example. The soldering agent may be human or animal fibrinogen, but

25 can be any polypeptide, and may be for example serum protein, albumin or fibronectin. The composition may also comprise thrombin and calcium.

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MATERIALS AND METHODSA. INTRODUCTION

Two laser welding studies according to the invention will be described below. In the first study, the energy source was a laser diode having a dominant wavelength at about 808 nm and the energy absorbing material was idocyanine green dye (ICG) dye having a maximum absorption at about 805 nm. In the second study, the energy source was an argon ion laser having a dominant wavelength within the range of 488-514 nm and the energy absorbing material was fluorescein isothiocyanate (FITC) dye having a maximum absorption at a wavelength corresponding closely to the argon laser dominant wavelength.

B. LASER DIODE/ICG DYE1. Fibrinogen Preparation

Human fresh frozen plasma was transferred to test tubes and placed in a freezer at -80° C for at least 12 hours. The tubes were then thawed at 4° C and centrifuged at 1000 X G for 15 minutes. The supernatant was decanted leaving the precipitate, fibrinogen. The viscosity of this solution allowed easy manipulation with forceps. Fibrinogen can be stored at -80° C for up to 1 year (7). For further details regarding the preparation of fibrinogen, attention is directed to U.S. Patent No. 4,627,879 which is incorporated by reference herein.

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2. Dye Preparation

Indocyanine green dye (Becton Dickinson, Baltimore, Maryland) was mixed with normal saline to make a saturated solution. The half life of the solution was approximately 10 hours. The dye has a maximum absorption at a wavelength of about 805 nm with an absorption coefficient = $2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

3. Laser System

Welding was performed with a System 7200 diode laser module coupled into a hand held focusing optic (Spectra-Physics, Mountain View, CA). This laser consists of a phased array of gallium-aluminum-arsenide semiconductor diodes. The major wavelength output of the laser diode is $808 \pm 1 \text{ nm}$. Additional bands of laser energy occur in the visible red spectrum and allow the operator to visualize the spot size of the laser during creation of the weld. The focusing optic allowed for a greater working distance, providing greater visibility of the anastomotic area. With the addition of the focusing optic the beam diameter is 2 mm at a distance of 4 cm. Laser power was measured at the output of the focusing optic with a Model 201 laser power meter (Coherent Science Division, Palo Alto, CA) and was maintained at 4.8 watts/cm^2 . The distance between the focusing lens system optic and welded tissue was maintained at approximately 4 cm during laser application.

4. In vivo Bursting Pressure Measurements

Newly created welds were evaluated in New Zealand White rabbits anesthetized with ketamine (35 mg/kg) and xylazine

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(5 mg/kg). After celiotomy a 16 gauge ridged, duck bill valve vessel cannula (DLP Inc., Grand Rapids, MI) was inserted into the distal abdominal aorta and secured in place with 3-0 silk sutures for measurement of intravascular pressure on a Datascope 2000 pressure monitor (Datascope Corporation, Paramus, NJ). Next, the abdominal aorta was cross-clamped and incised distal to the clamp to create a 7 mm longitudinal aortotomy. Small branch vessels were clamped as needed to prevent backflow bleeding. A 6-0 polypropylene suture was placed at each end of the incision and retracted in order to hold the tissue in place during welding. The field was irrigated free of all blood with sterile saline. Fine forceps were used to appose the edges of the aortotomy as needed.

For non-soldered welds (n = 11), the ICG was applied topically to the welding site through a 27 gauge needle immediately prior to laser exposure. For the laser soldered welds (n = 11), a drop of ICG was mixed with human fibrinogen and then placed with forceps onto the welding site prior to laser exposure. Only the minimum amount of ICG necessary to stain the fibrinogen was used; this allowed precise placement of the dye on the vessel surface.

Aortotomies were welded using a power density of 4.8 watts/cm² for the diode laser. When desiccation of the fibrin glue or tissue was noted, the laser energy was redirected to the next target site. The end point of welding was judged to have taken place when coaptation of the edges was observed and the tissue desiccation and

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retraction had just begun, or either the dye or the fibrin glue has clotted on the surface and no anastomotic defects were visible.

