The physical and biological properties of laser/light activated soldered micro-anastomoses

By

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The work on which this thesis is based is my own independent work except where acknowledged

J F Birch

October 2002

Dedicated to Julia my wife

"I want to know Gods thoughts – the rest are details"
Albert Einstein (1879-1955)

"The lyfe so short, my craft so long to lerne,
Th' assay so hard, so sharpe the conquering."
Geoffrey Chaucer (1346-1400)

"Of making many books there is no end,
and much study wearies the body...."
Ecclesiastes 12:12 (3rd Century, BC)

Synopsis

The ideal anastomosis is one that reproduces the continuity of the vessel, causing the least disruption of blood flow and retains the endothelial lining of the vessel without inducing a foreign body or immune reaction. Laser anastomosis caused aneurysm formation and led to the introduction of tissue solder. These reduced the complications but had found limited application in clinical practice.

In conjunction with Tissuemed™ it was hoped to produce a commercially viable tissue solder that could be used in the formation of vascular anastomoses. Due to the commercial nature of the project, much of the experimental design and rationale were dictated by the requirements of the regulatory authorities (LRQA) for CE marking.

Initial work focused on the precise proportions of MB in the solder, while PSA proportions were well reported in literature. Changes in the concentration of solder chromophores had not been investigated or reported by other groups. It was therefore proposed to characterise the solder strength as a function of MB concentration and solder absorption. The solder was seen to deviate from a linear Beer-Lambert relationship. As a result of this study, MB concentration of 0.24%w/w was chosen for inclusion into the solder to be submitted to LRQA.

The requirements of the regulatory authorities included data on the solder reabsorbtion, as well as histological evidence of its biocompatibility. In order to determine the lifespan of the solder *in vivo* radio-labeling was undertaken and histological markers, such as thrombosis, intimal hyperplasia and chronic inflammation, were investigated. The hypothesis for this experiment was that the solder did not remain in the body for more than 90 days and could therefore be classified as a temporary implant (EU Council directive 93/42eeu) and would not induce an adverse tissue reaction. As a result of this study it was noted that improved patency could be achieved with a lower laser power.

For reasons of cost and safety a move away from lasers was made. In place a filtered xenon arc lamp was constructed. Equivalence with the laser was demonstrated both *in vitro* and *in vivo*, showing that the lamp was able to produce a soldered anastomosis of similar burst strength and histological appearance to lasered anastomoses.

Previous studies had not looked at solders *in vivo* beyond 90 days. Since the onset of aneurysms from lasered anastomoses were a late event, it was essential to demonstrate that this had

been resolved with the use of solders. Three and six month anastomoses were produced and showed a complete absence of aneurysms or other complications.

The solder contains porcine albumin and therefore a measure of the antigenicity of this protein was determined by observing the immune response. A worst-case scenario was produced with pre-sensitised and naïve animals. No pathological effects were observed either systemically or in immune complex deposition in the kidneys. These results together with the previous data contributed to the solder and white light source being awarded CE marks.

Vascular compliance is an important factor in both short and long term patency. Therefore the physical characteristics of the solder were investigated by measuring the compliance compared to a continuous sutured anastomosis. It was determined that there was an increase in compliance associated with the use of solder over continuous but not interrupted suture techniques. The use of the solder as a sealant was seen to leave the sutured anastomotic compliance unchanged.

Finally, as a prelude to clinical studies, the use of solder as a haemostatic sealant was studied in cases of anastomoses formed using ePTFE graft material. There was a significant reduction in blood loss ($p<0.05$) and bleeding time ($p<0.05$) but the difference in overall haemostatic time was not significantly altered over simple compression ($p=0.065$).

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Much of the *in vitro* work was carried out at the Department of Chemistry, University of Loughborough under the supervision of Professor F Wilkinson. I am most grateful to him and to Dr S Worrall for her work in measuring solder absorption (Ch. 2) and Dr D Worrall for his help and advice.

The *in vivo* work carried out in this thesis was conducted both in the Comparative Biology Unit at the Royal Free Hospital and Biomedical Services at the University of Leicester and I would like to express my thanks to Mr D Moore at the Royal Free and the Staff at Biomedical services for their patience and invaluable assistance.

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Abbreviations

bFGF	Basic Fibroblast Growth Factor
BP	British Pharmacopoeia
BPT	Burst pressure testing
BSA	Bovine Serum albumin
BSE	Bovine Spongiform Encephalopathy
CA	Cyanoacrylic
CABG	Coronary artery bypass grafting
CE	Conformité Européene
cm	Centimetre
CJD	Creutzfeld-Jacob Disease
CO_2	Carbon dioxide
^0C	Degrees Centigrade
cGMP	Cyclic Guanosine Monophosphate
CMAP	Compound muscle action potentials
CSA	Conventional sutured anastomoses
CSF	Cerebrospinal Fluid
D	Diopters
EDTA	Ethylene-diamine-tetra-acetic acid
EMG	Electromyography
ePTFE	Expanded polytetrafluroethylene
EVG	Elastic Van Geison
FITC	Fluorescein Isothiocyanate
HSA	Human Serum Albumin
H&E	Haematoxylin and Eosin
HB EGF	Heparin Binding Epidermal Growth Factor
HIV	Human Immunodeficiency Virus
Ho:YAG	Holmium Yttrium Aluminum Garnet (Laser)
Hz	Hertz
ICG	Indocyanine Green
IH	Intimal hyperplasia
IM	Intramuscular
iu	International units
IV	Intravenous
J	Joules
Kg	Kilogram
KTP	Potassium Titanyl Phosphate

L2K	Laser 2000
LAFB	Laser Activated Fibrinogen Bonding
LRQA	Lloyds Register Quality Assurance
M-α-CA	Methyl-alpha-cyanoacrylate
MB	Methylene Blue
MHz	Mega Hertz
mins	Minutes
mg	Milligram
mm	Millimetres
mmHg	Millimetres of mercury
ms	Milliseconds
Nd:YAG	Neodynium:Yttrium Aluminum Garnet (Laser)
nm	Nanometres
NO	Nitric Oxide
NOS	Nitric Oxide Synthetase
O_2	Oxygen
%	Percentage
PBS	Phosphate Buffered saline
PC	Personal Computer
PCM	Percutaneous compliance measurement
PDS	Polydioxanone
PHZ	Para-anastomotic Hypercompliant Zone
PLS	Polychromatic light source
PSA	Porcine serum albumin
PSI	Pounds per square inch
PTFE	Polytetrafluroethylene
PTWC	Photothermal wound closure
RF	Radio Frequency
R5P	Riboflavin-5-phosphate
SCS	Standard Continuous Suturing
SEM	Scanning electron microscopy
TGF-ß1	Transforming growth factor beta 1
THC:YAG	Thulium Holmium Chromium Yttrium Aluminum Garnet
ULS	Unilink system
µm	Micrometre
W	Watts
WLS	White light source
w/v	weight for volume
w/w	weight for weight

Publications Arising from this Thesis

Published Papers

Methylene blue based protein solder for vascular anastomoses: An *in vitro* burst pressure study
Birch JF, Mandley DJ, Williams SL, Worrall DR, Trotter PJ, Wilkinson F, Bell PR
Lasers in Surgery and Medicine; 2000 26(3):323-329

Photon activated biological adhesives in surgery.
Mandley DJ, Birch JF, Williams SL, Trotter P, Wilkinson F, Davies GA
International Journal of Adhesives and Adhesion; 2000: 20: 97-102

Methylene Blue Soldered Microvascular Anastomoses *in vivo*
Birch JF, Bell PRF
European Journal of Vascular and Endovascular Surgery
Vol. 23, No. 4, April 2002: 325-330

Presentations Arising from this thesis

Minimal suture laser activated solder anastomoses *in vivo* (Oral)
The British Association of Plastic Surgeons (Summer Meeting)
International Convention Centre, Birmingham July 7th 2000

Minimal suture small vessel anastomoses using laser activated protein solder *in vitro* (Oral)
The British Association of Plastic Surgeons (Winter Meeting)
The Royal College of Surgeons, London Dec 3rd 1999

The compliance of laser tissue soldered anastomoses *in vivo* (Oral)
The Surgical Research Society
The Royal Free Hospital Dec 3rd 1999

The Haemostatic effects of activated protein solder as a reinforcement to anastomosis in ePTFE (Oral)
The International Symposium on Biomedical Optics
The Society for Photo-optical instrumentation engineers (SPIE)
San Jose, CA, USA Jan 22nd 2000

The compliance of laser tissue soldered anastomoses *in vivo* (Oral)
The International Symposium on Biomedical Optics
The Society for Photo-optical instrumentation engineers (SPIE)
San Jose, CA, USA Jan 22nd 2000

The haemostatic effects of Laser tissue solder as a re-inforcement to anastomoses with PTFE grafts
The International Symposium on Biomedical Optics
The Society for Photo-optical instrumentation engineers (SPIE)
San Jose, CA, USA Jan 26th 2003

Methylene blue solder re-absorption in microvascular anastomoses
The International Symposium on Biomedical Optics
The Society for Photo-optical instrumentation engineers (SPIE)
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Chapter 1

Introduction:

An historical review of sutureless anastomotic devices, laser and solder anastomoses

An historical review of sutureless anastomotic devices, laser and solder anastomoses

The development of the techniques used in forming sutured vascular anastomoses began in the eighteenth century with sporadic reports of individuals attempting to use a variety of techniques and materials. Early results were universally poor but with the pioneering work of Jassinowsky (Jassinowsky, 1891) and Carrel (Carrel, 1908) the triangulation technique of anastomosis was developed and attained widespread success. The sutures used were mainly silk, with synthetic materials becoming used in the 1930's. Since that time the major development in vascular anastomoses was the use of suaged needles that reduced vessel trauma and bleeding from suture holes. Microvascular surgery had been attempted for many years but was eventually reported successfully in 1960 by Jacobson et al (Jacobson, 1960) followed by other developments from O'Brien (Baxter , 1972) and Acland (Acland, 1973).

Sutures have the advantages of versatility, simplicity, strength and cost, being easily applied to most areas of surgery and with a track record in vascular surgery going back nearly sixty years. The vascular repair is common in surgical practice today, using either continuous or interrupted monofilament polypropylene sutures. Both of these techniques will result in an area of reduced compliance, which has been shown to be predictive of anastomotic patency (Baird et al, 1977). Interrupted sutures have less of an effect on compliance than a continuous method, but are rarely used in larger vessels. Sutures also cause foreign body reaction at the anastomotic site increasing their thromobogenicity and propensity to intimal hyperplasia. They also require the use of sharps increasing the risks of needlestick injuries in the post-HIV era and are difficult to use in areas of reduced access or via an endoscope.

For many years, attempts were made to perform vascular anastomoses without sutures. Even with the success of sutured repairs, the need for sutureless anastomosis has not diminished. On the contrary with the advent of minimally invasive surgery the need to be able to perform sutureless vascular anastomoses is as real as ever. Several techniques of clip anastomosis are still under investigation and those such as the Heartflo^{TM} and VCS clips have entered clinical practice. The ability to be able to anastomose blood vessels by endoscopy or other minimally invasive techniques would enable the surgeon to perform procedures never before possible with reduced trauma to the patient and the blood vessel.

1.0 Early vascular anastomotic devices

The descriptions of devices used in vascular anastomoses can be divided into Butt (Fig 1a), Lapped (Fig 1b) or Flange (Fig 1c) type joints. Butt joints were initially formed using tubes, which later evolved with the application of adhesives such as fibrin and cyanoacrylics. Lapped joints were formed with the use of ring devices of various configurations as are flange devices. In addition, staples and clips have also been used to form flange joints.

The search for the devices to aid anastomosis began in 1759 by Hallowell (reported in Watts, 1907) who thrust a pin through a tear in a brachial artery and tied a suture around it. As the pin was withdrawn the suture tightened closing the defect.

The further history of arterial repair techniques is described by Murphy (Murphy, 1897) who reports that the first attempt at suture repair was made by Broca in 1792, successfully repairing a longitudinal incision in an artery. The English surgeon Henry Lee (1865) used an undescribed method nonsuture of anastomosis, followed by Gluck in 1883 who used aluminum and ivory clamps. Tansini of Moderna (1890) tried to use horn clamps to close arteriotomy while the first report of suture repair in a clinical scenario was by Heidenhain (1894) who used catgut to repair a defect in an axillary artery. Murphy himself was more concerned with suture anastomosis, using fine silk and even Kangaroo tendon in some cases.

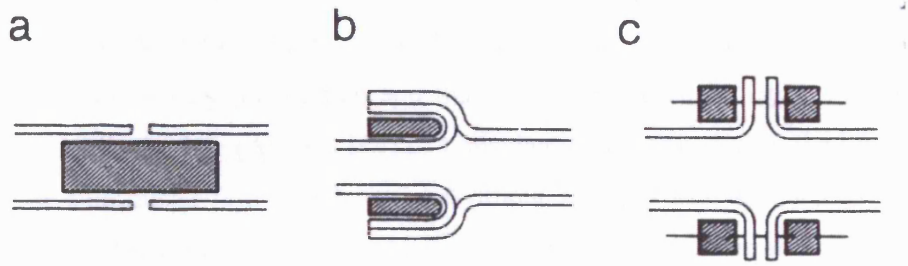


Figure 1: a Butt, b Lap and c Flanged joints

1.1 Butt Joints

Tubes - *Glass*

The first description of a nonsuture technique was by Abbe (Abbe, 1894). He questioned whether or not it would be possible to transplant limbs since it was known that suturing of nerves, tendons and veins was possible. He inserted thin glass tubes with an hour glass constriction, into arteries secured with a silk ligature. The limb in which this was performed survived but post mortem showed the tube free in the vessel with evidence of endarteritis. Glass cuffs were used by Queirolo (reported in Weiss, 1950) in 1895, where the portal vein was brought through the cuff and secured to the glass by a ligature, while in 1897 Nitze (Nitze, 1897) recommended the use of ivory rings.

Payr (Payr, 1900) used tubes constructed of glass celluloid, aluminum, hard rubber, or precious metals, settling for magnesium rings, as he believed that an absorbable material would be advantageous and that simple suture techniques would not withstand the pressure of larger vessels. However, unknown to Payr, magnesium forms a highly basic and histotoxic salt on hydration causing a violent tissue reaction and mysterious gas cysts that he describes, as reported by McBride (McBride, 1938).

Hopfner (reported in Watts, 1907) also undertook similar experiments with no greater success and concluded that transplantation had very little to offer. Despite this Ullmann used the Payr rings to transplant a kidney into the neck of a dog. The organ is said to have survived and produced urine but no mention is given as to the duration of survival.

In 1912, in the same year that he was awarded the Nobel Prize for Medicine, Carrel (Carrel, 1912) discussed the use of tubes for the joining and replacement of blood vessels. He conceded that glass intubation was not original and that it had been used by Brewer (reported in Carrel, 1912) for transfusion and Abbe (Abbe, 1894) in transplantation. Armed with the knowledge that foreign bodies may be introduced into blood vessels without accident but with thrombosis in some cases, Carrel hypothesised that thrombosis may be due to a preventable cause. He therefore used Parafinised rubber and this was reported as patent for 15 months. Further to this, plain and gold plated aluminum tubes were used, as well as the glass tubes used by Abbe. The results were mixed, with 100% thrombosis with glass tubes, while the aluminum tubes thrombosed due to corrosion. Gold plating had no better results and he concluded that the use of smooth edged gold or vein lined tubes may have better results. Johns (Johns, 1950) found the same ubiquitous thrombosis using glass rods.

Metal

Aluminum tubes were first used by Payr (Payr, 1900) and then by Carrel (Carrel, 1908) as described above. Tuffier (Tuffier, 1915) continued with parafinised silver tubes to bridge arterial defects in casualties of the first world war, a principle that was to be later taken up by Blakemore (Blakemore , 1942) during world war II. Tuffier had success for up to ten days in one case using this technique, but Makins (Makins, 1919) recommended that the tube should be removed at four days as the tubes are invariably thrombosed at that point. Blakemore (Blakemore, 1943) described the use of Vitallium lined tubes for the rapid non-suture approximation of vessels, and also for use in bridging defects in arteries and veins. The three problems to be overcome in these circumstances were the prevention of 1) thrombosis 2) infection and 3) pressure necrosis. Previous attempts to use tubes in this way had had almost no long term success. With the end of the war, Stewart (Stewart, 1947) described the clinical use of vitallium tubes in leg wounds with vascular injuries. Three out of the seven patients showed patent vessels with the tubes, while the other four went on to amputation. Johns later used vitallium to perform porto-systemic anastomoses with an 85% thrombosis rate (Johns, 1950).

Tantalum had been extensively used in neurosurgery for fracture fixation and was well known as an inert material, was used by Weiss (Weiss, 1950) to construct tubes. These were used in 25 canine femoral arteries as unlined, parafinised tubes, vein lined tubes and two-tube vein lined tubes. The two-tube vein technique showed an 86% patency rate, leaving Weiss to conclude that tantalum was as effective as vitallium in constructing non-suture anastomoses, corroborating Blakemores' findings.

The use of metal tubes in sutureless anastomosis was superceded by other methods until 1996. Mikaelsson (Mikaelsson, 1996) described intra-vascular cylinders of titanium with a male and female coupling arrangement for use in microsurgery. The vessels used were 1.1-1.3mm rat aortas and the prostheses were secured by dexon slings. The patency rate described was an impressive 100% at all time points, with histological evidence of complete endothelialisation after 17 days.

Soluble tubes

Smith (Smith, 1940) introduced the idea of a soluble dextrose tube, caramelised at 160°C, formed within a sterilised rubber stent and coated with gelatin. The vessels were secured over the tube while suturing occurred. While this technique relied on suturing of the vessel it demonstrated the progression of ideas in vascular anastomosis from mechanical to suture techniques.

At around the same time, Swenson (Svenson, 1947) developed fibrin tubes for non-suture anastomosis. This material had previously been used as a dural substitute, was easily made and well

tolerated by tissues being completely reabsorbed within 6-7 weeks. He reported a 100% patency rate at 3 and 6 weeks with no evidence of fibrin present at 6 weeks.

Ballinger (Ballinger, 1963) repaired vessels with absorbable stents made of gelatin in conjunction with 2-methylcyanoacylate. The tube dissolved in 15 minutes when exposed to blood at body temperature. The gelatin tubes acted to dilate the spastic artery and provided a rigid structure on which to perform the anastomosis. To reduce their brittleness the tubes were moistened in saline before use and secured the tube using silk ligatures. Anastomoses were formed both using conventional sutured anastomosis and adhesive. However, the use of the adhesive resulted in haemorrhage and haematoma formation as soon as the gelatin tubes dissolved.

Synthetic tubes

Blakemore (Blakemore, 1943) had first described the use of polymer tubes of unrecorded composition in several clinical cases. The condition of these patients was severe and results were poor, but it was pointed out that polymers had the advantage of flexibility over other materials.

In 1952 polymer tubes were used to repair defects in veins. To further the progression of surgery to the pancreas, duodenum and splanchnic bed, vein grafts were used by Daniel (Daniel, 1952), secured using a non-suture technique. After Hufnagel's (Hufnagel, 1947) work with Lucite (Methyl-methacrylate) and Teflon (Hufnagel, 1955) in bridging defects in arteries, attempts were made to reproduce the data in veins. Daniel (Daniel, 1952) used Lucite and Teflon to bridge defects in the portal vein. The results showed 100% occlusion rate with a 70% death rate. A second study used the tubes as stents in supporting the vessels while suturing of the vein took place and resulted in four patent grafts.