5 At the completion of the weld, the branch vessels were opened and backbleeding into the aorta was permitted for 3 minutes. Next, these branch vessels were clamped again and an infusion pump was used to advance normal saline through the previously placed angiocatheter into the distal aorta
10 and the pressure at the time of weld rupture was recorded. In an additional five rabbits, the above protocol was followed exactly using the ICG-fibrinogen combination; however, just prior to measurement of bursting pressure, 25,000 IU of urokinase (Abbott, Chicago, IL) in 0.5cc normal
15 saline was infused into the aorta for five minutes. This dose of urokinase was the amount needed to completely lyse 0.5cc of clotted rabbit blood within 5 minutes (8). Bursting pressure was then measured.

5. Control Survival Studies

20 Twenty two New Zealand White rabbits were prepared as above with a pair of 7 mm longitudinal abdominal aortotomies in each animal. The superior incision was closed with running 6-0 polypropylene suture and the inferior incision was welded closed. Each animal underwent ICG enhanced
25 fibrinogen soldering. A threshold power density of 4.8 watts/cm² was used for the diode laser. Following the welds, the inferior aortic cross-clamp was removed. If the welded anastomosis leaked, the hole was repaired with

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additional fibrinogen and laser exposure. After a leak-free anastomosis was created, the proximal aortic clamp was removed and the aorta was examined for evidence of thrombosis or leakage. At intervals between 1 and 90 days, the animals were sacrificed and weld sites harvested. Welded tissue was preserved in a 10% formaldehyde solution buffered to normal pH for HPS, Masson trichrome, or muscle specific actin staining. In addition, selected specimens were preserved in a 0.5% gluteraldehyde and 2% paraformaldehyde in 0.05M cacodylate solution (pH 7.38) for transmission and scanning electron microscopic evaluation.

6. Results

a. In vivo Bursting Pressure Measurements

The ICG dye enhanced welds required a laser application time of approximately one minute to close a 7 mm incision. Occasionally retreatment was required for residual weld defects noted after release of the cross-clamps. Immediately after welding, the bursting pressures of welds created without fibrin glue (262 ± 29 mm Hg, $n = 11$) were significantly less than welds soldered with the fibrin glue (330 ± 75 mm Hg, $n = 11$) (p less than .05). Suture closures did not burst at the highest pressures measured, up to 300 mm Hg; however, leakage through needle holes of the suture and small defects in the repair were noted at pressures above 145 mm Hg. The fibrinogen welds exposed to urokinase were not significantly weaker than nonperfused fibrinogen welds (290 ± 74 mm Hg, $n = 5$) ($p=ns$).

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b. In vivo ICG Welded Aorta and
Suture Control Survival Studies

5 Among the animals followed for 1 to 90 days, no evidence of
anastomotic rupture, thrombosis, or aneurysms occurred in
any of the sutured or fibrinogen-ICG solders. Immediately
after welding, the applied fibrinogen formed a smooth flow
surface onto which red blood cells and platelets adhered.
By the second day after surgery, sheets of fibrin were
10 identified crossing the aortotomy weld and the thrombotic
response had not increased significantly more than that seen
from the specimens harvested immediately after soldering.
Within 10 days a new intimal surface had regenerated over
the fibrin. By the second week after operation, histiocytes
15 were seen engorged with fibrinogen as the process of resorp-
tion started. By the third week after operation, the
internal and external elastic lamina remained disrupted, and
the initially acellular fibrin was densely packed with
proliferating cells which had eosinophilic cytoplasm
20 characteristic of myofibroblasts extending from just below
the intimal surface to the adventitia. This identification
was confirmed using a Smooth Muscle Specific Actin stain
(Enzo Diagnostics, New York, N.Y.) specific for myofibro-
blasts. These cells appear to have proliferated and laid
25 the matrix for the rapid regeneration of tissue at the weld
site. No foreign body reaction was noted. As the healing
response continued, fibrin deposited closest to the intimal
surface appeared to be resorbed by histiocytes faster than

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fibrin laying on the adventitial surface. By 90 days post
operatively, the fibrin had been almost completely removed
and only normal appearing myointimal cells remained between
5 the external elastic lamina and the intima. Outside the
external elastic lamina, occasional foci of fibrin
surrounded by histiocytes remained; however, in general only
fibrous tissue laid down after the welding was present. No
foreign body reaction was evident.