Hufnagel (Hufnagel, 1947) used Lucite for the permanent intubation of the thoracic aorta. The appeal of Lucite was its inert nature, low cost, simplicity of manufacture and apparent ability to resist thrombosis. Rigid tubes were used in this series of 15 anastomoses, with the common result that the ligatures cut through the vessel causing fatal haemorrhage. In 1955, Hufnagel (Hufnagel, 1955) continued with a description of a rigid nylon tube fixed with a serrated 'O' ring, which was itself secured with a silk ligature. Flexible prostheses were also used made of Dacron and Orlon, scored with a nylon 'V' ring and secured with a ligature. No systematic review of the results of these systems was given but mention is made of the clinical application of flexible grafts, and their use in 15 cases.

Egdahl (Egdahl, 1956) evaluated vein lined polyethylene tubes with a non-suture anastomosis to bridge aortic, femoral and carotid defects. The replacement of vein was also discussed with and

without a vein lining. The results in the arterial group showed 100% patency, while venous replacement with a vein lining in perforated tubes was superior to those with a solid tube. No objective data was given on patency and he concludes that while a non-suture method was of benefit in the arterial system, the use in the portal venous system was not recommended.

ADHESIVES

Cyanoacrylics

Adhesives in vascular surgery were first reported by Nathan et al (Nathan, 1960). After having found that several adhesives were well tolerated by tissues and were adherent to them, studies were made using Methyl-alpha-cyanoacrylate (M-α-CA). Several other compounds were tested, but only M-α-CA was found to be strong enough to repair tissue. The tissue repair histology reports stated that there was a "severe tissue reaction". Later studies focused on the closure of incisions in the aorta of which 10% of the animals died of fatal haemorrhage. In later procedures, this was prevented with the use of a Teflon patch. Histology again showed an intense fibrotic reaction, with small false aneurysms. Nathan described the ideal adhesive as being:

1) Capable of rapidly adhering to moist tissue
2) Sterilisable
3) Flexible
4) Biologically inert

According to this description M-α-CA was both histotoxic and brittle. Further reports of the histotoxicity of cyanoacrylic adhesives on blood vessel walls came from Weissberg and Goetz (Weissberg, 1964). This study used canine carotid arteries with M-α-CA painted on to the surface of the vessels. Fusiform dilations of the vessels were found at the site of application of the adhesive. Extensive necrosis has also been found at the site of application of the adhesive to liver and skin.

A new formulation of CA adhesive was described by Ota (Ota, 1965) composed of α-Ethyl-cyanoacrylic (Trade name-Aron-α-S2). Clinical results were published from 21 patients in which vascular prostheses had been implanted using a wrapping technique, to replace abdominal aneurysms, place vein patches or repair of vessels including the carotid. Various individual results were reported but no consistent or systematic presentation of data were given and α-Ethyl-cyanoacrylic was still described as less thrombogenic than M-α-CA. The advantage and utility of adhesives over and above fixed mechanical devices was emphasised.

The histotoxic side effects of cyanoacrylics were again well documented in Vinters (Vinters, 1985) review article, where the histotoxicity of the adhesive was blamed on the exothermic nature of the reaction, by products of the polymerisation reaction and breakdown products.

Fibrin

Fibrin powder was used to seal anastomoses by Bergel (Bergel, 1909) in 1909 but the move from cyanoacrylic adhesives took place in 1977 with the use of fibrin adhesive by Cioffi (Cioffi , 1977). The use of fibrin adhesive alone to form anastomoses was described by Karl (Karl, 1981) in 1981 while Gestring (Gestring , 1983) in 1983 used fibrin from the patients own blood. The use of this adhesive was described as simpler, faster, with increased patency. The use of autologous fibrin was intended to remove the threat of infection from HIV and hepatitis B. Ikossi-O'Connor (Ikossi-O'Connor, 1983) used a similar fibrin based system including factor XIII, fibrinogen, insoluble gelatin, aprotonin, and platelet growth factor with $CaCl_2$.

Sagi (Sagi, 1986) later used fibrin adhesive in conjunction with vicryl rings. The adhesive, Tisseel, was applied to the outside of the everted vessel on the rings, but these disintegrated within seconds of the clamps being removed. The additional use of polyethylene stents was described by Kamiji et al (Kamiji, 1989). The adhesive was composed of two solutions of fibrinogen and thrombin, aprotonin and calcium to complete the clotting cascade and applied as a monolayer or several layers. The anastomoses were studied at 4, 6 and 28 days, showing that all remained patent and that normal healing was apparent. Burst pressure testing of the vessels showed strength up to 250mm H_2O and was considered suitable for vessels down to 0.5mm or less.

The thromobogenicity of fibrin sealants was reported by Frost-Arner et al (Frost-Arner, 2001). A number of thrombin based sealants were used in this study to seal sutured microvenous anastomoses in a rat epigastric flap model. High levels of thrombin were seen to adversely affect flap survival, compared to favourable results with lower concentrations.

Kheirabadi et al (Kheirabadi, 2001) further tested fibrin sealants of different concentrations in a cardiovascular model. Rabbit aortic transections were sutured and sealed. These were subsequently tensile tested *in vitro*. The results showed that tensile strengths were not significantly different, but the use of 4 stay sutures did not prevent the animals from exsangunating.

Other advances in the use of adhesives was reported by Dumanian (Dumanian, 1995). Here a non-biological photopolymerisable adhesive was studied with respect to its mechanical and biochemical interactions with human blood vessels. The adhesive is based on polyethylene glycol 400 diacrylate cured with ultraviolet light and was used on human umbilical vessels transmitting saline. Results showed no significant increase in platelet deposition compared to sutured anastomosis. There was a significant reduction in the level of 'oozing' from the vessels after the application of the adhesive.

1.2 Lapped Joints

After the initial work performed by Payr into the use of rings, further work by other authors publishing similar data (Lespinasse, 1910; Landon, 1913), produced very similar results.

It was not until 1967 that Gottlob (Gottlob, 1937) proposed a new method specifically with small vessels (<2mm) and veins in mind. His technique combined the use of 'bushings' (rings) of PTFE and adhesive (Alkyl-cyanoacrylates). The bushings were secured to the outside of the two ends of the vessels by the glue. A sleeve was then used to join the two bushings together. The development of bone bushings and a collagen sleeve was described but problems arose with the application of cyanoacrylic adhesive to the outside of vessels. In arteries it resulted in medial necrosis, calcification and leucocytic infiltration. The endothelium was spared in arteries, but in veins lesions occurred. Therefore a technique was developed, in which the adhesive was only applied to the bushing. The technique was only used in an experimental setting and anastomoses formed in the thoracic and abdominal aortas in rabbits and cats. The poor long term results were put down to adhesive histotoxicity and the disadvantages of leaving foreign material.

No new devices appeared until a Russian publication in 1955, in which Donetsky (Donetsky, 1955) described a circular barbed ring for use in vessels of 2-20mm. The technique is an evertion one, and is described as being usable even in difficult locations. Both experimental and clinical use of the ring is reported in such cases as congenital defects of the heart and in arterial aneurysms. Patency data was not given.

Other ring devices using the same principle were described by numerous other authors including Carter (Carter, 1957), Goetz (Goetz, 1961), Guyton (Guyton, 1979), Daniel (Daniel, 1984), Euler (Euler, 1989) and Hamano (Hamano, 1996). The details of these many devices are omitted due to their similarity in design or application.

The most recent ring device was reported in 1999 by Qui et al (Qui, 1999). This ring was made of a co-polymer of lactic and glycolic acid and applied in an animal model. A patency rate of 100% was described compared to 83% of sutured anastomoses, although the anastomoses took upto 75 mins to perform.

1.3 Flange joints

The anastomoses of small blood vessels had reached a hiatus after the Second World War. Accepted practice was to use silk ligatures, which inevitably resulted in thrombosis in smaller vessels, so researchers endeavoured to produce an effective means of micro-anastomosis. Stapling was considered by many to be a viable route of investigation, being less traumatic and faster than conventional sutured anastomoses.

Staples

Androsov (Androsov, 1956) published details of a stapling device in the Journal of the American Medical Association. His aim was to reduce the time taken and difficulty of sutured anastomoses. The device was based around 'U' shaped clips, which are then bent to 'B' shaped staples. The vessels were aligned intima to intima, and vessels joined with diameters ranging from 1.3 to 15mm. Experimental and clinical applications of the device were described with reasonable results. However specifics were not given as to the precise long and short term patency rates, as well as the time taken for each procedure. The disadvantages of this system were the large number of parts of the stapler and its cost.

A similar device was described by Inokuchi (Inokuchi, 1958). This device also relied on 'U' staples, but in this instance the vessels were stapled to bushings. Evidence was given of the lack of platelet adherence when compared to manual suture techniques, though the technique of platelet counting or absorption was not given. In addition, no mention was made of the calibre of vessels that were joined, or of the time taken to perform the anastomosis. Further data was published in 1961 (Inokuchi, 1961a) based on an end to side arrangement of anastomosis. This was performed in 7 patients with a variety of conditions requiring porto-systemic bypass. Again the results were mixed and poorly reported. The thombogenicity of this system was later described (Inokuchi, 1961b) and compared to Carrels suture technique. Platelet deposition was greatest in the sutured anastomoses.

Another stapling device was described by Takaro (Takaro, 1960) with an emphasis on simplicity and versatility for the speedy application but showed no advantages over previously described devices.

Vogelfanger et al (Vogelfanger, 1958) described a different device that primarily addressed the problem posed by the anastomosis of vessels 2-3mm in diameter. A canine femoral model was used to

perform 157 anastomoses of 2-3mm. Of the arteries joined thrombosis occurred in 14% as opposed to 13% in veins.

The need for simpler devices was emphasised by Cooper (Cooper, 1962), which could staple smaller vessels more efficiently with fewer parts. Such devices were produced by Cooper and later Nakayama (Nakayama, 1962), for use with vessels of 1.5 to 5mm. Nakayama's device is further described and compared to Androsov's stapler by Bellman in 1967 (Bellman, 1967). The Nakayama device was described as less traumatic on the vessel and easier to use particularly in smaller vessels but no overall judgements were made as to the suitability of one system over the other.

The Vascular Closure Staple (VCS) system was reported in 2001 (Sultan, 2001). This device was reported as being used in femoral and carotid endarterectomy, as well as A-V fistula formation and the insertion of ePTFE grafts. This report states that the anastomotic time was significantly faster than suturing in femoral endarterectomy ($p<0.001$), ePTFE to femoral anastomosis ($p<0.001$) and carotid endarterectomy ($p<0.01$), but not A-V fistula formation ($p=0.26$). . More recently Birth et al (Birth, 2002) descrbed the VCS for use in the biliary tract *in vivo.* This was reported as superior to ssutures in terms of post operative biliary blood flow

A biodegradable ring device was recently described by Ichikawa (Ichikawa, 2001). In this study the device was used to form rabbit femoral arterial and venous anastomoses, which were mechanically tested. Evaluation of the anastomoses showed that arterial anastomoses were significantly stronger ($p<0.01$) but not venous anastomoses.

A number of further devices were described by Scheltes et al (Scheltes, 2000) and Mokros (Mokros, 2001), including staple devices for rapid coronary anastomosis. In these papers the devices were simply characterised rather than compared *in vitro* or *in vivo* situations

Unilink System

The final chapter in the history of Flanged mechanical anastomotic devices goes to the Unilink system (ULS) described by Östrup (Östrup, 1986). The ULS is capable of both end to end and end to side anastomoses and is composed of gauges to measure the vessels, the anastomotic instrument, ring pins, microhooks, and forceps for mounting the vessel wall. The anastomosis can be completed in as little as 2 minutes by an experienced operator as compared to 40 minutes using CSA.

The strength of the ULS was compared to CSA by Gilbert et al (Gilbert, 1988) and by Ragnarsson (Ragnarsson, 1990) and Zhang (Zhang, 1996). Early results in arteries showed the ULS to be significantly stronger than CSA, a situation than is reversed at 16 weeks. Venous anastomoses

showed no differences. The point is emphasised that medial and adventitial thinning occurs at the site of the ring with ULS contributing to its relative weakness at 16 weeks. Gilbert et al later looked at patency rates, anastomotic time and histological changes when a venous bridging graft is inserted using the ULS and CSA (Gilbert, 1989). The ULS had a 100% patency rate at 16 weeks compared to 85% with CSA. ULS anastomosis took 12 minutes (mean) versus 41.9 minutes for CSA. Histological findings were mixed in both groups with variable intimal hyperlasia. Full endothelialisation was present at 2 weeks for ULS and CSA but with evidence of giant cells and a foreign body reaction in ULS anastomoses. Arterialisation progressed from 2 to 16 weeks.

A long term evaluation of the ULS was reported in 1992 (Ragnarsson, 1992). Distal to the arterial anastomosis an area of fusiform dilation was noted in 8/20, and on both sides in veins while light microscopy showed no evidence of acute inflammatory cells but a foreign body reaction was evident. Distal to the anastomosis there was marked thinning of the vessel wall due to medial atrophy as previously encountered.

The clinical application of the ULS is restricted to a few studies. In 1989 Nylander et al (Nylander, 1989) described their experience in cases requiring microsurgery to the hand while Sasson et al (Sasson, 1994) used it to perform free flap tissue transfer. In each case high rates of patency were described with slight narrowing at the anastomosis but no gross morphological complications.

More recently, Cope et al (Cope, 2001) published a clinical series on the use of the Unilink system as applied to microvascular anastomoses in flaps. These were used to perform 99 venous and 4 arterial micro-anastomoses over a 5 year period. The anastomosis was successful in 99/104 anastomoses, with 5 requiring revision and 1 late thrombosis. Anastomotic time was described as less than 5 minutes in each case.

1.4 Laser Anastomoses

The use of lasers to perform sutureless anastomoses was predated by reports that vascular tissue could be welded together by high frequency electric currents commonly used in haemostasis (Sigel, 1963; Sigel, 1965). Sigel et al (Sigel, 1963) described the closure of incisions in arteries and veins with a greater than 50% success rate.

The introduction of lasers came with reports by Yahr and Strully (Yahr, 1967) who described the effects of lasers on vascular tissue. In the introduction to their early work it is curious to note the following passage:

"Animal tissues are generally transparent to irradiation (with lasers)...However when the character of the tissue is changed from a transparent substance to a black body, absorbed laser energy ....has a highly destructive effect."

This was written in the context of neodynium and ruby lasers, but is a prediction of the histological results that would be prevalent in a majority of laser anastomoses for the next two decades. They also mention in the same paragraph that the use of copper sulphate will substantially change the absorbtion characteristics of the tissue and is therefore the first description of the effects of chromophores on tissues. The focus of the work by Yahr and Strully was to produce a non-occlusive method of vascular anastomosis but they saw the laser as providing a means of arteriotomy rather than repair.

The first reported vascular repair using lasers, is attributed to Jain and Gorisch (Jain, 1979a, Jain, 1980b). These initial studies on micro-vessels were followed by clinical success in 1983 (Jain, 1984a). It was his intention to enable microvascular anastomoses to be performed with greater ease, speed, long term patency and fewer complications. Once established it was hoped that these techniques would enable the development of surgical procedures that are not possible using sutures.

Electrocoagulation

Thermal bonding of vessels was described in 1963 by Sigel and Acevado (Sigel, 1963) using a high frequency electric current to bond vessels joined by forceps. This gave a patency rate of 63% for repairs in a canine aortic and vena caval model. The repair was limited to a length of 7mm for arteries and 25mm for veins. In 1965, the same group (Sigel, 1965) described that the method of fusion was

coagulation of the vessel wall in which fibrilar detail within the wall remained intact. Full coagulative necrosis was seen to be detrimental to the formation of an anastomosis. No burst pressure studies were made, nor were there any comparisons with sutured anastomoses made.

Laser anastomosis of microvessels

Successful micro-anastomoses (<2mm) have been described using the Nd:YAG laser, the Argon laser and the commonly used CO_2 laser. This latter produces greater energy absorbtion and reduced scatter, to produce less thermal injury to the vessel. However the CO_2 laser is not easily delivered via a fibre-optic delivery system.

Jain et al (Jain, 1979) first used the Nd:YAG laser to repair circular, longitudinal and transverse incision later reporting end-to-end anastomoses (Jain, 1980b) and end-to-side anastomoses (Jain, 1983) with a patency rate of 90%. This was followed by a report of extra-cranial revascularisation performed in 5 patients in 1984 (Jain, 1984b). The results showed a 100% patency at 6-9 months on angiographic investigation with no evidence of aneurysm formation.

Enynia (Enynia, 1983) used the Argon laser in end-to-end microvascular anastomoses as did Pribil and Powers (Pribil, 1985). Immediate success was 100% but at 1 month 55% were stenotic and 77% developed aneurysms. Godlewski et al (Godlewski, 1986a) (Godlewski, 1986b) reported a 13% incidence of aneurysm formation while Krueger and Almquist (Krueger, 1985b) described similar results for rat iliac arteries.

The CO_2 laser was used by Quigley et al (Quigley, 1985, 1985b) who described the first microvascular anastomosis in rat femoral arteries. Histological findings revealed loss of endothelium, platelet aggregation, adventitial collagen necrosis and polymorphonuclear neutrophils infiltrating along the adventitia and the intima. There was also a well defined area of medial necrosis.

In a subsequent publication, the same authors (Quigley, 1986b) recorded a rate of aneurysm formation of 30% at 3 months compared to 0% from sutured controls. This high rate of aneurysm appeared to be related to permanent damage to the internal elastic lamina.

M^{c}Carthy et al (M^{c}Carthy, 1986) also performed similar work showing that aneurysm formation, coagulative medial and adventitial necrosis increased in laser anastomoses (22% Vs 4%) while Vale et al (Vale, 1986) saw a 12% aneurysm rate compared with 0% for sutured anastomoses. In contrast several other studies showed no tendency to aneurysm formation (Serure, 1983b; Sartorius, 1986, Frazier, 1985).

Anastomosis of rat carotid was performed by Bass et al (Bass, 1989) using the THC:YAG laser. These anastomoses were performed without solder or the use of stay sutures. The vessels were held in apposition over silicone stents during bonding. Thirty anastomoses were performed, of which 27 remained patent while separated. Nine vessels were burst pressure tested, showing an average pressure of 400mmHg. Histology claimed minimal thermal injury to the vessel wall due to the shallow depth of THC:YAG laser penetration. However, the longer term sequelae of the lasered anastomoses were not described.

1.5 Solders

The impetus to use lasers in repairing tissues arose from the need to perform fast consistent anastomoses with few complications and which were technically easier to perform. However the high rate of aneurysms and thermal damage to tissues was a consistent finding. The added technology required to perform these repairs was considerable when compared to sutured anastomoses but would have been justified had the lasers enabled the surgeons to perform procedures hitherto impossible or technically highly demanding. Parallel to this was the development of endoscopic and minimally invasive surgery. With these instruments allowing access to body cavities and spaces with little exposure and considerably less trauma, there was a need for a novel method of tissue repair.

Early Work

Early work, predating the use of solders, had found that blood applied to the surface of an anastomosis would give a stronger laser repair (Krueger, 1985a). Krueger et al (Krueger, 1985c) used the Argon laser and blood in peripheral nerve repair by forming a sleeve around the anastomosis. In this study an Argon laser emitting at 0.75 watts (W) for 0.5 seconds was used. A layer of blood 0.21mm had been found to result in 95% absorbtion of the laser energy in the laboratory, but in practice a few drops of blood were used. The rat external iliac artery and veins were repaired with a catheter used to splint the vessel while blood was applied and several bursts of laser energy were given until the repair had sufficient strength. Initial constriction of the vessels was described at the time of anastomosis. The vessels were allowed to heal for 2-6 weeks at which time the anastomosis was subject to histological analysis.