10 The suture closed aortotomies demonstrated ischemic changes
at the point of suture pressure on the aortic tissue. The
suture closures did not appear to induce thrombus adherence
to any greater extent than the laser welded closures;
however, tissue reaction to the sutures resulted in a
15 fibrotic build-up which distorted the intimal flow surface
by post-operative day 25. Comparable areas of soldered
aorta at the same time after operation demonstrated complete
re-endothelialization, with the aortotomy site completely
covered with intimal cells, and it was difficult to
20 distinguish this site from the surrounding normal rabbit
lumen.

C. ARGON ION LASER/FITC DYE

1. Dye Preparation

Ten milligrams of isomer I FITC (Sigma Inc. St. Louis, MO),
25 10 ml 0.9% sodium chloride irrigation solution at pH 4.5-7
(Abbott Laboratories, Chicago, IL) and 1 ml of 8.4% sodium
bicarbonate at pH 8.4 (Fisher Scientific, Fair Lawn, N.J.)
were mixed, resulting in an orange solution. Two to three

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drops (0.1 cc) of this solution were added topically to the weld site prior to exposure of the tissue to the argon laser. In control animals, a similar quantity of 0.9% sodium chloride was applied topically to the weld site.

2. Laser System

Welding was performed with a System 1000 surgical argon laser with a 1 mm spot size applicator (Coherent Medical Division, Palo Alto, CA). The laser was operated in the multiline mode. At low laser power the dominant wavelength is 488 nm.

The applicator was modified by mounting a focusing optic on the output end of the applicator (Becton Dickenson FACS IV part No. 53.10006-01, Becton Dickenson Immuno-cytometry Systems, Mountain View, CA). The focusing optic allowed for a greater working distance, providing greater visibility of the anastomotic area. With the addition of the focusing optic the beam diameter is 1.5 mm diameter at 6 cm. Laser power was measured at the output of the focusing optic with a Model 201 laser power meter (Coherent Science Division, Palo Alto, CA). The distance between the focusing optic and the welded tissue was maintained at approximately 6 cm during laser application.

3. In Vitro Dose-Response Curve

The adventitia of freshly harvested rabbit aorta was exposed to increasing output laser power ranging from 100 to 500 mW (power fluence range, 5.66 to 12.04 watts/cm²). For one group of exposures, a single drop of FITC was topically

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applied to the specimen through a 27 gauge needle. In a second group, no FITC was used. The tissue was exposed to the laser system described above for intervals of 15, 30, 5 60, 90, and 120 sec, and gross tissue changes were noted. The specimens were placed in 10% formalin and stained with hematoxylin, phloxin, and safranin (HPS).

4. In Vivo Bursting Pressure measurements

Ten New Zealand white rabbits were anesthetized with 10 ketamine (35 mg/kg) and xylazine (5 mg/kg). After celiotomy a 16 gauge ridged, duck bill valve vessel cannula (DLP Inc., Grand Rapids, MI) was inserted into the distal abdominal aorta and secured in place with 3-0 silk sutures for measurement of intravascular pressure on a Datascope 2000 15 pressure monitor (Datascope Corporation, Paramus, N.J.). Next, the abdominal aorta was cross-clamped and incised distal to the clamp to create a 7mm longitudinal aortotomy. A 6-0 polypropylene suture was placed at each end of the incision and retracted to hold the tissue in place during 20 welding. The field was irrigated free of all blood with sterile saline. Fine forceps were used to oppose the edges of the aortotomy as needed. For the FITC welds, the dye was topically applied to the welding site through a 27 gauge needle. Only the minimum amount of FITC necessary to stain 25 the welded area was used. Although it was impossible to control precisely the lateral spread of the dye once applied to the tissues, one to two drops of dye were usually adequate. Aortotomies were welded using power densities of

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3.8 watts/cm² with FITC and 7.6 watts/cm² without FITC. These power densities represented the minimum power levels at which reliable welds could be consistently obtained.

5 When desiccation of the aorta was noted, the laser energy was redirected to the next target site. The end point of welding was judged to have taken place when coaptation of the edges was observed and the tissue desiccation and retraction had just begun.

10 At the completion of the weld, an infusion pump was used to advance normal saline through arterial pressure tubing and the previously placed angiocatheter into the distal aorta. To minimize the number of animals sacrificed, we were able to perform two to three welds and bursting pressure
15 determinations on the aorta of each animal by progressively moving the aortic cross clamp distally after each bursting pressure determination.

5. In Vivo welded Aorta and Suture Control Survival Studies

20 Twenty-five additional New Zealand White rabbits were prepared as above with a pair of 7 mm longitudinal abdominal aortotomies in each animal. The superior incision was closed with running 6-0 polypropylene suture, and the inferior incision was welded closed. Eighteen animals
25 underwent FITC-enhanced welding; seven rabbits underwent laser welding without addition of FITC. A threshold power density of 3.8 watts/cm² was used for the non-FITC welds. Following the welds, the inferior aortic cross-clamp was removed. If the welded anastomosis leaked, the hole was

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repaired by additional laser exposure. After a leak-free anastomosis was made, the proximal aortic clamp was removed and the aorta was examined for evidence of thrombosis or leakage. At intervals between 1 and 75 days, the animals were sacrificed and weld sites harvested. Welded tissue was preserved in a 10% formaldehyde solution buffered to normal pH for PHS or Masson trichrome staining.

6. Results

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a. In Vitro Dose-Response Curve

For FITC stained in vitro rabbit aorta, the threshold for tissue blanching was 15 sec of 100 mW exposure. As the time of exposure and laser power density increased, the tissue acquired a reddish hue followed by further desiccation, contraction, and eventual charring at approximately 30 sec of 300 mW exposure. No holes were created until 90 sec of exposure to 400 mW laser energy. In the unstained aorta, no tissue effects were observed until 15 sec of 300 mW laser energy was applied. The initial tissue desiccation rapidly progressed to charring, and by 90 sec of exposure to this power, a hole had been created. The progression of tissue changes in the FITC-stained group in comparison to the unstained controls is demonstrated in Table 1 reproduced at the end of this specification. As compared with FITC-stained aorta, three times greater laser energy was needed to induce the same tissue changes on nonstained specimens. Also, the progression of tissue changes from initial tissue effects of the laser to charring and eventual hole creation was more gradual in tissue stained with FITC.

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b. In Vivo Bursting Pressure Measurements

Both FITC and non-FITC welds required laser application time of approximately 90 sec to close a 7 mm incision.

- 5 Occasionally a retreatment was required for residual weld defects noted after release of the cross-clamps. Mean bursting pressures immediately after weld creation were 164 mm Hg for FITC group compared with 147 mmHg for the non-FITC-welded group. Suture closures did not burst at the
10 highest pressure used (300 mm Hg); however, leakage through needle holes of the suture and small defects in the repair were noted at pressures above 125 mm Hg.

c. In Vivo FITC-Welded Aorta and Suture Control Survival Studies

- 15 Among the animals followed for 1 to 75 days, no evidence of anastomotic rupture, thrombosis, or aneurysms occurred in any of the suture or FITC welds. The weld site of FITC-enhanced argon ion laser welds was characterized by myointimal proliferation with a regenerated intimal surface.
20 Collagen deposit was noted at the weld site. One of the welds performed without FITC developed a small anastomotic dilation by 2 weeks postoperatively. No other abnormalities were noted in this group. Histologically, carbonaceous debris was usually visible on the surface tissue directly
25 exposed to the argon energy without FITC. After treatment without FITC, the argon ion laser induced a 50-100 um region of amorphous collagen surrounding the point of welding. A 300 um region of tissue manifested the loss of nuclear detail and cellular integrity characteristic of thermally

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damaged areas. No deposition of carbonaceous debris was evident on the FITC weld specimens. The suture closed aortotomies demonstrated ischemic changes at the point of suture pressure on the aortic tissue. These closures did not appear to induce thrombus adherence to any greater extent than the laser-welded closures. By 2 weeks after laser welding, a new endothelial surface had repopulated the intimal surface. Similar changes had occurred in the suture-closed aortotomies, although patches of denuded endothelium often remained.

D. DISCUSSION OF STUDIES

All three elements in the described welding system i.e. an energy source, an energy absorbing material and a tissue soldering agent meet the requirements of simplicity and safety for application in the clinical setting. Indocyanine green, fibrin glue, and diode lasers have been proven safe in clinical application (8,14,15). Indocyanine green has a large absorption coefficient at the 808 nm output of the diode laser. The diode laser is an inexpensively manufactured solid state semiconducting electronic device with heretofore limited medical applications due to low energy output capacities (less than 400 mW). When combined with topically applied indocyanine green dye, lasers at this wavelength can cause substantial and rapid tissue effects. The diode laser, for example, because of its low cost, small size, simple power requirements and durability, will replace other solid state lasers and popular gas discharge lasers in

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many applications. Finally, fibrin glue has been used as a hemostatic agent without significant complications. In elective surgery, fibrinogen can be harvested from the subject. Addition of thrombin and calcium have been required to achieve hemostasis intra-operatively (16,17). We were unable to create durable anastomoses using this clotting system. Following laser exposure, however, a durable fibrin coagulum is achieved even without the use of thrombin and calcium. Laser "spot welding" may be useful for repair of anastomotic leaks as well as during creation of sutured anastomoses.