All animals survived the procedure and histology showed a patency rate of 75% (Veins) and 67% (arteries). There was some partial occlusion of the remaining vessels with thrombus, but minimal

disruption of the vessel wall and endothelialisation. Examination of the control (sutured) vessels showed a greater degree of fibrosis with giant cells present.

Further work, using blood was published by Wang et al (Wang, 1988). In this study the rat femoral artery model was used to perform laser microvascular anastomoses with and without the aid of blood reinforcement. The anastomoses were subsequently burst pressure tested. A $C0_2$ laser was used with a spot size of 0.275mm and power of 80mW for 0.2 second pulses. The subsequent analysis only took place in patent and non-aneurysmal vessels. The initial burst results showed that at time zero, laser only anastomoses had a burst pressure strength of 279mmHg compared to 746mmHg for those reinforced with blood ($p<0.05$). The difference between the two groups was seen to decrease with time, showing no significant difference between 1 hour and 3 days when the burst pressure result was identical. After this blood reinforced anastomoses were seen to be stronger at day 7. Reference to previous bench studies described the fact that in laser-only anastomoses, re-welding was required in 68% of anastomoses compared to 24% in those reinforced with blood. Summary of the chronology of solder development is seen in Table 1.1.

Author	Year	Protein	Chromophore	Laser	Tissue
Poppas	1988	Egg albumin	Nil	CO_2	Rat Urethra
Vance	1988	Blood		Argon ion	Coronary Arteries
Wang	1988	Blood		CO_2	Rat Femoral artery
Chuck	1989	nil	FITC	Argon ion	Rabbit aorta
Oz	1989	nil	ICG	808 diode	Rabbit aorta
Oz	1990	Fibrinogen	ICG	808nm diode	Rabbit aorta
Cirkrit	1990	Fibrin	nil	CO_2	Jugular vein
Libutti	1990	Fibrin	ICG	808nm diode	Colon
Bass	1991	Fibrin	ICG	810nm diode	Canine Bile duct
Poppas	1993	HSA	FeO_2/Fluorescein	KTP-532	Canine Ureters
Oz	1993	Fibrin	ICG	808nm diode	Umbilical veins
Poppas	1993	HSA	Fluorescein	KTP 532	Canine Urethra
Bass	1994	Fibrin	ICG	810nm diode	Canine Bile duct
Kirsch	1994	Fibrin	ICG	808nm diode	Ureter
Menovsky	1994	HSA	nil	CO2	Rabbit tibial nerve
Khadem	1994	Fibrinogen	R5P	Argon ion	Rabbit cornea
Kirsch	1995	HSA	ICG	808nm diode	Rabbit urethra
Menovsky	1996	Egg Albumin	nil	CO2	Rabbit dura mater
Poppas	1996	HSA + bTGF	Thermal feedback	1.32μ laser	Pig skin
Kirsch	1996	HSA	ICG	8-8nm diode	Rabbit urethra
Lauto	1997	BSA	ICG	800nm diode	Rat tibial nerve
Kilkelly	1997	HSA	Fluorescein	CO2/Nd:YAG	Rat tendo achilles
Lauto	1998	BSA	ICG	810nm diode	Rat tibial nerve
Massicote	1998	HSA	nil	1.32μ laser	Pig skin
Lauto	1999	BSA 2 layer	Carbon black	GaAs diode	Rat tibial nerve
Birch	2000	PSA	MB	670nm diode	Splenic artery
Birch	2002	PSA	MB	670nm	Rabbit carotid

Table 1.1 A Chronological Summary of laser activated solders

Solders

The term solder was first coined by Poppas (Poppas, 1988a) and superceded 'welding' as the term given to the fusion of intrinsic tissue proteins to bond tissues together. In order to better understand the development and application of solders the following discussion will deal with them according to the area of surgery in which they were applied, namely urology, vascular, gastro-intestinal, nerve repair, skin repair, tendon repair, ophthalmology and otolaryngology.

Urology

The use of solders began in 1988 with the production of solder based on egg albumin. Poppas et al (Poppas, 1988b) used 100mg egg albumin in 1ml of saline to reinforce the suture line of urethral repairs in rats. The results were compared to conventional suturing (CSA) and laser assisted micro-suture repair using a milliwatt CO_2 laser and showed success rates of 50% (CSA), 58% (Laser) and 90% (solder) respectively. No chromophore was used in this study. The observation that the application of blood to the edge of a wound produced a stronger initial wound than lasering alone led to the idea that an extrinsic protein would produce stronger welds. This was similar to the observation by Jain who used vein to cover defects in the anastomosis formed by Nd:YAG laser (Jain, 1980a) and later blood in clinical practice (Jain, 1984b). The Nd:YAG laser was used initially as this is seen to penetrate tissues further while the milliwatt CO_2 laser does not. With the later findings that medial damage caused the side effects of medial necrosis and aneurysm formation (Quigley, 1986a; Quigley, 1985a) the CO_2 laser was seen as an advantage in not causing thermal injury.

It was not until 1993 that the use of albumin in protein solder was described. Poppas et al (Poppas, 1993b) continued their development of laser repair of the genitourinary tract by supplementing the closure with an albumin solder. The lithogenic nature of sutures had meant that the formation of a sutureless anastomosis would improve the morbidity associated with ureteric anastomosis. *Ex vivo* canine ureters were used to study the solder reaction. Different solders were used containing 40% human albumin with or without various chromophores. These were either iron oxide or fluroscein. As with other studies, the burst pressure strength of these repairs was noted and compared to laser only anastomoses. The results showed that laser only anastomosis could not be formed while the use of solders enabled the production of anastomoses capable of withstanding burst pressures of upto 301 mmHg (Fluroscein only and fluroscein/albumin solder). Other groups using iron oxide showed pressures of 271mmHg, while fluroscein solder alone gave a result of 281mmHg. Albumin

solder alone without chromophore gave a burst pressure strength of 215mmHg. The use of this solder was again discussed in relation to its application in laparoscopic surgery, ending with the statement that the surgical procedures had been performed experimentally and that it would only be a matter of time before the techniques would be applicable in humans.

A description of the preparation of human albumin for laser tissue solder was made by Poppas et al in 1993 (Poppas, 1993). The human albumin prepared in this paper was sourced from a sterile solution of 25% albumin and prepared by filtration and centrifugation. It was found that solutions above 45% were too viscous to use while those below 40% did not provide strong enough bonds. The storage of the albumin under a vacuum allowed the albumin solution to be kept for up to one year with no loss of viscosity. The elimination of viral infection was performed by a pasteurisation technique of heating the albumin to 60^0C for 10 hours. Some discussion is also made of the underlying process behind the coagulation of albumin activated by laser. Thermal re-modeling has some evidence to support it while unfolding and polymerisation also have support. Observations described in the paper indicate that the level of power required for bonding is significantly lower than previously described for laser tissue bonding when solder containing fluorescein is used.

This was followed by another study by the same group (Poppas, 1993b) in which a 40% human albumin/fluorescein solder was used to perform patch graft urethroplasty in a canine model. This new technique was examined from the point of view of operative time, burst strength and histological appearance. Operative time was reported as being 42% faster (13.3min Vs 23 mins) with burst strength at 39.5 mmHg compared to 10.2 mmHg for sutured repairs ($p<0.005$). There was a 50% rate of fistula formation for sutured repairs compared to 0% for soldered bonds. Histologically the sutured group showed a greater fibroblastic response with giant cell formation consistent with a reaction to suture material. In the soldered group less inflammation was noted with no giant cells and viable graft mucosa.

Work on the re-implantation of ureters was conducted by Kirsch et al (Kirsch, 1994). This was performed with a view to undertaking the procedure laparoscopically. The solder used was an ICG/fibrinogen combination similar to that previously described. In a canine model the solder was used to close vesicle muscle flaps over submucosal ureters. These were compared to contralateral controls closed with Vicryl sutures. The closures were examined at 7, 14, and 28 days using pyleography, which confirmed patency up to pressures of 100mm of water. The repairs were removed, sectioned and tensile tested to destruction showing that soldered repairs withstood greater stress than the controls although the numbers used were only a single case at each time point and could not be

subjected to statistical analysis. In addition although the procedure was intended for laparoscopic use the repairs were performed as open procedures. Therefore the endoscopic application of this technique could not be discussed or the results used to indicate the benefits or use of this technique laparoscopically.

Further work by Kirsch et al (Kirsch, 1995a) was described in 1995. In this study a 50% albumin (no chromophore) solder was produced to fix bladder mucosa to urethroplasty strictures and fistulae. A rabbit model was used in which oval urethral defects were formed. Some were repaired by bladder mucosa patch graft fixed with laser activated solder while others were repaired with sutures. The time taken for repair was noted to be significantly reduced (13.8 vs 24.0 mins respectively), while there was an significant increase in immediate burst pressure from 20-80mmHg. The difference between these figures dropped and both showed leak pressures of over 500mmHg after 21 days. Tensile testing also showed a higher result in the solder group at 3.16KgF/cm^2 compared to 0.57 KgF/cm^2.

Following on from this work Kirsch et al (Kirsch, 1995b) published a similar report into the use of ICG/albumin solders in bridging defects in the tunica albuginea in the correction of congenital penile curvature. A canine corporoplasty model was used and patch grafting performed using saphenous vein or tunica vaginalis fixed with PDS suture or contralateral solder repair. Again burst pressures showed that solder repair was stronger (108.7 Vs 35.3mmHg) and quicker to perform (9.8 Vs 17.1 mins). Longitudinal burst pressure testing also showed greater strength in the solder group ($p<0.05$). Failure occurred in 2 solder and 1 suture repair, while histology showed minimal differences in inflammatory response with foreign body reaction only seen in the controls.

This was followed some years later by an experimental study into the repair of vasovasotomy in the rat. Seaman and Kirsch (Seaman, 1997a) performed surgical vasovasotomy in the rat using an albumin/hyaluronate/ICG solder activated with an 808nm laser diode. An 80% patency rate was achieved using sutured controls compared to 90% for soldered repairs. Mean operative times were significantly shorter but there was no significant difference in granuloma formation between the two groups.

The first clinical urological experience of solder repair was reported by Kirsch et al (Kirsch, 1995a). In this study a 42% albumin /ICG solder activated using an 808nm laser diode, at a power density of 15.9W/cm^2, was used to reinforce repairs in congenital urological defects. The procedures undertaken were urethroplasty, urethral diverticulectomy, patch graft urethroplasty and pyeloplasty. The results of this surgery were reported up to 9 months post operatively. The patients had an age

range of 3 months to 38 years (mean 8.0). Using the solder added 7.9 minutes to the overall anastomotic time of 28.3 minutes. The application technique used involved using an initial priming layer followed by further supportive layers. In each case the amount of solder applied was measured but not reported in the paper. Intraoperative leak pressure measurements of the repairs were significantly higher in the soldered versus the sutured repairs (94.2 +/-24.1 mmHg Vs 20+/-2.9 mmHg). Suture disruption was seen to occur in 2 of the soldered repairs and no intra or postoperative complications were reported. The report of a weakening of sutures and suture disruption was not surprising given the reports of suture strength diminishing on exposure to laser (Kirsch, 1996a). However once the solder is in place the sutures contribute little to the strength of the repair.

This latter study (Kirsch, 1996a) examined sutures such as chromic catgut, plain catgut, polygalactin (dyed and undyed), polydioxanone and polyglyconate and tested their strength after exposure to laser. The laser used was an 808nm laser diode at a power density of 15.9W/cm^2 and spot diameter of 2mm, for 10, 30 and 60 seconds. The sutures were tied into loops and covered with ICG/albumin solder. Those sutures covered with solder and exposed to laser showed a significant decrease in suture strength. Conversely the sutures that had no coverage of solder, showed no significant change in tensile strength. This effect was more marked in those sutures that had green or violet dye present in them but the effect was not statistically significant.

This work was followed in 1996 by a study into full-tubed skin graft urethroplasty for anterior urethral replacement (Kirsch, 1996b). The absence of suture holes, associated with soldered repairs, was anticipated to reduce the complication rate such as fistula formation and stenosis. The procedures took place in 11 rabbits where urethrectomy was performed and skin flaps raised to reconstruct it. The skin flaps were formed using standard microsuture techniques or by the use of solder. Post operatively intraluminal burst pressure strength was tested using methylene blue as an indicator. The soldered repairs had a dramatically higher initial burst pressure than sutured controls (149. 2 Vs 22.4 mmHg). In addition, the time taken to perform the repair was significantly reduced at 19.5 vs 31. 2 minutes ($p<0.003$). Of the complications recorded, a fistula occurred in 1 control animal but in none of the soldered animals. Strictures occurred in 4 control animals and 5 soldered repairs. Retention was noted in all of the remaining animals of both groups after the stents had been removed. Histology at this time revealed extensive squamous keratinisation, hyperplasia and periurethral fibrosis. From the current study it could not be stated that soldered urethral repair would result in a lower rate of fistula due to the overwhelming incidence of stricture formation. There was a similar incidence of stricture formation in

both treatment groups, with the control group showing 4 strictures while the soldered group showed 5 strictures.

The source and type of the protein used in laser tissue solder repairs is discussed by several authors. Previously fibrinogen was taken from human cryoprecipitate but this is associated with a small but significant risk of infection with either hepatitis B virus or HIV, not to mention the possibility of prion pathogens as yet unclassified. In addition to the source and type, the concentration and preparation of the protein used is discussed.

The protein formulation of solders for clinical application was investigated by Poppas et al (Poppas, 1996). It was seen that 50% albumin solders significantly improve wound strength when compared to lower concentrations. This was prepared from 25% human albumin and concentrated by a lyophilisation technique. In addition solders were prepared using chromophores such as ICG, FITC and methylene blue. Spectrophotometric analysis of the solders showed that there was no change in the absorption characteristics of the solder in the presence of 50% albumin. This technique was later used to produce solders to study the effect of adding tissue growth factors and their effects on tissue repair.

Poppas et al (Poppas, 1996) used a porcine skin model due to its similarity to human skin morphology, with the creation of 2cm full thickness incisions. The repair techniques included standard suturing with polypropylene (5/0) which were compared to solders containing growth factors. For solder repair the sutures were placed prior to soldering and removed afterwards. The solder used was composed of 50% human albumin containing ICG, activated using an 808nm laser diode (power of activation not given). Prior to use, the growth factors were heat tested to determine their stability since soldering generates temperatures of 60-80^{0}C. The growth factors included Transforming Growth Factor Beta (TGF-β_1), Heparin Binding Epidermal Growth Factor (HB-EGF) and Basic Fibroblast Growth Factor (bFGF) obtained from human sources. It was found in an *in vitro* assay that heating these growth factors did not result in a significant loss of their bioactivity.

No immediate or 7 day differences were noted in the wounds created in terms of their gross morphological appearance or their tensile strength. At time 0 and 3 days there was no difference between the groups. However by day 5 the use of 10μg of TGF-β_1 in the solder had significantly increased the wound strength over sutured repair. Human albumin solder was therefore shown to be a possible carrier for tissue growth factors. In addition, the use of TGF-β_1 has been shown to significantly increase the tensile strength of skin wounds. The inference is that the use of these growth factors accelerates wound healing although no mention is made of the gross appearance of the wound

created. An attempt was made to quantify the amount of new collagen formed and although this was seen to be higher than standard suturing, was not statistically significant.

Further work by Kirsch et al (Kirsch, 1997a) centred on the closure of dermal wounds as a model of sutureless hypospadias repair. An albumin-hyalurante-ICG solder was used in this instance in order to provide an immediate leak free closure during hypospadias repair. Despite previous clinical work on a similar problem, this issue was again re-addressed by Kirsch (Kirsch, 1995b) specifically looking at the variables of power density, solder composition, chromophore composition, application site and technique used. Mention is made of previous results pertaining to the use of albumin solders in clinical practice stating that there was no significant reduction in the fistula rates of those repairs formed reinforced with solder (19%), compared to those that were not re-inforced (24%).

In this study a rat urethroplasty model was used with three treatment regimes tested. These were 1) no closure after skin flap bisection, 2) conventional suturing or 3) soldering, each examined after 3,5,7,10,14,or 21 days. Tensile strength was seen to be superior in the soldered group up to 5 days but was not significantly different after 7 and 10 days from sutured repairs. However the appearance of external scarring was significantly different with soldered repairs showing less scarring and sutured repairs having a visible furrow together with foreign body reaction, poor tissue alignment and tracking of epithelial cells into the lamina propria. Measurements of temperature rise were taken using a thermocouple and gave readings of 45-101^{0}C in the superficial skin. The conclusions from this study were that soldering improved tensile strength up to 5 days and that the use of a chromophore such as ICG reduces thermal injury by acting as a heat sink. The claims of accelerated wound healing were not made since there was no data relating to the length of the inflammatory phase, with an earlier onset of fibroplasia.

Further work on reconstruction of the urinary tract was performed by Wright and Poppas (Wright, 1997). Due to the widely ranging reports of solder concentration, laser wavelength and power and chromophore type, the results of many studies were not comparable with each other. In addition many different model types were used to assess these parameters. The question asked in this study was whether there was an optimal combination of wavelength and albumin solder to produce what is described as photothermal wound closure (PTWC). Wavelengths from the infrared and visible spectra were used in combination with chromophores where they were required. Lasers emitting at wavelengths of 532nm, 808nm, 1320nm 2100nm and 10600nm were used with the 532nm and 808nm lasers used in conjunction with fluorescein and ICG chromophores respectively.

Initially a 40% human albumin solder was used to repair ureterotomies with the various lasers. Secondly using the wavelength that gave the strongest repair, the concentration of the albumin solution was varied to produce 38%, 45% and 50% solders (w/v). These also tested *in vitro* to repair longitudinal ureterotomies in canine ureters. Once complete the ureterotomy repairs were burst pressure tested and histology performed.

Using the 40% solder the Nd:YAG (1320nm) laser gave the strongest repair (221mmHg) followed by 808nm and 532nm lasers (ie the chromophore dependant solders). A comparison of this 1320nm laser with varying concentrations of albumin showed a progressive increase in the repair strength from 25% to 50%, showing an almost sigmoid curve.

Histologically there were marked differences between the different laser wavelengths used. The 2100nm (Holmium:YAG) and 10600nm lasers caused superficial thermal injury with poor deep tissue approximation. The 532nm and 808nm lasers showed minimal tissue change with obvious full thickness solder denaturation. The 1320nm (YAG) laser showed a consistent change through the whole layer of the ureter. The weld characteristics of these lasers appeared to match their tissue penetrance despite the use of chromophores in two cases. The ideal laser wavelength was described as one in which penetration matches the thickness of the tissue, there is efficient absorption with minimal scatter and the heat output is controllable to avoid thermal injury. From this point of view the 1320nm laser was seen as being ideal.

While these reports were positive about the use of solder, Barrieras et al (Barrieras, 2001) compared fibrin sealant (Tisseel), solder (50%albumin/ICG) and sutured pyeloplasties. The results of burst pressure testing showed that soldering had a significantly higher burst pressure than Tisseel ($p=0.034$) but a high rate of urinoma complications. They concluded that Tisseel and solder procedures are not superior in the long term to sutures.