We have observed that argon ion laser welding of biliary tissue is facilitated by the bile and blood staining of the tissues [6]. Here we employ an exogenous compound to photosensitize vascular tissue for laser welding. Under identical experimental conditions, the use of FITC enabled welds to be formed at one-half the threshold laser power density as compared with non-FITC welds.

The threshold laser power density for welding without FITC is in agreement with previous reports (22). By using FITC, this threshold can be decreased. The lower energy required for welding minimized thermal damage to surrounding, healthy tissue. Additionally, because of the topical application of FITC, the energy deposition is localized to the area of the weld. The circumscribed zone of coagulation injury was apparent on the adventitial surface of the vessel without underlying thermal damage.

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The specificity of energy deposition is further enhanced by use of argon ion laser power outputs below 0.2 watts. In this range, an all-lines mode argon laser energy output is predominantly a 488 nm wavelength. This wavelength closely corresponds to the maximum absorption for FITC. At higher powers other wavelengths contribute to the total output and can be nonspecifically absorbed by surrounding tissue, leading to thermal injury.

Blood also appeared to act as an engaging endogenous chromophore. Welds were easier to create and appeared more durable if the aortic tissue was blood stained. In the bursting strength experiments, total elimination of blood from the field by copious irrigation prior to welding made welding without FITC much more difficult. With use of FITC, the lack of blood in the field was much less important. The FITC dye also helps to improve visualization of the weld site because of its bright green fluorescence as observed through standard argon ion laser safety goggles used to filter out laser light (Laserguard, Glendale, CA).

Extrinsic dye to enhance selective delivery of laser energy to tissue has been used in photoradiation of cancers, ophthalmologic vessel ablation, and diagnostic fluorescence imaging (10, 11, 12, 24). Use of exogenous dyes on target tissues may prove superior to use of endogenous chromophores by allowing greater control of local chromophore concentration and energy delivery.

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A variety of mechanisms may be responsible for fibrinogen soldering. The inability of urokinase to prevent welding with fibrin glue suggests that enhanced clotting of rabbit
5 blood with exogenous fibrinogen is not the dominant mechanism of soldering. More likely, heated fibrinogen undergoes covalent crosslinking independent of the coagulation cascade and is transformed into an insoluble, durable biologic glue holding together edges of the host
10 tissue. The amorphous coagulum is rapidly infiltrated with myofibroblasts and covered with host endothelial cells. Fibrin may be stimulating this rapid regeneration of vessel wall (18). As the fibrin glue is resorbed by histiocytes, myointimal proliferation continues until a new media has
15 been created. The internal and external elastic lamina, on the other hand, are not reconstituted. As has been reported by others (19,20), no foreign body reaction is evident. In effect, the fibrin solder consists of a biodegradable scaffold upon which the divided vessel heals. Use of dye
20 enhanced fibrin glue also reduces injury to tissue surrounding the weld and may allow more rapid proliferation of myofibroblasts and the deposition of the extracellular matrix necessary for regeneration of the vascular tissue at the welding site. The foreign body response to suture
25 material (19) and the irregular flow surface caused by the sutures are avoided. Other advantages include superior bursting strength, less red blood cell death and less plaque adherence to the intimal vessel wall. As our techniques

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have improved, the laser soldering method has also proven faster than sutured and conventional laser weld anastomoses, especially in smaller sized vessels.

5 Despite theoretical advantages, laser tissue welding has been hindered by technical obstacles and an inability to achieve consistent bonding. Use of dye enhanced protein solders obviates these difficulties and make clinical applications of laser welding technology feasible.

10 In the case of the 808 nm diode laser energy output, the output wavelength lies within the "optical window" of the vessel wall (13), thus failing to show any tissue effects even at the highest energy outputs available (9.6 watts/cm²). In this way, despite elevation of fibrinogen temperature during welding, the underlying vessel wall is not damaged. The solder-dye mix also provides a convenient means to apply the dye in a controllable fashion.