The most recent report on the use of soldering in clinical practice was reported by Kirsch in 2001 (Kirsch, 2001). In this series, 138 boys were operated on using either sutures (84) or soldered repairs (54) to perform hypospadias repair. The solder used comprised of 50% HAS, 2.5mg/ml ICG and was activated with an 808nm laser diode. Demographically the two groups were similar with significantly reduced anastomotic time for soldered repairs. Complications were 4.7% for solder and 10.7% for sutured repairs, which -was not statistically significant ($p=0.21$).

Vascular

In 1988 Grubbs et al described the use of fibrin glue to re-inforce the suture lines of micro-anastomoses formed in the rat femoral model using a CO_2 laser (Grubbs, 1988a). These were compared with standard suture anastomoses and laser alone anastomoses. The reinforced and suture alone groups showed significantly higher burst strengths and patency rates than laser alone. In addition the rate of aneurysm formation was lower with fibrin reinforcement. However the fibrin group showed medial damage similar to the laser only group.

Later in 1988, Vance et al (Vance, 1988a) described the technique of vascular anastomosis using an Argon laser and a chromophore (haemoglobin) after it was noticed that carbonisation occurs at the anastomotic site. These anastomoses were performed on porcine coronary arteries *in vitro*. The application of a chromophore allowed a larger reduction in the incident laser energy density using only one supportive stay suture. These anastomoses were able to withstand pressures of 90-300mmHg (mean 203 mmHg) with powers of 300 to 500mW. Histology of these samples showed limited tissue damage.

It was not until 1989 that Chuck et al (Chuck, 1989b) described the use of synthetic pre-sensitising dyes in the formation of vascular anastomoses to reduce incident Argon laser power. Fluroscein isothiocyanate (FITC) was used to produce a dose-response curve *in vitro* and subsequently used to repair aortotomies in rabbit aorta. These were compared to repairs without chromophore and it was seen that there was no significant difference between the bursting pressures of the two groups but a decreased incidence of collateral thermal damage in the chromophore group. It was in this paper that the suggestion was first made that chromophores and protein glues be combined with laser to produce soldered anastomoses.

Later in 1989, the use of Indocyanine green (ICG) was described by Oz et al (Oz, 1989) in vascular anastomoses. In this study a laser diode was used to repair rabbit aortotomies. These were compared to animals in which suture and laser only repairs had been made. Using ICG, these welds were able to withstand pressures of 262 mmHg, while sutured anastomoses burst after 145 mmHg. Histology was not performed on these specimens but there was no significant difference in the incidence of side effects.

The first combination of chromophore and protein to form a solder was reported by Oz et al in 1990 (Oz, 1990). This was also the first appearance of the term 'solder' or 'soldering'. Rabbit aortotomies were repaired and immediate results showed that the burst pressures of the repairs created without solder were significantly weaker than those with solder (262 mmHg Vs 330mmHg;$p<0.05$).

The use of suture alone did not result in significant burst pressures but did leak at pressures significantly below those of laser or soldered repairs. After 90 days the soldered repairs showed no ruptures, thromboses, foreign body reactions or aneurysms.

The use of a similar fibrin solder was reported later in 1990 by Cikrit et al (Cikrit, 1990). In this study the $C0_2$ milliwatt laser was used to perform end-to-end anastomoses in the canine jugular vein. In those veins repaired with 'laser only', the anastomotic failure rate was 50%. This decreased to 18% in those anastomoses reinforced with fibrin solder. Overall, there was an 82% patency rate in the laser repaired veins, compared to 93% seen in the contralateral, sutured controls. In addition burst strength was seen to be improved by the use of fibrin glue. A transmural necrosis was seen in all groups, extending further in the laser only group, while the sutured controls showed a greater inflammatory response.

Further work using fibrin based solder was reported by Oz in 1993 (Oz, 1993b). In this study the application of solder/welding techniques was applied to the anastomosis of synthetic vascular prostheses and vascular graft materials. Glutaraldehyde tanned human umbilical veins were therefore prepared and venotomies repaired using laser assisted fibrinogen bonding (LAFB). The results of repairs using sutures, laser alone or LAFB were compared. The solder used in LAFB contained ICG mixed with fibrinogen and was applied using forceps. The laser used was an 808nm laser diode ($4.8W/cm^2$). The repairs were burst pressure tested and it was seen that sutured repairs gave a similar result to LAFB (126.6mmHg Vs 111.6mmHg). There was no evidence of collateral thermal injury in the graft. Further to this *in vivo* analysis of the technique was undertaken in canine femoral arteries using the vein graft as an interposition graft or as an A-V fistula. This study showed that A-V fistula anastomoses can be formed without the aid of haemostatic sutures. Again the anastomoses were free of thermal injury.

The use of solder strips was reported by Small et al in 1997 (Small IV, 1997). In this *in vitro* study an 805nm laser diode was used to compare the relative attributes of liquid and solid solder patches. These were composed of 60% human albumin with ICG chromophore (0.5% w/v). The patches were 60-70% porcine gelatin (denatured collagen) and 0.25% ICG (w/v). No reason was given for the differences in ICG concentration between the patches and the solders. Variations in the patch thickness and laser power/exposure time were also compared. The solders and patches were tested for burst pressure strength on sections of porcine aorta mounted in a circular pressure rig. The samples were pressurised and a record taken on PC. This was continued until leakage occurred. The results showed that the strongest repairs were obtained using the 1.0-1.3mm patches (481mmHg) whereas the

weakest bonds were produced in the high power short exposure solder group (194mmHg). Overall the solders were difficult to assess because solder thickness was difficult to maintain and reproduce. The solder formed a cast over the wound and in some cases plugged the gap produced in the aorta. There was much greater reproducibility in the patch group as the thickness and distribution was kept constant and this study demonstrated the effectiveness of this mode of solder application. Similar effects were produced by Trickett et al (Trickett, 1997b) and Lauto (Lauto, 1997b) who performed nerve repair successfully.

A more recent report of vascular applications of solder repairs was reported by Phillips et al (Phillips, 1999). In this study, a comparison was made between the use of sutures, clips (VCS), laser and solder repairs for vascular anastomoses with a view to developing a technique that could be applied to minimally invasive coronary bypass surgery. This was performed in a canine carotid model using 50% albumin (no chromophore) and a 1.32μm laser at powers of 300-400mW. The procedures were performed and tested for anastomotic time, leak pressure and tensile strength, as well as histological appearance in the soldered repairs.

The results (Table 1.2) showed a significant decrease in anastomotic time compared to clips and sutures of laser and solder repairs. However there was no significant difference in leak pressure strengths while the sutured repairs had the highest tensile strength. Histology showed that there was thermal injury through 30% of the vessel wall of those performed with solder. There was no evidence given that these techniques could be applied to endoscopic techniques, or perform the end to side anastomoses commonly seen in coronary surgery.

Type of Anastomosis	Anastomotic time (min)	p value
Nd:YAG + 50% HAS	8.4 +/-0.7	Vs Suture – 0.001
1.9μm laser	7.8 +/- 0.3	Vs Suture – 0.001
Vascular clip	8.3 +/- 3.3	Vs Suture –0.17
Suture	13.8 +/- 1.0	

Table 1.2 Comparison of Solder, Laser, Clip and Suture anastomoses

A novel method of vascular solder anastomosis was described by Mandley (Mandley, 1995) using porcine albumin and methylene blue. The rationale for this choice of protein was its availability,

similarity to human albumin and the absence of major viral or prion pathogenic contamination. Similarly methylene blue had been chosen for its ability to fade. This is a well recorded phenomenon that results in conversion to a leuco form on exposure to light in its absorbtion spectrum (590-700nm). Thus a fading, self-regulating solder had been formed reducing the possibility of thermal injury and a mechanism of visual feedback for the surgeon. The report by Mandley discusses the activation of this solder by an argon ion laser requiring a three phase electrical supply and water cooling. Subsequent work by this group had used a much small laser diode that could run on 240v mains electricity.

More recent in vitro investigation and comparison of solders and laser anastomoses was reported by Ott et al (Ott, 2001). In this study, porcine arteries and veins were apposed over a catheter and anastomosed either using 50% albumin/ICG solder, or a Ho:YAG laser. Histology showed both techniques to cause thermal injury up to 3mm from the anastomosis, with no difference in burst strength, although tensile testing showed soldered repairs to be stronger. Lauto also compared the 808nm diode laser with an Nd:YAG laser for vascular anastomosis (Lauto, 2001). *In vitro* investigation showed lower temperature with the diode laser, while *in vivo* studies showed less thermal injury with the diode laser and a 100% patency rate. The Nd:YAG laser had a 75% thrombosis rate and a greater degree of thermal injury. Longer term measurements were not made as the study was ended at 21 days.

Gastro-intestinal

The first report of solders used to reinforce colonic anastomoses was described by Libutti et al (Libutti, 1990). Solder was prepared using human fibrinogen and ICG activated using a laser diode (808nm). The beam had dimensions of 2mm at a distance of 4cm. Eight single layer colonic anastomoses were formed in 6 dogs using interrupted silk sutures. The solder was applied in a paintbrush manner and activated. Subsequently the anastomoses were burst pressure tested and showed that suture only anastomoses had an immediate leak pressure of 137mmHg (+/- 22mmHg) while solder reinforced anastomoses leaked at 326mmHg (+/- 67mmHg) ($p<0.001$). Examination of the repair showed no evidence of thermal injury. It is claimed in this study that precise approximation of tissues is not required as the solder fills the gaps, and their burst pressure results seem to bear this out in the immediately formed anastomosis. However the effects on the long term strength of the repair cannot be concluded from this study.

The application of this technology to the realm of laparoscopic surgery was speculated upon by Bass et al (Bass, 1991b). In this study human fibrinogen was used to make a solder containing ICG.

Canine choledocotomies were repaired through a laparotomy using laser activated fibrin solder of different constituents. The laser used was a gallium-aluminum-arsenide laser emmitting at 810nm. These were compared to sutured controls and the repairs burst pressure tested. The mean burst pressure of the fibrin lasered group was 104mmHg (+/-99 mmHg). Using stabilised fibrinogen the handling characteristics were seen to be improved and the burst pressures rose to 264mmHg (+/-7mmHg). As in other studies the sutured controls did not burst but showed leakage at pressures significantly below those of the soldered group (88mmHg +/- 76mmHg). Histology showed the fibrin coagulum to be an eosinophilic amorphous mass with no thermal injury effects seen in the adjacent tissues. An important discussion point was made that the application of urokinase to the coagulum does not result in the bond breaking down. Therefore a mechanism other than the coagulation cascade must be responsible for the formation of cohesive and adhesive bonds.

Further work using a fibrinogen solder centred around choledochotomy repair in a canine model. Bass et al (Bass, 1994a) performed a 0.5 cm incision in the common bile duct and repaired it using ICG doped fibrinogen solder activated using an 810nm laser diode. The immediate leak pressure was 264mmHg compared to 83mmHg for sutured controls. This had increased to 364mmHg by 2 days and 510mmHg by 7 days. The results for the sutured controls were not included in the report. Histology showed the solder as an eosinophilic amorphous coagulum with minimal thermal changes. Day 2 showed some mononuclear infiltration but no mucosal regeneration. By day 7 only scant amounts of solder remained and there was no evidence of stenosis. The focus of this study was again to produce a technique that could be translated into laparoscopic use. Current devices included staples and slip knot ligatures but lack of access, complications and technical difficulty preclude these techniques being more widely used.

In looking at new strategies for applying solder to increase the immediate strength of the wound, Lauto et al (Lauto, 1999) used solid albumin solder. In this study a 2 layer bovine serum albumin solder film was produced as a bilayer; one with carbon black as a chromophore and the other clear. The total thickness of the bilayer was 0.110-0.150 mm and a low intensity diode laser emmitting at 810nm at a power of 200 +/-20mW was used to activate the film. The solders tested were composed of either one layer of solder (no white albumin layer) or two layers. In addition variations were made in carbon black concentration and laser power levels. The film was applied to canine small intestine to determine the initial tensile strength while temperature measurements were made on both sides of the repair using topically applied thermocouples.

The results showed that there was a higher initial tensile strength from the two layered films but that a reduction in laser power would reduce the strength to the same as a one layered film. Temperature measurement showed that there was less of a temperature gradient for the two layer film than for the one layer film.

This latter observation is important in that this implies a significant heating effect from the laser itself rather than heat generation from the chromophore. Comment from the author states that because of the reduced pathlength there was an underestimation of the temperature difference in the one layer group whereas the two layer group was accurate. Another observation made was that the solder film rehydrated on the surface of the intestine forming a liquid. In this case the films are simply a novel method of delivering the liquid solder.

Nerve repair

The application of the solders to nerve repair came in 1994 by Menovsky et al (Menovsky, 1994a). This was an *in vitro* study using rabbit tibial nerves to investigate bonding strength in nerves using a CO_2 laser and various solders. These were composed of egg albumin, dried albumin solution, fibrinogen, human albumin, fibrin glue and blood cells. In addition the effects of varying power were investigated from 50 to 150mW. The results were compared to laser only nerve repair and sutured repair. The strongest repairs were produced using the dried albumin solution increasing the strength nine fold compared to laser only repair (2.4g to 21.0g). However immersing the anastomosed nerve in 20% saline for 20 minutes significantly reduced the strength of the bonds from 21.0g to 9.8g. The use of a 20% egg albumin solution produced bonds that were not significantly stronger than laser only welding, or soldering with fibrinogen, red blood cells and fibrin glue. This technique of tensile testing seemed inappropriate as the tensile strength of a nerve repair is in no way indicative of the final outcome of the repair. Indeed the stronger bond may have resulted in excessive tissue damage. The significant questions raised by this study are the immunological response of a host to foreign protein based solder and the persistence of the solder at the site of an anastomosis.

The use of lasers to repair nerves had previously been reported, but the application of chromophores to the repair site did not occur until 1996 with a study by Menovsky et al (Menovsky, 1996). The primary aim of this was to determine the mechanism by which laser tissue welding and soldering worked. Dura mater and peripheral nerves were studied *in vitro* having been welded using a CO_2 laser at 100mW (0.1s pulses) with a spot size of 320μm. This was performed with and without the use of egg albumin. In all of the specimens used there was evidence of adequate tissue bonding, but the

repairs were seen to be much stronger in the solder group. After welding both the dura and the epineurium had a similar appearance on SEM with a depression at the site of the incident laser exposure. The fine loose structure of the collagen fibril was lost and individual fibrils had a homogeneously blurred and swollen appearance. In the solder repairs the surface of the dura and epineurium could not be seen. However the appearance of the solder was that of a dense homogenous mass elevated from the surface. Nearer the site of repair the solder clung to the tissue as an outer stent. In addition the solder had penetrated between the collagen fibrils and fused solid. From these findings it was concluded that fusion is primarily a thermal effect occurring at temperatures of 60-120^{0}C. Other proposed mechanisms of solder activation and tissue welding have included denaturation of the structural proteins (Okada, 1987), dehydration of proteins (Fenner, 1992), collagen to collagen fusion (White, 1988), crosslinking of proteins (White, 1988), formation of non-covalent bonding between collagen (Bass, 1991a; Bass, 1992) and interdigitation of collagen fibres (Schober, 1986). The critical parameters to the success of soldering are discussed here as involving solder thickness, fluence rate (ie laser power/power density) and solder viscosity although no mention is made of the degree of hydration of the tissues to be repaired.

In terms of nerve repair the use of solders may have an inhibiting role in preventing the spouting of new axons should solder enter the defect. In this instance the production of fibrous tissue between the ends of the repair would inhibit growth, as well as the production of adhesions to surrounding tissues. The conclusions from this study were that thermal coagulation was responsible for laser soldering, with cell membranes becoming disrupted and forming a micro-solder of intracellular contents, which together with denatured collagen results in tissue adhesion.

In vitro work on laser activated protein solder strip repair of nerves was performed by Trickett et al (Trickett, 1997a). This was compared to laser epineurial repair with an 808nm laser. The rat sciatic model was used *in vitro* without stay sutures in 2 groups of 6 animals. The first used direct laser fusion while the second used solid protein solder strips composed of albumin and ICG. The results were analysed with respect to tissue apposition and axonal damage. Direct welding produced weak bonds with even mild manipulation resulting in disruption. Further, upon irradiation of the epineurium, this would coagulate and retract exposing the axons to the laser resulting in nerve damage. This was confirmed on histology showing damage up to 50μm from the edge of the wound. The solder strip repair resulted in nerve repair that would withstand some tension. Histology confirmed that the solder was denatured and that no axonal damage occurred. This study emphasised the use of the laser diode as a cheap, compact, efficient and reliable tool of solder activation.

In vivo nerve repair was first described by Lauto (Lauto, 1997a) in 1997. A rat model was used in conjunction with solid protein bands. These were composed of bovine serum albumin and ICG activated by a laser diode at 800nm (90mW) with a spot size of 200μm giving a power density of 300W cm^{-2}. The solder bands were prepared by mechanical pressure to form thin slabs 3mm x 0.5mm x 0.15mm.

The rat tibial nerve was transected and left for 3 mins to allow axoplasmic extrusion to take place. The nerve was then repaired using either four 10/0 sutures or four laser solder protein bands. Of the 24 procedures in each group, 5 per group were submitted to tensile testing. The remaining rats were kept for 3 months after which time EMG recordings were taken. Soldered nerve repairs were significantly faster to perform than suturing while tensile testing showed that soldered repairs were significantly weaker than sutures (21 +/-5g Vs 50+/-10g). At 3 months, all nerves were intact with CMAP showing no significant differences in either group. The CMAP in the unoperated side was significantly higher. Histology showed little difference between the two groups with the protein bands reabsorbed, no evidence of acute or chronic inflamation and similar degrees of myelination.

Solder repairs in this study showed the single advantage of a shorter operating time at the expense of a reduced initial tensile strength. Solid protein bands were claimed to have the advantages of increased self life, ease of gamma sterilisation, ease of handling over liquid solders and would not obstruct the regenerating axons as liquid solder may. The conclusions of this study were that the protein bands could be used to repair nerves of less than 1mm and were an improvement over laser nerve repair.

The nerve model was used later by Lauto et al (Lauto, 1998) to investigate the dependence of repair strength on solder protein concentration. Previous studies had relied on liquid solder giving inconsistent results. In this study, Lauto uses solid protein bands to repair ex-vivo rat tibial nerves and vas deferens, testing the tensile strength of the repairs. The solder used was composed of bovine serum albumin (58% or 68%) in combination with ICG (0.25%). The solder mixture was formed into strips in a parallel plate vice and pressure applied. A strip thickness of 0.15mm was used. The laser in the study was an 810nm diode laser with a power range of 90- 140mW. The spot size was 200μm with exposure times in the region of 1-3second bursts.

Five sets of repair groups were created with a combination of laser power (90 Vs 140mW) and protein content of the strips (58% Vs 68%) used in the two tissue types. The irradiation time was also varied to ensure that each repair received the same dose of laser energy (J/mg). The results showed that for nerves the higher protein concentration resulted in a higher tensile strength and that the same was

true for the vas deferens. Although a relatively confusing study to understand and interpret, broad conclusions could be drawn, that the higher the protein concentration, laser power and surface area of the repair the higher the tensile strength. By increasing the thickness of the band there is a diminishing return in tensile strength as the penetration of the laser is limited after a critical thickness.

Ophthalmology

Further developments in 1994 saw the comparison of heat and light activated solders. Khadem et al (Khadem, 1994) investigated the use of riboflavin-5-phosphate (R5P) as a chromophore in a fibrinogen based solder activated with a blue-green Argon laser (488-514nm) for 2 minutes at 0.6W. This was compared to a FITC/albumin solder as a heat activated solder for the closure of corneal incisions.

Several experimental conditions were set including different solder compositions and post-solder re-hydration. Subsequently the corneal repairs were burst pressure tested. The results showed that the strongest repairs were formed using R5P with 18% Fibrinogen immersed in water for 40 minutes ($p<0.005$). This study was undertaken after it was seen that heat activated soldered resulted in distortion of the tissues. This led to the development of singlet oxygen mediated protein crosslinking. These photo-oxidation reactions are classified as Type I (sensitiser triplet binds to substrate to produce free radicals) and Type II (sensitiser reacts with oxygen first to produce singlet oxygen). In type I reactions the substrate radicals then undergo further reactions including that with molecular oxygen to form the superoxide anion $O\cdot_{-2}$. These photosensitisers are known to cause nondisulphide covalent crosslinks in susceptible proteins in an oxygen dependant way. The addition of a singlet oxygen quencher to the reaction (Sodium Azide) was seen to result in significantly weaker solder repairs indicating that the reaction of R5P/Fibrinogen may rely on type II reactions with a lesser role for type I reactions. It was claimed in this study that there was a temperature rise of only 5^0C (measured by thermocouple) detected in the albumin/FITC solder, a figure significantly lower than those reported by (Serure, 1983a). In this study an argon laser was used for 80 seconds, with blood as a chromophore, showing a temperature rise of up to 80^0C.

Additional work was performed by Goins on reinforcement of radial keratotomy(Goins, 1997), and keroplasty in human cadaver eyes (Goins, 1998). The first of these studies performed in rabbits, compared the use of R5P/fibrinogen based photodyanamic glue in closing incisions made during laser radial keratotomy. The solder was inserted and activated with an Argon blue green laser. Control groups consisted of no treatment or closure with sutures. The histological appearance of the wounds

were assessed at 1 and 8 weeks and showed a reduced propensity for wound dehiscence in the solder treated group with a prominent epithelial response. The control groups however showed less organised epithelium and late disorganization. Keratometry of the eyes showed increased flattening of the eyes in the control group at 6.5 diopters (D) compared to 1.0D in the solder treated group. There was also less late corneal flattening in the solder group.

The latter study (Goins, 1998) was performed in human cadaver eyes and compared the strength of R5P/fibrinogen solder repaired corneal closure with 10/0 nylon sutures. Sutures in ophthalmic surgery are associated with toxic or allergic reactions and immune deposits while broken sutures are a cause of microbial keratitis, which has an 11% incidence of total blindness. By using tissue solder it was hoped to minimise these complications along with reducing operating time, operative desiccation and a reduced incidence of corneal rejection. A 7mm central corneal trephination was performed and the button removed. This was then resutured using either 4 sutures and R5P based tissue solder, or 16 nylon sutures. The resulting repairs were burst pressure tested. The controls showed a leak pressure 124mmHg (+/-70-180mmHg) with iris prolapse occurring at 185mmHg (90-300mmHg). A single sutured control was used and gave wound leakage at 70mmHg and prolapse at 300mmHg.

Otolaryngology

A similar study to that by Kirsch et al (Kirsch, 1995b) in urethroplasty was conducted by Wang et al in the trachea (Wang, 1995). The possibility of using free mucosal stamps in the repair of mucosal defects in the airway to prevent stenosis was investigated. A canine model was used and defects were produced in the trachea using a CO_2 laser. Control wounds were left open while the treatment groups underwent either 'trapdoor' closure or patch graft repair fixed using laser activated solder. The solder used was 25% albumin with ICG chromophore activated with an 810nm laser diode. Histology showed the solder treatment group to have regenerated squamous cells in 7 days and ciliated epithelium by 2 weeks. There was no thermal injury from the diode laser. Coverage in the control group took 4 weeks to occur.

Tendon repair

Kilkelly et al described the technique of tendon solder repair in 1997 (Kilkelly, 1997). The aim of tendon reconstruction is a solid repair that will glide easily over surrounding tissues. This study set out to determine whether this was possible with the application of thermal lasers.

The tendo-Achilles of the rat was chosen as a model and four injury management groups were produced. Group 1 received a modified Kessler repair while Group 2 were repaired using a CO_2 laser at 34mW with a 25% human albumin solder. No chromophore was used. In Group 3 an Argon laser was used at 200mW with a solder composed of 10% fluorescein and 25% human albumin. The last group were repaired using the Nd:YAG laser, however no successful repairs were made using this technique. The tendons were tested biomechanically and histologically.

At time zero the repairs that had been formed using the Argon and CO_2 lasers could not be tested and disrupted immediately. By 14 days, the laser repairs were up to 74% (Suture) 59% (CO_2) and 64% (Argon) of the uninjured tendon strength. Histology showed an increased amount of inflammation in the Argon and CO_2 laser treated groups relative to the sutured repairs. This study demonstrates that Laser/solder tendon repair is possible but requires significant refinement before it will be applicable to the clinical scenario. The obvious failings of this study were the omission of a chromophore in the solder mix and the use of albumin at a low concentration associated with low strength repairs in vascular tissue (Wright, 1995). The fact that this has previously been determined casts doubt over the validity if this study and conclusions that may be drawn from it.

Skin repair

Despite the many reports of the use of solders to repair skin (Poppas, 1998, Menovsky 1998, Chan, 1998b, Chan, 1998c, Suh, 1998) few have done this exclusively. Chan et al (Chan, 1998b) set out to investigate the effects of tissue hydration on the efficacy of tissue soldering in a rat dermis model alongside baboon articular cartilage. This was performed in response to the observation that solder repaired wounds were subject to rehydration and breakdown on exposure to isotonic or hypotonic solutions. In this respect the application of solder to areas where these solutions may be present, would result in weak bonds, particularly the renal tract, joints or even skin. With this in mind the study set out to examine the solder-tissue bond using scanning electron microscopy. The solder used was composed of 25% human albumin, 0.5% sodium hyaluronate with 2.5mg/ml ICG in de-ionised water. This was activated using an 808nm diode laser with a spot size of 1.5mm and a power of 500mW.

The tissues were prepared to expose the underlying type I collagen and the surfaces dried. A full thickness incision was made and the solder applied. The solder was activated using a sweeping motion and the tissue immediately divided in two perpendicular to the original incision. One half was placed in 1% glutaraldehyde while the other was soaked in PBS for 1hour, 1, 2 or 7 days. A sample size of n=1 was used for each treatment.

Although it is nearly impossible to form any conclusions with such a small sample size, the results showed that solder attached in 4 out of 5 specimens in those samples fixed. Close to the tissue surface the solder gave the appearance of globules 2-3μm in diameter. The solder activated closer to the laser had a more homogenous appearance. Soaking in PBS for 1 hour resulted in solder detachment from the cartilage. Longer periods of soaking resulted in even greater degrees of detachment. Air dried solder fixed immediately and showed no evidence of globule formation.

In skin, a similar picture arose with a two layer appearance and globule formation adjacent to the tissue. In addition air vacuoles, attributed to steam, were seen in the solder.

The conclusions were that the solder was not completely coagulated at the tissue surface although some hydrated specimens were stable. The appearance of vacuoles in the solder implies that there was a sufficient temperature rise to generate steam. The asymmetry of these vacuoles also implies a temperature gradient across the solder layer with the upper layer exposed to more heat than the lower one. The presence of vacuoles may have insulated the lower layers from the heat generated in the solder above. The control of temperature is a critical factor in solder repair of temperature sensitive tissues with techniques for controlling suggested as 1) using a smaller droplet size 2) reducing chromophore concentration and 3) applying temperature control feedback to restrict laser energy.

This work was followed on by Chan et al (Chan, 1998c) with the development of a microjet device to deliver solder in droplets of varying sizes. Solder repairs by this technique were then tested for rehydration. The tissues used were rat dermis and bovine intima specimens. Solder used in this study was diluted 25% human albumin with 0.25% ICG and 0.5% sodium hyaluronate, with laser activation by 808nm diode laser. The microjet device was capable of producing droplets of 0.2-0.3μl with a pulse duration of 6.4ms, a frequency of 1Hz and back pressure of 70psi. Five microdrops were deposited on each tissue type prior to fixation or rehydration. Both the control and rehydrated solders were well attached to the tissue surface on SEM. Some vacuoles were seen in the coagulated solder, as well as thermal injury for up to 300μm as assessed by the fusion of collagen fibrils. This was true for both tissue types. By using a much smaller droplet size the thermal decay rate was reduced to 1.7ms. Despite this thermal injury was still seen and may be explained by the observation that as each

successive droplet was applied the concentration of ICG increased making it a more efficient producer of thermal energy. The microjet device was limited to 2Hz due to solder heating causing clogging of the nozzle. The use of piezo-electric devices may overcome this and will enable the delivery of nanolitre quantities of solder.

A definitive study into the effects of soldering on skin was performed by Suh et al (Suh, 1998). Using the rat dorsal skin model incisions were closed either by soldering the dermis, suturing the dermis and solder to the epithelium, or just suture closure. Initial results showed no significant difference between the repair methods, while at day 3 suture and dermal soldering was stronger ($p<0.05$) than solder/suture combination. By 10 days solder only closure exceeded both suture and solder/suture combination. No estimation was made of the degree of scaring at the incision, but histology showed a minimal fibrotic reaction with full epithelialisation by 7 days in the solder only group.

Further work on the soldering of articular cartilage was described by Judy et al (Judy, 1998). In this abstract, they describe the use of 1,8-napthalamide dye based solder. In this study sheep articular cartilage was soldered with encouraging results *in vivo*. With time (unspecified) newly synthesised cartilage was formed, with type I and II collagen seen.

1.6 Critical areas of research and development

Throughout the use of solders certain themes emerge as common to the experience of many authors. These include the use of particular proteins and chromophores causing various results in terms of time reduction, improvements in ease of use and increase in strength.

Originally blood was seen to act as a reinforcement for anastomoses increasing the strength of lasered repairs (Krueger, 1985a), (Wang, 1988) as well as the initial observations by Jain that muscle could be used (Jain, 1979b). From this point on the search began for an appropriate protein with which to reinforce the wound repairs. Several different sources of protein have been described in protein solders. These include fibrin, egg albumin, human albumin, bovine albumin and porcine albumin.

Fibrin

Early work concentrated on fibrin with Grubbs et al (Grubbs, 1988b) using cryoprecipitated fibrinogen, thrombin, and aprotonin. This study in rat femoral vessels, showed no significant difference in patency although initial burst pressures were higher in the fibrin reinforced group. The first combination of a chromophore with a protein was reported by Oz et al (Oz, 1990) who used human fibrinogen with ICG to repair rabbit aortic arteriotomies. Burst pressure testing showed the use of fibrinogen to significantly increased the strength of the repairs compared to lasered anastomoses (262 mmHg Vs 330mmHg:$p<0.05$). Sutured repairs did not burst but leaked at significantly lower pressures (165mmHg). Post operatively there were no aneurysms or medial necrosis seen in the solder group.

Fibrin as a solder was used by Libutti et al (Libutti, 1990) to form colonic anastomoses. Again ICG was used as the chromophore and leak pressure analysis showed fibrin to significantly improve the strength of the repairs without thermal injury.

A similar arrangement was used by Cikrit et al (Cikrit, 1990) to repair canine jugular veins. Human fresh frozen plasma was used as the source of the fibrin however in this case the adhesive was applied after the veins had been lasered. Thus the fibrin was not used as a solder, but as a separate reinforcement.

A comparison of fibrin glue and laser activated fibrinogen was performed by Bass et al (Bass, 1991c). Fibrin glue applied alone to canine choledocotomies produced poor pressure results (104+/-99mmHg) compared to laser activated fibrinogen (264+/-7mmHg). The leakage pressure of sutured repair is seen to be 88 +/-76mmHg. In a similar study, the same group repaired longitudinal

choledocotomies *in vivo* (Bass, 1994b). These showed higher burst strengths for fibrinogen soldered repairs increasing with time.

Oz et al (Oz, 1993a) showed similar results but used ICG as a chromophore in umbilical vein anastomoses but sutured repairs were as strong as those formed with laser activated fibrinogen bonding (LAFB).

Albumin

The use of albumin was first made by Poppas et al (Poppas, 1988a) who used egg albumin coagulated with a CO_2 laser. Their early observations with blood led to experimentation with fascia, muscle and egg albumin leading to the finding that a high concentration of egg albumin provided the most effective repairs.

Menovsky et al (Menovsky, 1994b) also studied the use of egg albumin, human albumin, fibrin, fibrinogen and blood as solders for repairing nerves. No chromophores were used in these studies and human albumin was seen to form the strongest repairs with rehydration seen after 20 minutes. Blood and egg albumin (10%) were not seen to increase repair strength. Later the same group (Menovsky, 1996) used egg white to repair nerves and dura mater, but without chromophores.

Human albumin was used by the same group in 1993 (Poppas, 1993b) to repair ureters. On this occasion the 41% albumin solder was mixed with chromophores such as fluorescein or iron oxide. The use of albumin solder alone in the absence of a chromophore required significantly higher laser power.

Later Poppas et al (Poppas, 1993a) discussed the preparation of human albumin by a lyophilisation technique. Solders of 40-45% (w/v) were the easiest to prepare and the purification technique was seen to reduce the likely transmission of HIV or Hepatitis B Virus. A later report described the technique of producing a 50% human albumin solder (calculation technique unknown) (Poppas, 1996).

This human albumin solder was again used in genitourinary repair by the same group to perform urethroplasty in a rabbit model (Poppas, 1993a) and later by Kirsch et al to perform re-implantation of ureters (Kirsch, 1994), bladder mucosa patch graft urethroplasty (Kirsch, 1995a), venous patch graft corporoplasty (Kirsch, 1995d), full tubed skin graft urethroplasty (Kirsch, 1996c), vasovasostomy (Seaman, 1997a) and ureter repair (Wright, 1997). These were all performed using the human albumin based ICG solder. This group was also the first to publish data on the use of solders to reinforce suturelines in clinical practice. In 1995 a series of ten patients had genitourinary

reconstructions reinforced with 42% human albumin/ICG based tissue solders. The conclusions were that these were safe to perform with no post-operative complications.

Fibrinogen was combined with various chromophores and compared with 35% albumin (source unknown) by Khadem et al (Khadem, 1994) for repair of corneal incisions. The use of solder in this series was seen to result in stronger repairs but no *in vivo* data was presented. Later work by the same team looked at solders in radial keratotomy (Goins, 1997) and later looked at penetrating keratoplasty in cadaveric eyes (Goins, 1998) with the same conclusion as the first study.

The use of albumin solders to deliver growth factors was studied by Poppas et al (Poppas, 1996). The strength of the skin repairs studied were enhanced by the presence of TGF-$ß_1$, which was shown to be heat stable.

After the use of albumin with chromophores, the next major development in solder technology came with the report of solid bands of albumin for nerve repair by Lauto et al (Lauto, 1997b). Previously all repairs had used liquid solders of various consistencies. These had presented problems with application and ease of handling. As solder thickness has been seen to affect the strength of the repair, it was also difficult to precisely apply a certain thickness. In the case of solder films this is much easier to perform and the results from Lauto et al show that while being significantly faster, the technique was no less effective in nerve regeneration. Other patches were used by Trickett et al (Trickett, 1997b) in nerve repair and by Small et al (Small IV, 1997) in aortic patches *in vitro*. A direct comparison was made here with liquid solders along with an investigation of the effect of solder film thickness on repair strength. The results showed that while there was no difference between the liquid and solid solders in terms of burst pressures, the solid solders gave more consistent results with an increase in strength seen with an increase in solder thickness and duration of laser application. This was also confirmed by Chan et al (Chan, 1998a) who looked at scanning electron micrographs of soldered dermal repairs. These showed significant variation in the thickness of liquid solder repairs with globule formation adjacent to the solder/dermis interface.

Investigations into the optimal concentration of albumin in the solder were performed by Lauto in 1998 (Lauto, 1998). This study again looked at solid solder patches at concentrations of 58% and 68% by weight. Using *ex vivo* tibial nerves or vasa deferentia the repair strengths were compared. The conclusion was that a higher concentration of solder results in a stronger tensile repair ($p<0.05$). Previous reports into the use of liquid solders had similar conclusions, with studies performed by Wright and Poppas (Wright, 1997) and Kilkelly et al (Kilkelly, 1997).

Further to this the effects of rehydration have been investigated. Lauto et al (Lauto, 1997b) looked at the rehydration effects of various solder compositions. These strips were made of bovine serum albumin (BSA) and were the first description of such a solder. Immersion of three different concentrations (56%, 66%, 70% and 75% (w/w)) of BSA in saline solution showed that there was a significantly reduced solubility of the 75% solder. These same solders used in rat intestine, were seen to produce anastomotic strength related to the surface area of the repair.

Recent advances in solder application have also been made with a report by Chan et al (Chan, 1998c) into the use of a microjet device to apply solder to the anastomotic line. A diluted solder was required for this device, which produced droplets of 0.2μl in bursts of 1droplet per second into an area irradiated by laser. This ensured that the droplets were coagulated immediately. Scanning electron micrography revealed that there was less evidence of globules at the tissue solder interface indicating a more consistent solder activation. As a consequence of this there was less evidence of solder rehydration on SEM.

The use of porcine albumin has been reported by Mandley (Mandley, 1995) and is based on the premise of safety. Recent concerns into the use of human and bovine sources led to the finding that porcine albumin was both readily available and could form anastomoses capable of withstanding high pressures.

Most recently, the use of two layered solder strips has been reported by Lauto et al (Lauto, 1999). These strips are composed of one layer containing carbon black as a chromophore while the other is undyed albumin (BSA). The use of the two layer strips was intended to reduce the laser intensity required to produce a weld. In this study there was seen to be a reduction by half of the laser power required to produce welds of a similar tensile strength to strips of one layer with no chromophore.

The use of non-human sources of albumin was considered from a safety point of view by this group. An *in vitro* study into methylene blue based tissue solder used porcine serum albumin as a base (Birch, 2000). Although the incidence of viral infection is remote when using human albumin based solder the risk is nevertheless present. In addition recent events pertaining to the rise of Bovine Spongiform Encephalopathy (BSE) and links to Creutzfeld-Jacob Disease (CJD) precluded against the use of bovine albumin.

Chromophores

The use of chromophores was first conceived along with the use of extrinsic proteins after the observations made on the influence of blood on lasered repairs (Krueger, 1985a; Vance, 1988a; Wang, 1988). The use of an exogenous chromophore was first reported by Oz et al (Oz, 1989) and Chuck et al (Chuck, 1989a) who used ICG and FITC respectively. In both of these cases the chromophore was used alone and applied topically. FITC absorbs over a spectrum of 470-520nm with a peak at 490nm. This makes it compatible with the argon ion laser that emits at 488nm. In the report by Chuck et al (Chuck , 1989b) it is stated that the inclusion of blood in the operative site made welding much easier and resulted in stronger repairs. Oz et al (Oz, 1989) on the other hand used ICG with a peak absorbtion at 805nm and a diode laser emitting at 808nm with a power of 300mW ($9.6W/cm^2$).