While two different laser/dye combinations have been described, namely an 808 nm wavelength near infrared diode laser with indocyanine green (ICG) dye, and an argon ion laser with fluorescein isothiocyanate (FITC) dye, other energy sources and energy absorbing materials may be used. The energy source need not be a laser, but could be any type of energy source such as for example an X-ray source, radiowave source, microwave source or non-coherent visible light source. The energy absorbing material need not be a dye, but could be any energy absorbing substance in any form which is used to absorb energy from energy sources. As in

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photodynamic therapy or diagnostic fluorescence imaging, a laser and dye combination can be chosen such that the laser's output wavelength matches the absorption peak of the dye, thereby providing efficient and target-specific laser energy delivery.

In the case of the 808 nm diode laser the output wavelength lies within the "optical window" of the vessel wall. That is, the laser fails to show any tissue effects even at the highest power outputs available (9.6 watts/cm^2). In this way despite elevation of fibrinogen temperature during welding, the underlying vessel wall is minimally damaged. The soldering agent-dye mix also provides a convenient means to apply the dye in a controlled manner.

Other dye/laser combinations which have been successfully tested are FITC dextran/argon ion laser, methylene blue, acridine orange, or rose bengal/dye laser, and Kodak Q-switch II/Nd:Yag (1064 nm) laser. Other possible dye/laser combinations include, for example, hematoporphyrin derivative/argon ion laser and tetramethyl rhodamine isothiocyanate/krypton ion laser.

It is preferable to use a dye that is water soluble, such as the FITC dye disclosed above. The dye and energy absorbing material are preferably mixed before application to the tissue; otherwise the dye will tend to run freely to areas which are not desired, such as inside an anastomosis. The soldering agent may be any substance which after exposure to the energy source above, especially in

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conjunction with one of the energy absorbing materials, is transformed into a biological glue which will hold tissue together. The solders may be synthetic or naturally occurring, biodegradable or permanent, and may require combination with solvents and gels in order to acquire the appropriate consistency for the welding process. In effect, the solder constitutes a biodegradable scaffold upon which the divided tissue heals. Use of dye enhanced solders also reduces injury to tissue surrounding the weld and may allow more rapid proliferation of myofibroblasts and the deposition of the extracellular matrix necessary for regeneration of the vascular tissue at the weld site. The foreign body response to suture material and the irregular flow surface caused by the sutures are avoided.

The soldering agent may have as a base composition human or animal proteins, such as bovine proteins. Serum proteins such as albumin or fibronectin may also be used. The tissue to be welded may already have a natural soldering agent such as collagen or elastin. These and other additives may change the viscosity of the overall soldering agent composition and make it easier to apply.

One may also wish to add one or more drugs to the energy absorbing material/soldering agent mixture so that the mixture may also serve as a slow release drug delivery system. One such drug may be a growth factor to improve wound and tissue healing.

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The present invention provides new means of apposing tissue to allow more natural healing. The techniques developed are quicker and in some instances safer than sutured repair.

5 The potential applications of the present invention include:
(1) primary tissue soldering, especially in vascular, colon, fallopian tube, tracheal, esophagus and neural anastomoses,
(2) repair of sutured anastomoses when leakage occurs, especially in cardiovascular surgery when the protein's
10 clotting mechanism is disrupted, (3) management of oozing surfaces, for example injured liver, renal, or splenic tissue following trauma, (4) repair of organs and endothelial layers within said organs, such as a colon lining, and
(4) secondarily to strengthen a sutured area. Additional
15 uses for the invention will occur to those skilled in the art and will become more widespread once the present technique becomes more widely available.

The method according to the present invention can be practiced on any living subject, human or animal. The
20 preferred subject is a human being undergoing surgical operations.

The relative quantities of the energy absorbing material and soldering agent which contact the tissue should be sufficient to the effect welding of the tissue. It is
25 possible that the tissue already has a natural soldering agent present so that such agent need not be added exogenously.

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Contacting the tissue with a soldering agent thus includes presence of a soldering agent endogenously and/or adding a soldering agent exogenously.

5 Similarly, the energy imparted to the energy absorbing material should be sufficient to effect heating of the tissue to result in welding. For example, when the energy is being imparted using a laser, factors such as the temperature and size of the tissue area to be welded will
10 vary, and therefore the amount of energy absorbing material, soldering material and energy level of the laser required should necessarily take these factors into account.

As used herein, the term welding includes welding in the conventional sense, as well as bonding and soldering.

15 Although a preferred embodiment of the invention has been disclosed and described herein, it should be understood that the present invention is in no sense limited thereby and its scope is to be determined only by that of the appended claims.

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TABLE 1. Dose-Response Curve*

Milliwatts	Seconds				
	15	30	60	90	120
5	Argon laser with FITC				
100	1	2	2	2	2
200	2	2	2	2	2
300	3	4	4	4	4
400	4	4	4	5	5
10	500	5	5	5	5
	Argon laser without FITC				
100	0	0	0	0	0
200	0	0	0	0	0
300	3	3	4	5	5
15	400	5	5	5	5
	500	5	5	5	5

*0, no observable effect; 1, blanching; 2, red; 3, curling of tissue after dessication; 4, carbonaceous deposition; 5, perforation

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of welding a tissue of a subject comprising:

contacting the tissue to be welded with an exogenous energy absorbing material having an absorption peak at a particular wavelength and which enhances energy absorption by the tissue at said wavelength relative to energy at other wavelengths, and an exogenous tissue soldering agent in sufficient quantities to permit a tissue weld to be formed; and

imparting energy having a characterising wavelength corresponding to the particular wavelength of the energy absorbing material and absorbable by the energy absorbing material to the energy absorbing material in an amount sufficient and under conditions so as to cause heating of the tissue and welding of the tissue.

2. The method according to claim 1, wherein the imparting of energy comprises imparting electromagnetic energy to the energy absorbing material.

3. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a preformed mixture of energy absorbing material and a tissue soldering agent.

4. The method according to claim 1, wherein the imparting of energy comprises imparting laser energy to the energy absorbing material.

5. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a dye.

6. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a dye having a dominant absorption peak at a particular



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wavelength and wherein imparting of energy comprises imparting laser energy having a dominant wavelength corresponding to the dominant absorption peak of the dye.

5 7. The method according to claim 1, wherein the imparting of energy comprises imparting of energy having a frequency component in the visible spectrum to the energy absorbing material.

8. The method according to claim 1, wherein the
10 imparting of energy comprises imparting energy having a dominant wavelength of about 808 nm to the energy absorbing material.

9. The method according to claim 8, wherein the
15 contacting of the tissue comprises contacting the tissue with a mixture of indocyanine green (ICG) dye and a soldering agent.

10. The method according to claim 1, wherein the
20 imparting of energy comprises imparting of energy having a dominant wavelength of about 488 nm to the energy absorbing material.

11. The method according to claim 10, wherein the contacting of the tissue comprises contacting the tissue with a mixture of fluorescein isothiocyanate (FITC) dye and a soldering agent.

25 12. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising human or animal fibrinogen.

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13. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising fibrin glue which
5 comprises fibrinogen combined with at least one fibrin glue activator.

14. The method according to claim 13, wherein the fibrin glue activator comprises thrombin.

15. The method according to claim 13, wherein the
10 fibrin glue activator comprises calcium.

16. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a tissue soldering agent comprising a cryoprecipitated suspension which comprises fibrinogen and Factor XIII, and a
15 fibrin glue activator comprising thrombin.

17. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising a polypeptide.

18. The method according to claim 1, wherein the
20 contacting of the tissue comprises contacting the tissue with a soldering agent comprising a serum protein.

19. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising albumin.

20. The method according to claim 1, wherein the
25 contacting of the tissue comprises contacting the tissue with a soldering agent comprising fibronectin.

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21. The method according to claim 1, wherein the imparting of energy comprises imparting laser energy continuously to the energy absorbing material.

22. The method according to claim 1, wherein the imparting of energy comprises imparting laser energy discontinuously to the energy absorbing material.

23. The method according to claim 1, wherein the contacting of tissue comprises contacting the tissue with an energy absorbing material and a tissue soldering agent applied into or on the surface of the tissue.

24. A tissue soldering composition for use in welding tissue comprising an exogenous energy absorbing material having an absorption peak at a particular wavelength and which enhances energy absorption by the tissue at said wavelength relative to energy at other wavelengths, and an exogenous tissue soldering agent in relative amounts sufficient to effect welding of the tissue when applied to the tissue and when a sufficient amount of energy is imparted to the composition.

25. A tissue soldering composition according to claim 24, comprising dye and fibrinogen.

26. A tissue soldering composition according to claim 24, wherein said energy absorbing material is indocyanine green (ICG) dye.