Parallel to this work Vance (Vance, 1988b) had employed the histological dye basic fuschin as a chromophore for the Argon laser in end to end porcine coronary artery anastomoses *in vitro*. The power density required was reduced by 50% and thermal damage was decreased, as technical consistency improved. Burst pressure testing produced results of 165-250mmHg (mean 211) with chromophore and 90-350mmHg (Mean 222mmHg) without chromophore. A subsequent report by Vance (Vance, 1990) gave consistent burst pressures of 300mmHg in 2mm rat arteriotomies and a 29% rate of aneurysm formation in keeping with other reports (Quigley, 1985, Quigley, 1986).

The combination of chromophore and exogenous protein was reported in 1990 by Oz et al (Oz, 1990), with the use of both ICG and fibrinogen in rabbit aortotomies. The fibrinogen welds were seen to be stronger than the application of ICG alone. Again the solder was activated using an 808nm laser diode at a power density of $4.8W/cm^2$. A similar ICG/fibrinogen solder was later applied by different groups to colonic anastomoses (Libutti, 1990), choledocotomies *in vitro* (Bass, 1991b) and *in vivo* (Bass, 1994a), umbilical vein grafts (Oz, 1993b) and ureteric re-implantation (Kirsch, 1994). Variations on this theme appeared in 1993, with the introduction of human albumin/ICG solders (Poppas, 1993b). This solder was used in bladder mucosa patch graft urethroplasty (Kirsch, 1995a), laryngotracheal mucosa transplantation (Wang, 1995), venous patch graft corporoplasty (Kirsch, 1995d), the first clinical genitourinary reconstructions (Kirsch, 1995a), full tubed skin graft urethroplasty (Kirsch, 1996b), skin (Poppas, 1996), vasovasostomy (Seaman, 1997a) and nerve repair (Trickett, 1997b). The convenience of using ICG alongside a laser diode made this combination cheap and easy to use. The strength of the repairs were often seen to exceed those of the sutured controls with few reported side effects. However the chromophore ICG does not display any significant degree of fading although there is a colour change from one shade of green to another. Thus this chromophore

will continue to absorb long after the solder is activated and generate further heat at the site of the anastomosis. Thermal injury may occur although this has not been reported in longer term studies where histology has been examined.

Alongside developments in the use of ICG came the use of FITC. This chromophore was first reported by Chuck et al (Chuck, 1989b) but was not used with an exogenous protein until 1993 when Poppas et al applied it to urethral repair in *ex vivo* canine ureters (Poppas, 1993b). Absorbing at 490nm FITC originally required the use of an argon laser, but in this study a KTP-532 laser was used. In a latter report by Poppas et al (Poppas, 1993b), a KTP-532 laser was also used to perform patch graft urethroplasty. At 6 weeks, histology showed no significant thermal injury and a significant reduction in urethrocutaneous fistula formation compared to sutured repairs. Subsequent to these reports the use of FITC was not further reported.

Aside from the use of conventional chromophores, one system had been reported based on the use of R5P and its ability to cause protein crosslinking. Khadem et al (Khadem, 1994) used R5P in conjunction with an 18% fibrinogen solder for repairing penetrating corneal incisions. As previously mentioned this photosensitiser is presumed to act by causing nondisulphide covalent crosslinks in susceptible proteins in an oxygen dependant way. This study revealed that burst pressures of 80-260mmHg could be achieved. Later *in vivo* (Goins, 1997) and cadaveric studies (Goins, 1998) confirmed that this photosensitiser/fibrinogen solder was effective in repairing wounds formed in ophthalmic surgery.

Photofading is an attribute exhibited by several chromophores, but none to the extent of methylene blue. This is a photoreduction reaction resulting in the formation of a leuco form of the dye. The molecule can be re-oxidised back to its absorbent form but requires the presence of oxygen. The fading of methylene blue although not reported in solder literature has the potential to enable both the production of a self regulating solder, that fades as it is activated, and a mechanism of visual feedback that indicates to the surgeon when solder activation is complete.

New techniques of anastomosis only gain acceptance if they are technically easier to perform, quicker, have better long-term results and allow access to procedures not previously possible. It is on these criteria that all techniques of anastomoses are judged. Comparisons between different studies are difficult due to the large number of variables, including the differences in application and animal model used, but some comparisons can be made if the studies include sutured controls. Due to the relatively recent developments in solder surgery few references exist to the speed with which the

anastomosis can be performed focussing more on the issues of improved patency, burst strength and reduced complication rates.

Speed

Reports of the speed with which soldered anastomoses can be performed are few as emphasis is placed on anastomotic strength and complications associated with soldering. These are summarised in table 1.3. In testing anastomoses, it is impossible to compare different techniques in a blinded fashion. This leaves the inherent problem of comparing soldered and sutured anastomoses in an objective way.

Reports pertaining to the speed of soldered anastomoses are few but nearly all report reduced anastomotic time. In performing urethroplasty anastomotic time was significantly reduced by Kirsch et al (Kirsch, 1995c; Kirsch, 1997b) along with corporoplasty (Kirsch, 1995d), full tubed skin graft urethroplasty (Kirsch, 1996d) and clinical urinary tract reconstruction (Kirsch, 1995b). Lauto also reported on the reduced time to perform nerve repairs (Lauto, 1997a) although no figures are given. In attempting to apply solder anastomotic techniques to laparoscopic surgery Phillips (Phillips, 1999) found that there was no difference in the time taken to perform vascular clip anastomoses and laser anastomoses but that these were both faster than sutured repairs.

Author	Laser	Application	Speed (mins)		p Value
			Solder	Suture	
Kirsch '95	808nm diode	Urethroplasty	13.8 (+/- 2.5)	24.0 (+/- 5.3)	<0.01
Kirsch '95	808nm diode	Corporoplasty	9.8 (+/-2.3)	17.1 (+/-5.1)	<0.05
Kirsch '95	808nm diode	Hypospadias repair *	7.9 (+/-3.0)	20.3 (+/-9.4)	>0.05
Kirsch '96	808nm diode	Urethral reconstruction	19 (+/-2.5)	31.2(+/-3.3)	<0.003
Kirsch '97	808nm diode	Hypospadias repair	7.7(+/-0.77)	4.9(+/-1.1)	<0.001
Phillips '99	Nd:YAG	Vascular anastomosis	8.4 +/- 0.7	13.8 +/- 1.0	0.001
Phillips '99	1.92μ	Vascular anastomosis	7.8 +/- 0.3	13.8 +/- 1.0	0.001
Phillips '99	VCS	Vascular anastomosis	8.3 +/- 3.3	13.8 +/- 1.0	0.17
Kirsch '01	808nm diode	Hypospadias repair*	8.5 +/-0.8	26.7 +/-1.7	<0.001

*Human study

Table 1.3 A Comparison of anastomotic times

Patency

Comparisons with lasered anastomoses performed by Grubbs (Grubbs, 1988b) showed no significant change in patency with the use of fibrin glue. A later study by Cikrit (Cikrit, 1990) did show a significant increase in patency and decreased disruption. Very few other reports exist on the patency of sutured versus soldered anastomoses. Seaman (Seaman, 1997b) reported on the patency of vasovasotomy repairs with significantly improved results for soldered repair.

Strength

The burst strength of soldered anastomoses is first described by Vance et al (Vance, 1988a) performing coronary artery anastomoses with no significant increase in burst strength. Increased burst strength was recorded by Grubbs (Grubbs, 1988b) Wang (Wang, 1988), Chuck (Chuck, 1989b), Oz (Oz, 1989; Oz, 1990) and Cikrit (Cikrit, 1990) in early vascular soldered repairs. Colonic (Libutti, 1990), bile duct (Bass, 1991b), occular (Khadem, 1994) and ureteric (Poppas, 1993c) anastomoses were also seen to be stronger. Studies in nerve repair showed no increase in tensile strength and soldered repairs were significantly weaker than microsuture repairs. Tendon repairs using 25% albumin were also seen to be weaker than sutured repairs, although were stronger than lasered repairs. These differences were not significant compared to the native tendon. Later work on the use of solder patches showed a higher burst strength associated with patches of higher protein concentration. This was also noted by Lauto (Lauto, 1998) in testing tibial nerves and vasa deferentia. The highest burst pressures reported are attributed to methylene blue based solder in porcine splenic arteries (Birch, 2000). Here pressures exceeding 1100mmHg were seen although this was in an *in vitro* scenario.

Histology

Vance et al (Vance, 1988a) described limited tissue damage with the application of a chromophore (Fuschin). Grubbs et al (Grubbs, 1988b) described a reduced incidence of aneurysm formation ($p<0.001$), while Chuck et al (Chuck, 1989b) reported a lack of carbonaceous material, normally seen without chromophore treatment, with FITC. Oz (Oz, 1989) reported similar endothelialisation in both soldered and sutured anastomoses, while a later paper (Oz, 1990) reported no aneurysms, ruptures or thromboses in soldered repairs. Cikrit et al found similar findings with fibrin solder (Cikrit, 1990). Thermal injury was absent in colonic anastomoses performed by Libutti (Libutti, 1990) and the addition of growth factors enhanced the histological appearance of the repairs formed in skin incisions (Poppas, 1996).

1.7 Preceding work with alternative chromophores

The use of methylene blue was commenced by Mandley (Mandley, 1995) but this came after the investigation of numerous other chromophores. Eosin and EDTA had been used in conjunction with an argon ion laser to some effect. However, these chromophores are not listed in the British Pharmacopoeia (BP) and would not be suitable for submission as a component in a medical implant or device (Solders are classified as a medical device or implant (European Union council directive EU 93/42eec). In addition, the feature of photofading is exhibited to a lesser degree than MB.

The concept of photochemical fading had been introduced in the literature as a possible solution to the problem of defining a precise end point for laser welding and soldering. Various reports had indicated end points such as solder discolouration (Vance, 1988c), turning brown or white, blanching and drying (Basu, 1988). However, none of these were precise enough to prevent laser over-exposure and thermal injury. It was therefore determined to investigate photochromic substances that change colour on exposure to light. In this context, Basic Fuschin and Eosin Y solders were investigated but were not found to exhibit enough fading to act as an indicator. The chromophores listed in the BP were MB, Evans blue and fluorescein. Of these, fluorescein absorbed in the red/ orange spectrum while Evans blue did not exhibit fading to the same degree as MB. Other chromophores not listed in the BP were investigated but none were seen to have advantages over methylene blue.

Further investigation showed that methylene blue gave a significant quantum yield and was initially combined with bovine serum albumin. This solder was seen to produce *in vitro* anastomoses that could withstand pressures of 200-400mmHg. In the light of the events surrounding the incidence of BSE and possible links to CJD, the use of bovine albumin was stopped and alternative sources sought. These included porcine albumin and recombinant human albumin. The latter protein had been developed in an experimental setting and had not previously been used in clinical products. In addition, electrophoretic testing had shown the protein to be impure containing a large proportion of high molecular weight complexes. Porcine albumin was attractive because it was easy to acquire in both sterile and non-sterile forms.

It was subsequent to this that methylene blue was chosen as the basis of a solder product that could be taken to market. With this in mind, it was intended to further investigate the solder to determine the optimal constituents, as well as design experiments, the results of which would form the basis for a submission to LRQA as the designated regulatory authority for medical devices. A successful submission would result in the awarding of a CE mark with the authority to use and market that product as a safe medical device.

1.8 Summary

The search for a technique of sutureless vascular anastomosis has continued for nearly two centuries. Early attempts with mechanical devices, tubes, rings, adhesives, staples and coupling devices met with varied success, but inevitably left foreign material at the site of the repair.

The fundamental techniques of sutured anastomosis, were described by Carrel (Carrel, 1912) at the turn of the century and began the advent of vascular surgery. With increasingly complex surgical procedures, the need arose to perform anastomoses in smaller vessels and in areas of restricted access. Microvascular anastomoses were perfected in the 1960's (Jacobson, 1960) and laparoscopic surgery has enabled many procedures to be performed with minimally invasive techniques. With these and other areas of reduced access the need for non-suture methods of tissue repair and vessel anastomosis is as great now as ever.

The advent of laser surgery offered the hope of fast, strong, easily formed and patent anastomoses that could be performed by minimally invasive techniques. However a high rate of aneurysm formation and medial necrosis excluded this technique. The finding that blood could reinforce lasered anastomoses led to the use of extrinsic proteins such as fibrin and albumin from human sources. In parallel with these developments, chromophores were used to concentrate laser power in the hope that a reduced power would result in fewer complications.

Solders were produced based mainly on albumin in combination with a chromophore such as ICG or FITC. Such solders found wide application, from urological reconstructions to skin and tendon repair. Progress into the clinical arena has occurred in some cases but has been hampered by several common problems. The generation of heat by the laser/solder combination continues to be a problem resulting in thermal injury, while the use of lasers restricts the application of lasers in terms of safety and cost.

The use of proteins such as human albumin raises many problems. Fears of HIV and CJD (Will, 1996, Collinge, 1999) contamination have prevented their use in a medically implanted device despite their resilience to pasteurisation. Similar concerns also exist regarding bovine albumin (Diringer, 1998). Porcine albumin has been utilised by one group (Mandley, 1995) and has been effective in forming vascular anastomoses capable of withstanding high pressures. This is a readily available protein, which is easily sterilized.

Finally, the choice of chromophore in the solder is critical but few choices exist regarding a chromophore that will provide the surgeon with visual feedback or self regulate heat generation.

Methylene blue has been described as photofading, giving an indication to the surgeon of solder activation, while reducing the generation of heat in doing so. In addition evidence suggests that anastomoses formed using methylene blue are capable of with standing supraphysiological pressures and therefore have the potential to form high quality, strong anastomoses with no thermal injury and fewer complications. With this solder it was intended to produce a commercially viable solder that would gain a CE mark as a medically implantable device. In order to satisfy LRQA as to the safety of solders and to characterise the solders physical and biological properties, these experiments were undertaken in combination with other bench mark tests of sterility, purity and tracablity.

Chapter 2

In vitro absorption and burst pressure testing of Methylene Blue based protein solder

In vitro absorption and burst pressure testing of Methylene blue based protein solder

2.1 Introduction

Mandley (Mandley, 1995) used Eosin and EDTA in conjunction with an argon ion laser to produce anastomoses capable of withstanding supraphysiological pressures. Later methylene blue was used and found to exhibit photofading. However results from methylene blue soldered vascular anastomoses have not been reported.

The argon ion laser primarily used in the work by Mandley, required a three phase electrical supply and water cooling (5 litres/min), rendering it less adaptable for many clinical applications. It emits at a wavelength of 488-514nm, which overlaps with the absorption profile of blood and may explain the thermal injury reported by some authors (Krueger, 1985). The introduction of laser diodes was a significant advance in the application of lasers to clinical soldering because of their utility, simplicity and relatively low cost, without loss of chromophore absorption. Their application was however restricted by their emission spectrum and its correlation with the absorption spectrum of a suitable chromophore.

In the case of methylene blue such a laser diode exists and its emission profile can been seen in figure 2.1. The diode emits at a wavelength of 670nm, while MB absorbs over the range 590-700nm with peak absorption at 668 and 609nm. The two peaks can be seen in this absorption profile and relate to the formation of MB dimers and trimers, changing the absorption profile.

The chromophore in a protein solder is considered to act as a heat generator, absorbing incident photon energy, becoming excited and generating thermal energy. This in turn causes polymerisation. The mechanism of this reaction may include crosslinking, disulphide bridge formation, or unraveling and tangling of the protein chains. For those chromophores with an appreciable triplet state quantum yields, there may also be a component involving Type I or II photosensitisation reaction, producing singlet oxygen, leading to the oxidation of albumin residues (Shen, 1995). There is also the possibility of a photochemical reaction of the chromophore with solder components. However little is known of the precise nature of the cohesive and adhesive bonds formed with laser activated solder.

Early work (Mandley, 1995) using this combination had relied on a concentration of methylene blue equating to 0.15%w/w activated at a power of 180mW (power density of 22.9W cm^{-2}). This resulted in a burst pressure strength of 200-300mmHg. However, the effects of varying chromophore concentration had not been investigated or reported. It was therefore intended that the relationship between burst pressure strength, chromophore concentration and absorption [1] be investigated.

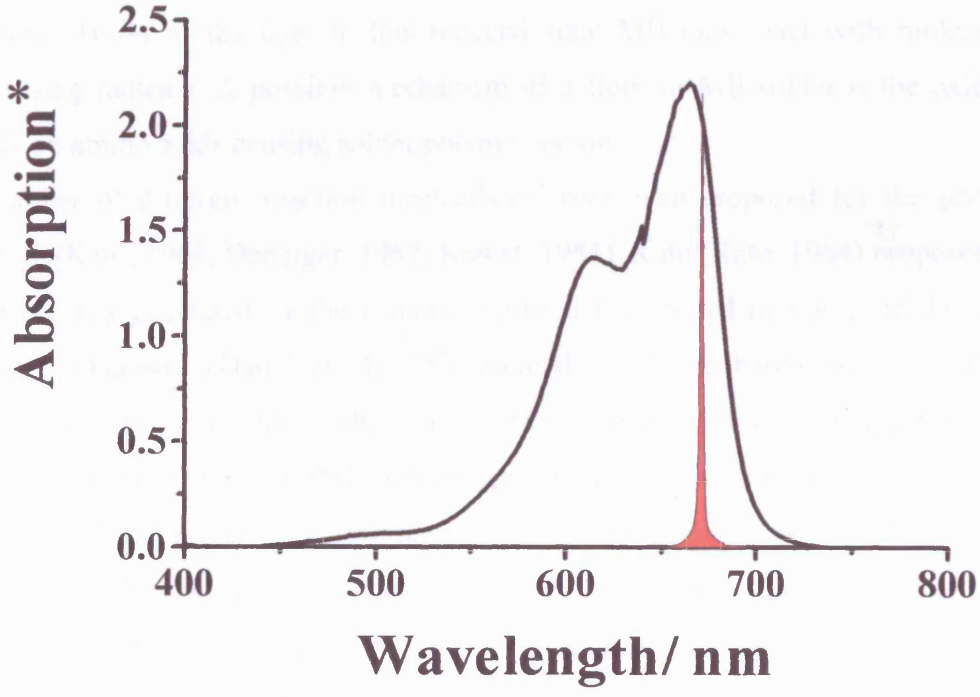


Figure 2.1: The Absorption spectrum of Methylene Blue (Black) and laser emission profile (Red)

2.2 Methylene Blue

Chemistry & Photochemistry

Methylene blue (MB^+ Cl^-) was first described by Caro (Caro, 1877). It is a member of the thiazine group of dyes (Fig 2.2), has a relative molecular mass of 319.85. MB and absorbs strongly in the visible region of the spectrum (Fig 2.1) with an extinction coefficient of $7x10^5 dm^3 mol^{-1} cm^{-1}$ at

* Absorption is a ratio and therefore no units are given.

664nm in plain aqueous solution. At low concentrations ($\approx < 10^{-6}$ mol/dm^3) it exists in the monomeric form with a peak absorption at 664nm. As the concentration is increased, dimers and higher aggregates are formed, the dimers having an absorption maximum at 615nm. This is seen at lower concentrations as a shoulder in the absorption profile (Fig 2.1).

In 1961, Usui et al (Usui, 1961) found that methylene blue could be photoreduced to the colourless 'leuco' form following irradiation with visible light. This leuco form can be oxidised back to MB by atmospheric oxygen, although this reaction is not quantitative and is accompanied by a slight permanent degradation of the dye. In this reduced state MB may react with molecular oxygen to produce oxidising radicals. A possible mechanism of action for MB solder is the oxidising effect of these radicals on amino acids causing solder polymerisation.

A number of different reaction mechanisms have been proposed for the photoreduction of methylene blue (Kato, 1964; Danziger, 1967; Kamat, 1981). Kato (Kato, 1964) proposed that the semi-reduced species was generated by the reaction between the excited monomer triplet and the ground state monomer. Danziger (Danziger, 1967) presented a scheme based on the irradiation of MB monomer or dimer (Fig 2.3 - A) resulting in the formation of a dimeric charge transfer species (B) leading to the formation of leuco MB (C) or regeneration of the ground state (A).

Kamat and Lichtin (Kamat, 1988) have suggested a mechanism whereby an electron is ejected from excited MB. This electron becomes solvated and reacts with the dye and hydrogen, forming semireduced MB ($MBH^{+}\bullet$). Semireduced MB (B) can then revert to ground state MB (A), leuco MB (C) or combine with semi-oxidised MB ($MB^{2+}\bullet$) giving 2MB. It is this photobleaching reaction which makes methylene blue an attractive chromophore for incorporation into a laser activated solder. This gives the potential not only for a visual end-point to laser irradiation, but bleaching at the irradiation wavelength resulting in a reduction in incident light absorption. This, it is hoped, provides protection from over-irradiation and thermal damage to the tissue.

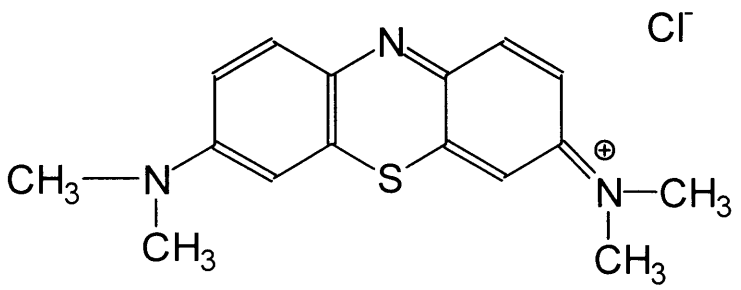


Figure 2.2: The Structure of Methylene Blue

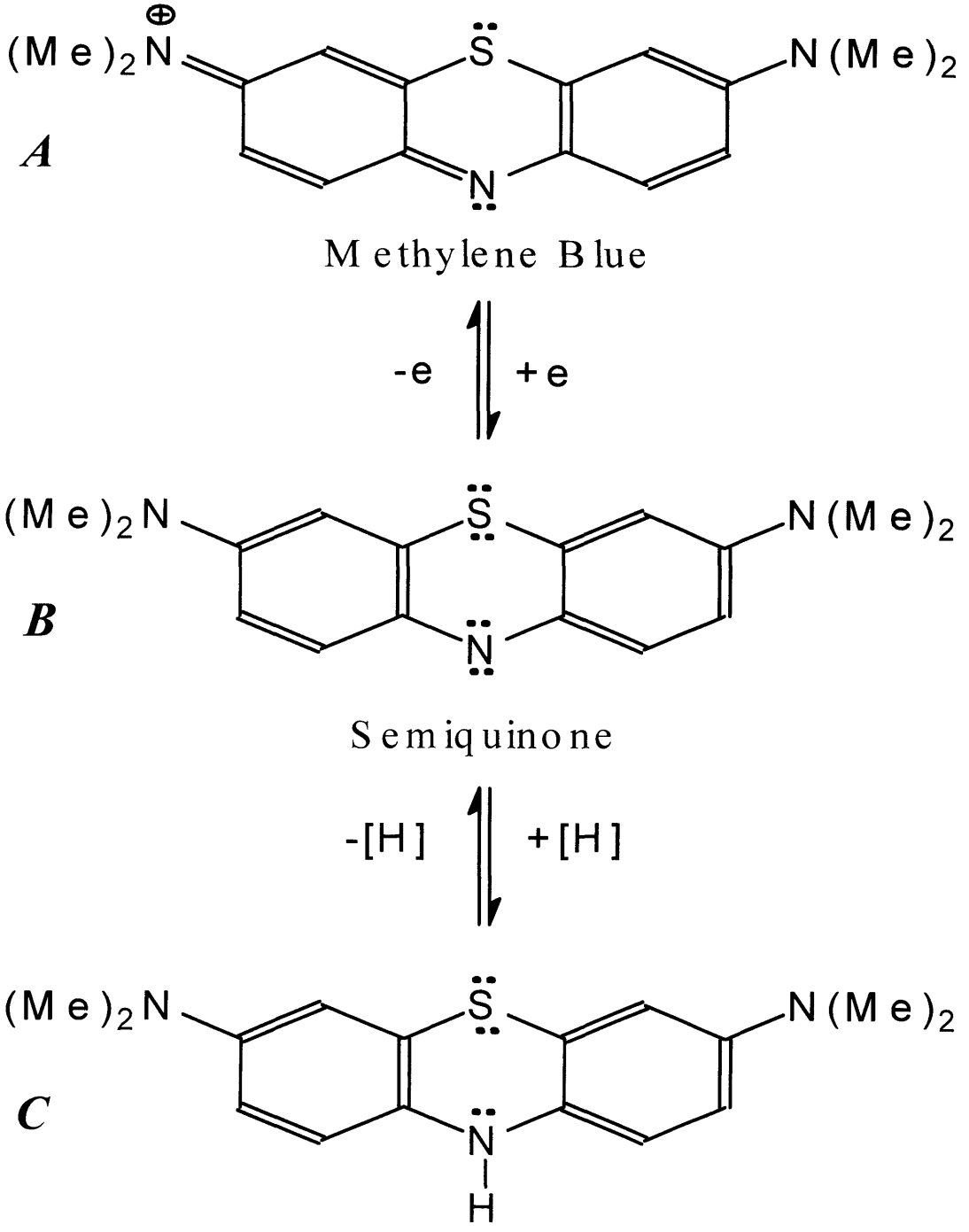


Fig 2.3: The photoreduction of Methylene blue

Pharmacology and Clinical Use

Methylene blue was described as the first synthetic antimalarial agent by Ehrlich in 1891 (reported in Vennerstrom, 1995). MB has since been described as a nitric oxide synthetase inhibitor (Johnson, 1989), a cGMP inhibitor (Johnstone, 1993), also showing anti-muscarinic and anti-cholinergic effects (Pfaffendorf, 1997). Nitric oxide synthetase inhibition has been used in the treatment of refractory hypotension in septic shock and anaphylaxis (Evora, 1997a; Zhang, 1995; Hata, 1995; Evora, 1997b). These latter effects were studied in experimental canine models of endotoxic shock, showing significant changes in both pulmonary and systemic arterial pressure after infusion of MB at levels of 2-10mg/kg (Zhang, 1995; Daemen-Gubbels, 1995; Preiser, 1995).

Barber et al (Barber, 1995) reported that the application of Methylene blue to the exterior of saphenous vein, as practiced in CABG procedures, may eliminate acetylcholine induced relaxation, mediated by NO. Bentz et al (Bentz, 1991) agreed with these findings of reduced vascular relaxation and although increased platelet deposition was claimed, the results were shown not to be statistically significant.

Other clinical uses of MB include the localisation of parathyroid glands (Bland, 1985), the eradication of renal stones (Wein, 1976; Boyce, 1967), the treatment of eczema herpeticum (Chang, 1975b; Chang, 1975a), the treatment of methaemaglobinaemia (e.g. in dapsone poisoning) (Ferguson , 1997; Berlin, 1984) and in addition MB has been patented as an anti-HIV agent (Floyd, 1996).

There is some evidence that the injection of large quantities of MB into the intra-thecal space to locate the cause of CSF rhinorrhoea has caused progressive paraplegia and other neurological deficits (Gerrard, 1980; Poppers, 1970). Other adverse reactions include the induction of jejunal atresia (Daemen-Gubbels, 1995) and haemolysis (Cowett, 1976; Crooks, 1982) after intra-amniotic injection, as well haemolysis in conditions of unstable haemoglobins (Sills, 1994).

2.3 Materials and methods

Solder preparation

The solder was prepared using reconstituted dried ingredients. Porcine albumin powder (Sigma Aldrich Chemicals, USA), and methylene blue powder (Sigma Aldrich Chemicals, USA) were mixed together and hydrated with 'Water for Injection' BP (Phoenix Pharmaceuticals, UK). (Selected due to

together and hydrated with 'Water for Injection' BP (Phoenix Pharmaceuticals, UK). (Selected due to its inclusion in the BP), and 2% w/w glycerol (Martindale pharmaceuticals, UK). The resultant solution was mixed for 30 minutes and left to stand for 2 hours. The final albumin concentration was kept constant at 41% (w/w) (Mandley, 1995), while the absorption of the solder was varied by changing the MB concentration (0.15-0.6% w/w).

Activating system

Activation was performed using a laser diode system (Laser Module - HPM250/3139, Laser 2000, Ringstead, Northants, UK. (Fig 2.4)) coupled to a silica optic fibre (50μm core diameter) at a wavelength of 670 nm, 180 mW power and a focused spot diameter of 1mm at 40mm (22.9 W cm^{-2}). Laser power was measured using a Coherent power meter (Model 210, Coherent, USA).

Absorption measurement

Absorption spectra of undiluted solder were recorded in a 100μm pathlength cell using a Xenon arc lamp as the illumination source and a gated intensified photodiode array detector (EG&G Princeton Applied Research). The solder was irradiated at chromophore concentrations of 0.15-

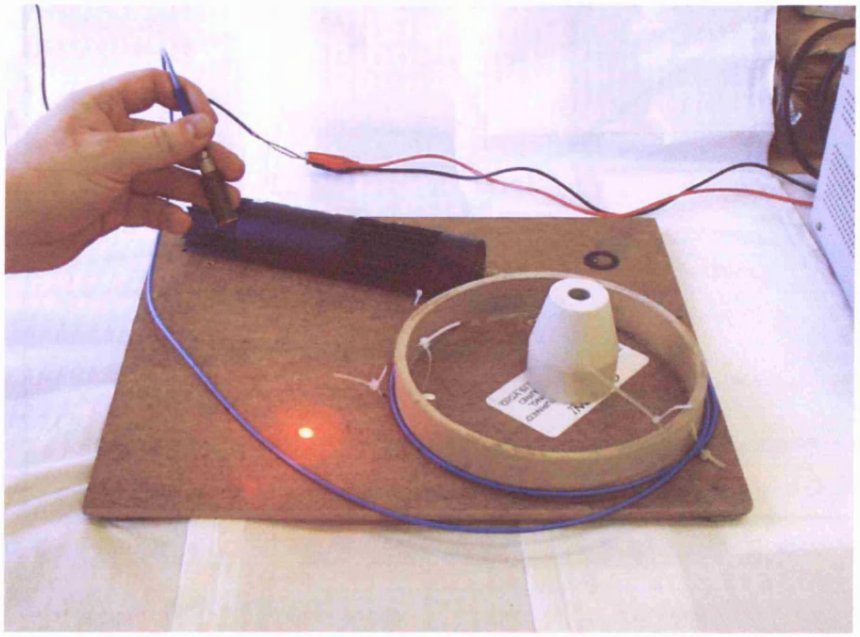


Figure 2.4: The Laser 2000 system

0.60%w/w and the proportion of incident light to transmitted light was recorded. The resultant plot of chromophore concentration against absorption (Fig 2.5) was used to determine the absorption of the solders used to anastomose vessels. For each data point a single measurement was made and plotted as the redline (Fig 2.5). This was compared to the calculated relationship according to the Beer-Lambert law, expressed as the black line (Fig 2.5). The spectral profile of the laser diode was measured using the gated photodiode array system. The use of this apparatus has previously been described (Worrall, 1998).

Anastomotic technique

Porcine splenic arteries (2-4mm) were harvested, cleaned, sectioned and two stay sutures applied (8/0 polyamide). The vessel was secured on a fenestrated needle and the stay sutures used to appose the vessel edges. Volumes of 1-2µl of solder per side were used and activated with the laser. This procedure was repeated as a second layer was applied. The solder was irradiated for 5 seconds per mm (+/-100µm) spot and chromophore fading observed as the end-point. Typically, the number of spots on each side of the vessel was approximately 5, with a small area of overlap between each spot. This process was repeated 6 times for each concentration of chromophore and the results tabulated and plotted (Origin™, Microcal, UK).

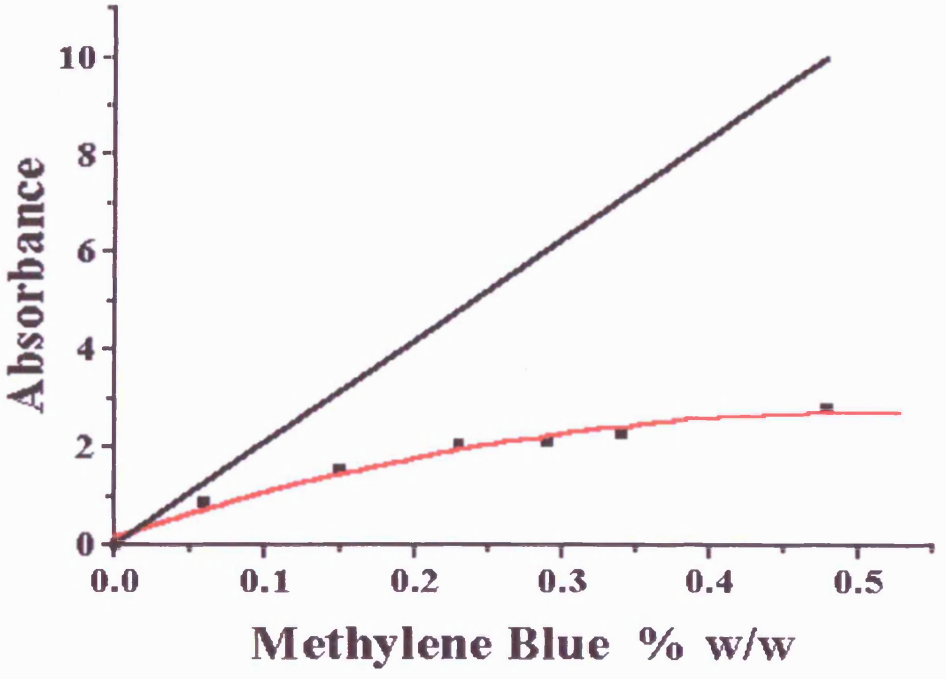


Figure 2.5: Methylene Blue absorption as a function of concentration (Red) with predicted Beer-Lambert

Burst Pressure Testing (BPT)

The completed anastomosis was pressure tested using a syringe driver, pressure transducer (0-30psi) (RS Components, UK) and PC (See Appendix C). The needle and vessel were mounted between the transducer and the syringe pump, and the PC set to acquire data. A digital manometer was also used to corroborate the results. The vessel was observed for signs of leakage and the maximum pressure was recorded and plotted. Side branches occasionally leaked, and these were occluded by ligation.

2.4 Results

The results are summarised in figures 2.5, 2.6 and 2.7. The results of the measurement of absorption versus chromophore concentration (Fig 2.5) shows the linear concentration-absorption curve (Black line), calculated by extrapolation to infinite dilution, and the measured values of absorption at the stated concentrations (Redline). Clearly there is a significant deviation from linearity, possibly due to aggregate formation, which occur at higher concentrations.

For burst pressure testing, a sigmoid 'dose-response' type curve is seen to changes in concentration and absorption of the solder, with a plateau after a chromophore concentration of 0.27%

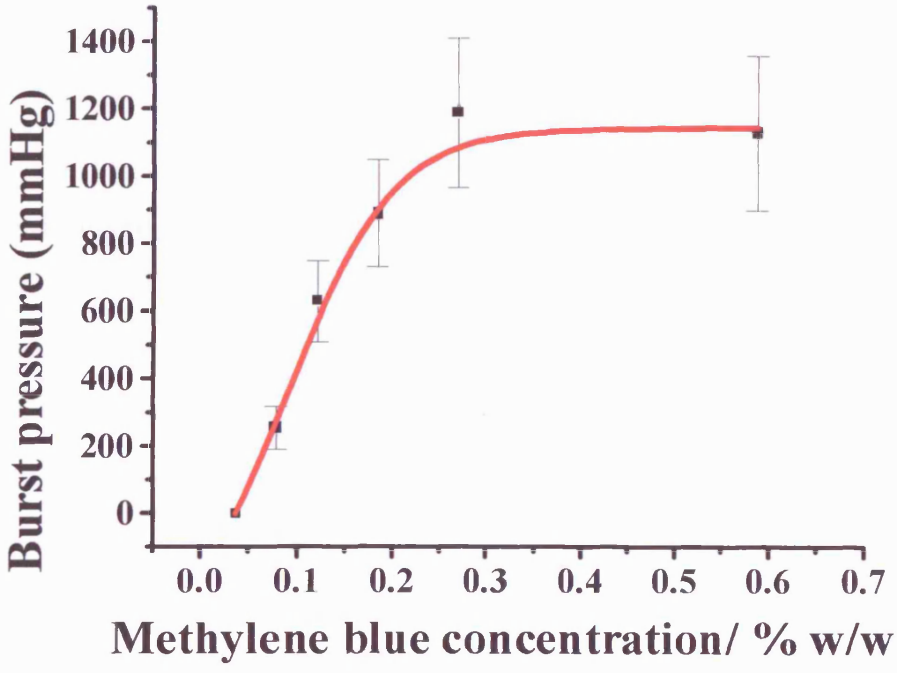


Figure 2.6: Burst pressure as a function of solder concentration (+/- sem)

w/w (Fig 2.6.), corresponding with an absorption of approximately 2 at 670nm. This equates to approximately 99% of incident energy absorption.

The relationship between solder absorption and burst pressure is therefore altered due to the non-linear relationship between concentration and absorption, resulting in the curve of figure 2.7. The burst pressures achieved in this study show levels not previously recorded in comparable experiments (Kamiji, 1989, Sagi, 1986, Wang, 1988, Poppas, 1993b) (1188+/-222mmHg) indicating that the potential strength of soldered vascular anastomoses is greater than previously anticipated.