27. A tissue soldering composition according to claim 24, wherein said energy absorbing material is fluorescein isothiocyanate (FITC) dye.

28. A tissue soldering composition according to claim 24, wherein said soldering agent comprises human or animal fibrinogen.



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29. A tissue soldering composition according to claim 24, wherein said soldering agent comprises a polypeptide.

5 30. A tissue soldering composition according to claim 24, wherein said soldering agent comprises a serum protein.

31. A tissue soldering composition according to claim 24, wherein said soldering agent comprises albumin.

32. A tissue soldering composition according to claim 24, wherein said soldering agent comprises fibronectin.

10 33. A tissue soldering composition according to claim 24, wherein said soldering agent comprises thrombin and calcium.

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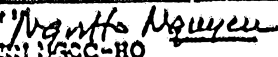
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INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05125

.. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC 5: A61N 5/06 A61B 19/00;		
US: 128/343, 397, 398, 898; 606/8		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System ¹	Classification Symbols	
	606/8	
US	128/395, 397, 398, 898	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁴		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁰ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁴
X	Lasers in surgery and medicine Vol. 9, Vol. May, 1989, Saur, "The first sutureless End-to-End Bowel Anastomosis" pages 70-73	1,2,4,5,12-14 17,18,20,21,23-25 28-30,32,33
X	The Journal of Urology; Vol. 139, February 1988, Poppo et al, "Laser Welding in Urethane Surgery: Improved Results with a protein solder" pages 415-417	1-6,12-20,23, 24, 28-33
X	Journal of Surgical Research, Vol. 45, Vol. July 1988, Grubbs, Jr. et al, "Enhancement of CO ₂ Laser Anastomosis by Fibrin Glue" pages 112-119	1-16,12-18,20, 22-25,28-30,32, and 33
		5-11, 21-23,26 27
<p>⁸ Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ¹	Date of Mailing of this International Search Report ¹	
30 November 1990	26 FEB 1991	
International Searching Authority ¹	Signature of Authorized Officer ¹	
ISA/US	 NGUYEN NGOC HO INTERNATIONAL DIVISION	
In David Shay		

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US,A 3,769,963 (Goldman) 06 November 1973 column 2 lines 10-32	5-11,21-23,26, 27
Y	US,A 4,774,339 (Haugland) 27 September 1988 see column 1, lines 51-65	5,6,10,11,21-23 27
Y	US,A 4,805,623 (Jobsis) 21 February 1989 see column 23, lines 6-9	5-9,26
Y	US,A 4,457,992 (Bhattacharjee) 03 July 1984 see column 5, lines 40-45 and column 6, lines 30-33	5-9,26
Y	Journal of Vascular Surgery, Vol. 6, No. 5, November 1987, White et al, "Argon laser-welded arteriovenous anastomoses" pages 447-453	5-9,10,11,21-23 26,27

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers _____, because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim numbers _____, because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this International application as follows:

1. As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.
2. As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
3. No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remarks on Protest:

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.

STANDARD ABSTRACT AND ABRIDGEMENT WORKSHEET
 PATENT

P/01/005

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ACTION BY

LODGEMENT/ ACCEPTANCE	21 Application No. 64071 1990	Serial No. 646406
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EXAMINER	24 E.P.D. (if different from earliest date at INID 32)		
	31 Priority Document No(s) (earliest first)	32 Date Of Filing	33 Country Code
/...../.....

54 Title (if title on application form unsuitable)

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60 Cognate Application Nos.

61 Additional To Application No.

62 Divisional Of Application No.

57 Brief Summary Of Inventive Concept And Other Noteworthy Disclosures
 (or identify a suitable extract in the space provided)

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Examiner's Initials

Senior Examiner's Initials

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Claim No. Or Claims * Δ 0	Explanatory * Δ 0 Extract On Page →	Lines →	* Δ 0
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ACTION BY

54. Title

56. Related Art (in chronological order, most recent first)

CC	Publ'n Level	Patent No.	App. No. If AU	Class Marks If AU

57. Brief Summary Of Inventive Concept (or identify a suitable extract in the space provided below)

Extract Inventive Concept From Specification At Page → Lines →

Claim No. Or Claims	Lodged On	Amended By Items
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Examiner's initials
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Senior Examiner's Initials

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Request Application

Specification Drawings

ACCEPTANCE