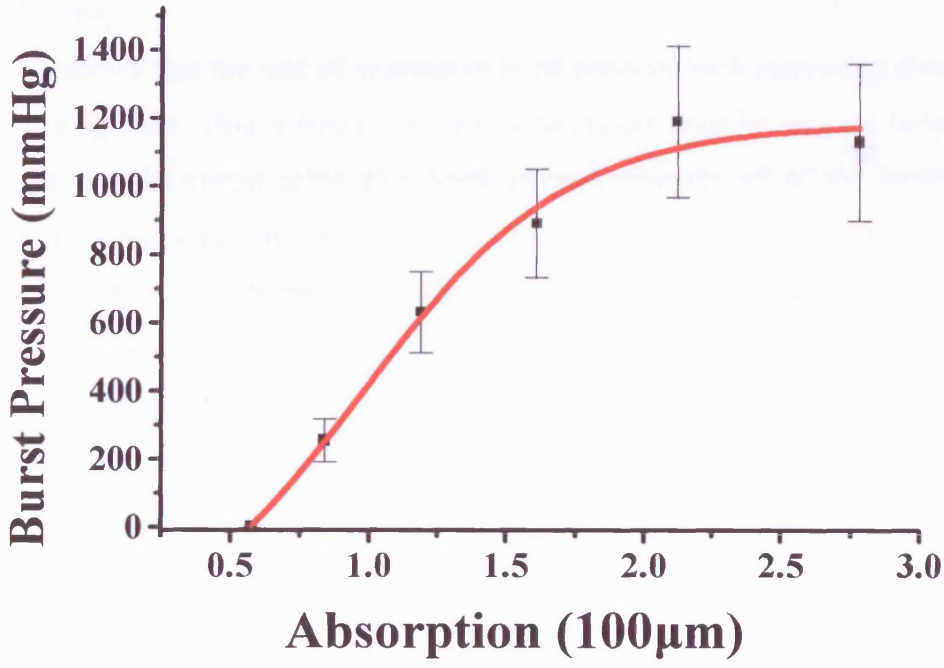


Figure 2.7: The burst pressure as a function of Solder absorption

2.5 Discussion

The results of this investigation show a sigmoid 'dose-response' type curve of concentration against burst pressure (Fig 2.6.). A similar relationship to that observed here has previously been described by Judy et al (Judy, 1994) where the relationship between external light energy and shear strength is shown for dura mater repairs *in vitro*. This was performed using an argon ion laser or a filtered xenon arc lamp in conjunction with brominated 1,8-napthalimide dye for photochemical

The result of concentration against absorption however, shows a flatter curve and the increase in anastomotic strength rises more evenly (Fig 2.7.). The explanation for his lies in an understanding of the behaviour of MB in a solder. Measurement of absorption (@ 670nm; 10mm pathlength) against MB concentration (Fig 2.5) shows a significant deviation from the Beer-Lambert law at high concentrations. This deviation is thought to be due to the well known monomer-dimer-trimer equilibria observed for MB (Braswell, 1968) while at higher concentrations dimeric and trimeric forms dominate the equilibrium mixture. Since only the monomeric form absorbs significantly at 670nm, the change in the relative concentrations between these molecules affects the absorption of the solder used in this study (Braswell, 1968).

Figure 2.7 shows that the rate of increase in burst pressure with increasing absorption slows as absorption becomes greater. This is because a finite temperature must be reached before solder curing is achieved. Once the absorption achieves a level where essentially all of the incident photons are absorbed, a maximum initial heating rate will be achieved. However, even beyond the level where 99% of photons are absorbed, (an absorption of 2) there is still some increase in burst pressure with increasing concentration. This can be explained by the photochemical reaction of MB and the formation of the 'leuco' form, with loss of absorption at 670nm. In addition, at lower concentrations the proportion of dimers present in the mixture is lower. Hence, at lower concentrations, this reaction results in a rapid decrease in the proportion of photons absorbed with few monomers formed from dimers. At higher concentrations, although the same rate of concentration loss is expected, since there is a greater initial absorption, there is a significant irradiation time before there is a significant decrease in the proportion of incident photons absorbed. Consequently, the time for which heating is sustained is greater.

This indicates that in order for optimal burst pressures to be achieved, sufficient thermal energy must be generated. The period required will be a function of the absorption of the solder and the state of hydration of the vessel, which alters heat capacity and heat conduction at the anastomosis. Dehydration of the solder is also a significant factor in the curing reaction. The degree of tissue hydration is therefore critical but difficult to control, affecting both the formation of cohesive bonds (solder to solder) and adhesive bonds (solder to adventitia). The optimal conditions for these interactions are poorly understood at present and further investigation is required to refine solder vascular anastomosis (Marx, 1997).

Attempts have been made to avoid thermal damage by using a temperature controlled feedback system incorporating infrared detectors (Poppas, 1996) or thermocouples (Self, 1996), but these can only give an estimate of the surface temperature reached. They are also difficult to align due to the small size of the anastomotic area. Using a system based on a photochemically bleaching chromophore, such as that described here, temperature rise is potentially limited as photobleaching will reduce absorption and limit the heat build-up, preventing over-exposure. Careful control of the chromophore concentration will allow control over the peak temperature reached, and over the length of time for which this is sustained, although no attempt has been made to quantify this effect in this study.

MB was used in this study to produce a tissue adhesive that provided a visible end point and potentially a self regulating absorption switch. This solder is able to produce anastomoses capable of withstanding high pressures and can therefore be applied in the *in vivo* situation to determine its suitability for clinical use.

Chapter 3

Short term *in vivo* investigation of methylene blue soldered microvascular anastomoses

In order to address the issue of soldered anastomotic viability *in vivo* and provide satisfactory evidence for the regulatory authorities (based on the criteria stated in European Union council directive EU 93/42eec), this study included the addition of radiolabelled albumin (I-125). Iodine-125 (half life: 60.2 days) was covalently bonded to porcine albumin (see Appendix B) and mixed with the solder solution. Radio-iodine has been used over many years to determine protein turnover using either I-125 or I-131 (Jarnum, 1971). Iodine-125 labeled human albumin is regularly used as a radio pharmaceutical tool for the determination of plasma volume. Radio-iodine has the advantages of not affecting protein metabolism and the label is rapidly excreted after metabolic breakdown. Labeling with chromium (Cr-51) has the effect of causing protein denaturation and is lost from the protein with time. Labeled albumin has been reported in human studies over a 21 day period (Sterling, 1951), with similar results reported by Matthews (Matthews, 1957). Most significantly, McFarlane (McFarlane, 1958) reported a different rate of catabolism of I-131 and I-125 over a 22 day period. The conclusion from this is that the rate of iodine clearance is a good indicator of protein catabolism.

In parallel with the surgery, a series of blank standards were prepared with a known mass of solder. To correct for isotope decay these blanks were gamma counted and used to interpret the counts remaining in the soldered anastomoses. This allowed data interpretation in terms of the actual amount of the solder remaining after a particular time. The anastomoses were formed with a known amount of solder and allowed to continue for up to 60 days before being sacrificed. The explanted vessels were then placed into a gamma counter and the amount of solder remaining expressed as a percentage. In this way the proportion of albumin left at the site of the anastomosis could be determined, as well as a determination of the effects of changing power density on the patency of soldered microvascular anastomoses.

In addition to gamma counting, the explanted vessels were fixed in formalin and examined for histological markers of healing, endothelialisation, inflammation, foreign body reactions, intimal hyperplasia, medial necrosis and aneurysm formation.

3.2 Materials and methods

Radiolabelling of albumin

The technique of radio-iodination of albumin is described in Appendix B.

Solder preparation

See Chapter 2. The MB concentration was kept constant at 0.24% w/w.

Activating system

Activation was performed using a laser diode system (Laser Module - HPM250/3139, Laser 2000, Ringstead, Northants, UK) coupled to a silica optic fibre (50μm core diameter) at a wavelength of 670 nm, 180 mW (22.9 W cm^{-2}) or 90mW (11.4 W cm^{-2}) power and a focused spot diameter of 1mm (+/- 100μm) at 40mm. Laser power was measured using a Coherent power meter (Model 210, Coherent, USA) (see appendix C).

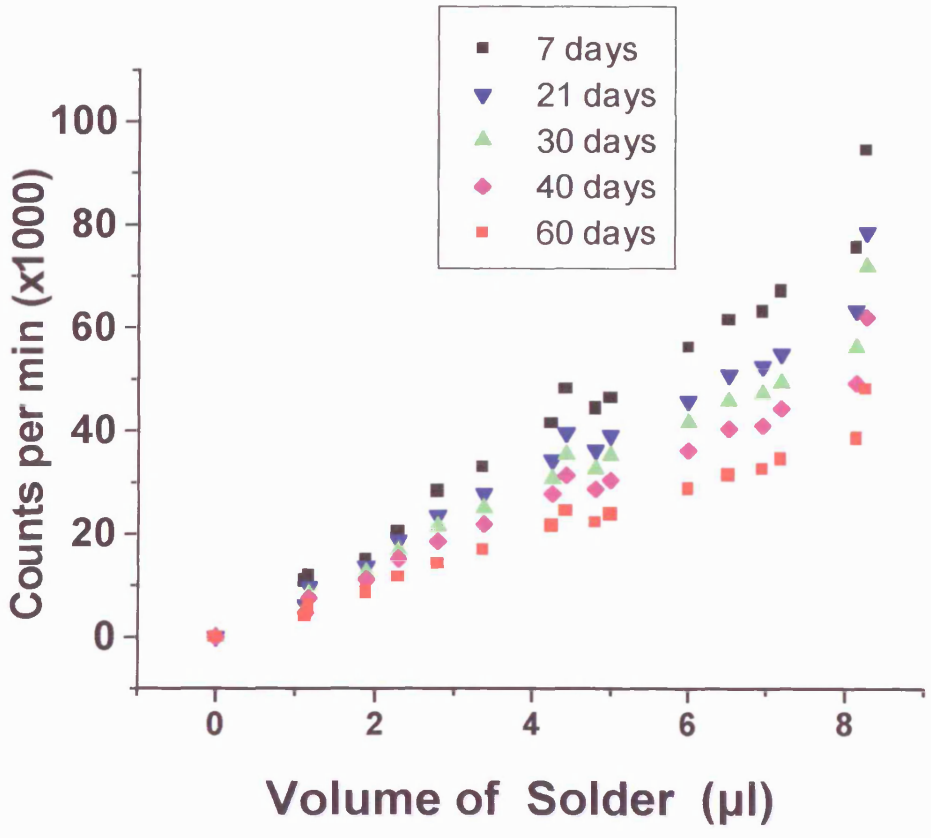


Figure 3.1 Radio-activity against solder mass for blanks

Surgery

Thirty male New Zealand white rabbits (2.5-3.5 Kg) were divided into two groups, a) High Power (180mW, 22.9 W cm^{-2}) and b) Low Power (90mW, 11.4 W cm^{-2}). Each animal received a premedication dose of 0.3ml/Kg of Hypnorm (Fentanyl citrate 0.315mg/ml, Fluanisone 10mg/ml - 0.3mg/kg) IM 15-20mins prior to anaesthetic. The animal was anaesthetised using inhalational anaesthetic induction and maintenance (Halothane 5% reducing to 2% and 1.5 Ltrs O_2) and monitored with an oxygen saturation probe.

The animal was placed supine, the skin shaved and prepared with aqueous chlorhexidine and povidone iodine, and draped for surgery. A midline incision was made and the left carotid exposed and prepared for anastomosis with haemorrhage controlled using bipolar diathermy. Heparin was administered (1000-1500 iu, IV). The vessel was clamped using an Acland 3V clamp (Fig 3.2), transected and three stay sutures applied (8/0 Polyamide). Solder was applied to one face of the anastomosis at a time and activated using the Laser 2000 (L2K) system at a power setting of either 11.4 W cm^{-2} or 22.9 W cm^{-2} for 5 seconds per spot (Fig 3.3). Typically, 4-5 spots of irradiation were required for each face. Two layers of adhesive were applied to each surface (Fig 3.4). The clamps were then removed (Fig 3.5). With the observation of constriction in an anastomosis, papaverine (5mg/ml) was applied. Once haemostasis was achieved, the wound was closed with 3/0 Vicryl. The animals were kept for 7, 21 30, 40 or 60 days, after which time the animal was terminated, the vessel inspected for patency, thrombosis or disruption and removed.

Gamma counting

The vessels were mounted on card and placed in counting tubes filled with 10% formal saline. They were counted in a gamma counter (Auto gamma detector, PW 9530, Philips, UK) in parallel with standard blanks, to produce a correction for each sample and enable the mass of albumin left to be determined. The blanks were composed of three cards each corresponding to a particular mass of solder. On gamma counting these the mean count was taken as the data point for the graph. The results of the standard blanks are displayed in figure 3.1. This table shows the counts for each of the blanks over the given time periods e.g the line composed of black squares, shows the counts for the blanks on day 7 after the procedure.

The raw data for these graphs can be found in Appendix B Tables B2 and B3.

Histology

After gamma counting the vessels were fixed in 10% neutral formalin. Sections of four microns were stained with Haematoxylin & Eosin for general morphology and with elastic Van Geison to assess the collagen and elastic elements of the tissue. The sections were examined in a blinded fashion, for thermal injury, thrombus formation, giant cells, endothelialisation, constriction, neutrophil infiltration, medial disruption and intimal hyperplasia. Grading of IH was not performed due to financial restraints and the fact that it was not anticipated that such significant levels would occur.

Statistical Analysis

The data points relating to the number of counts found at each time point were analysed using Fishers' Exact Test (calculated on Prism™ (Graphpad software Inc, UK)) for non-parametric data. A p value of 0.05 was considered significant.

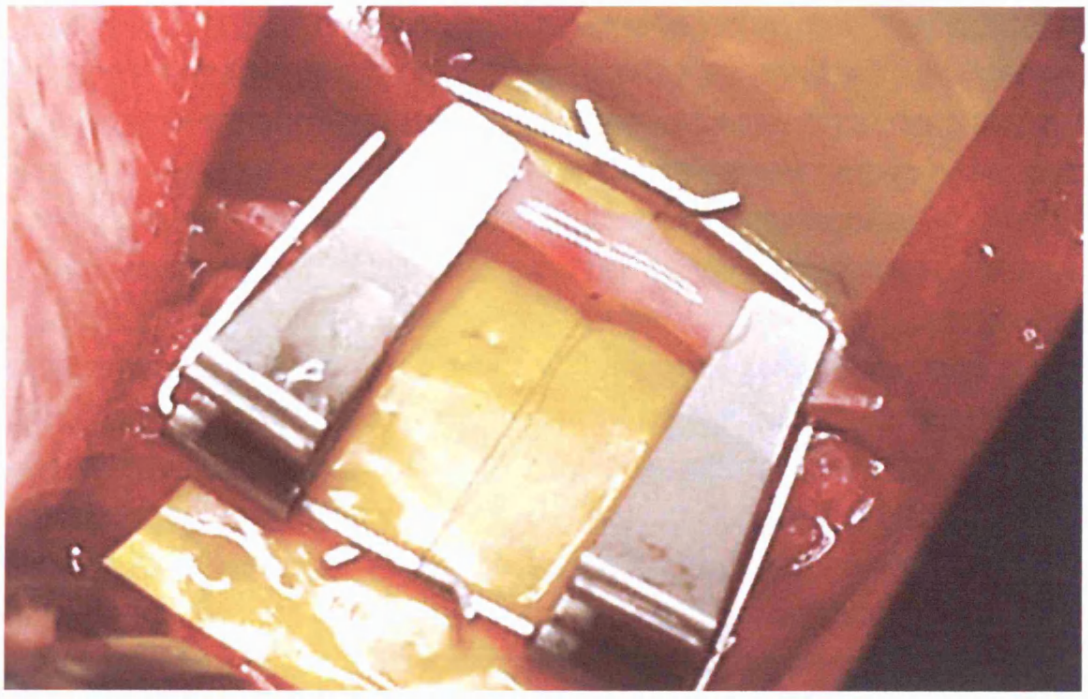


Fig 3.2: Acland clamp applied to rabbit carotid

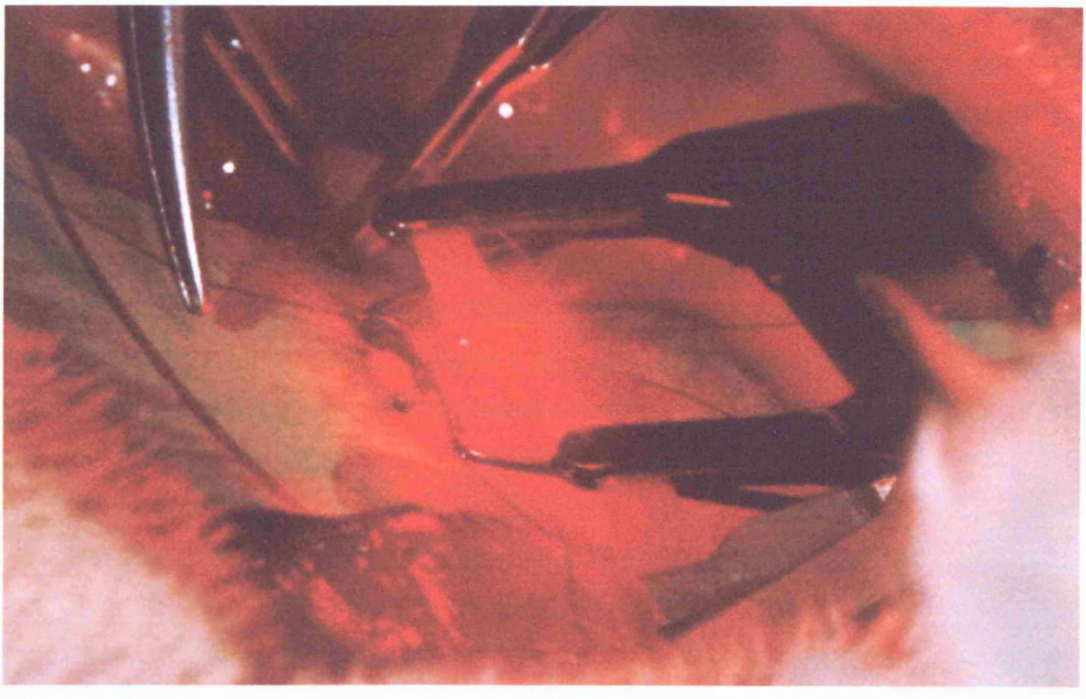


Fig 3.3: Laser activation of solder

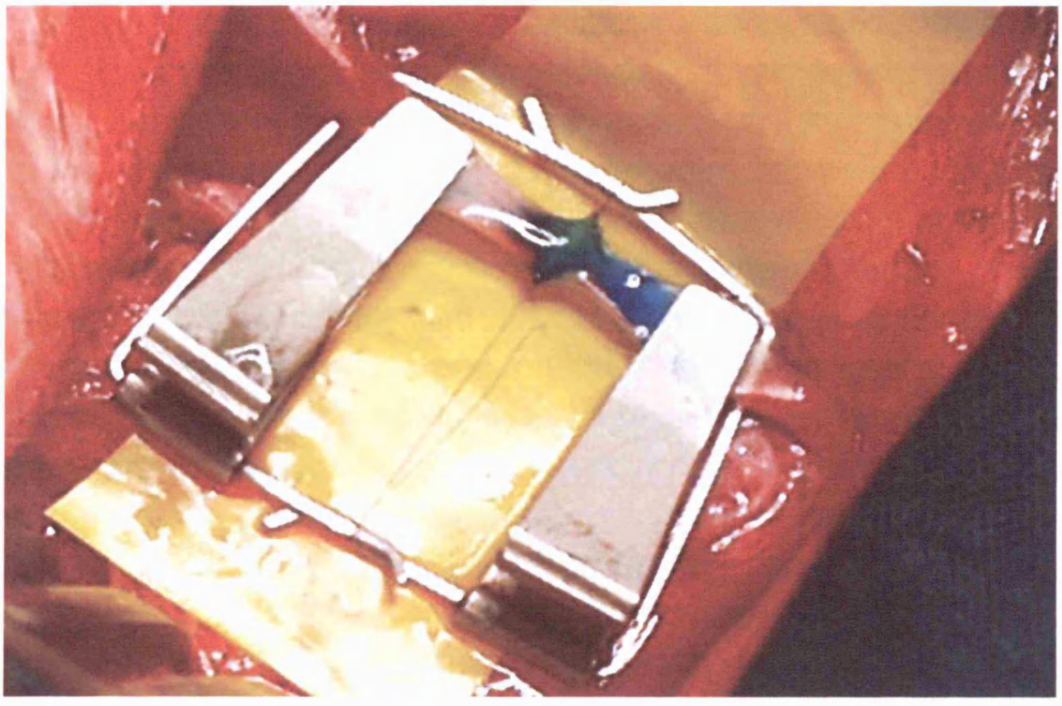


Fig 3.4: Solder activated on one face of carotid

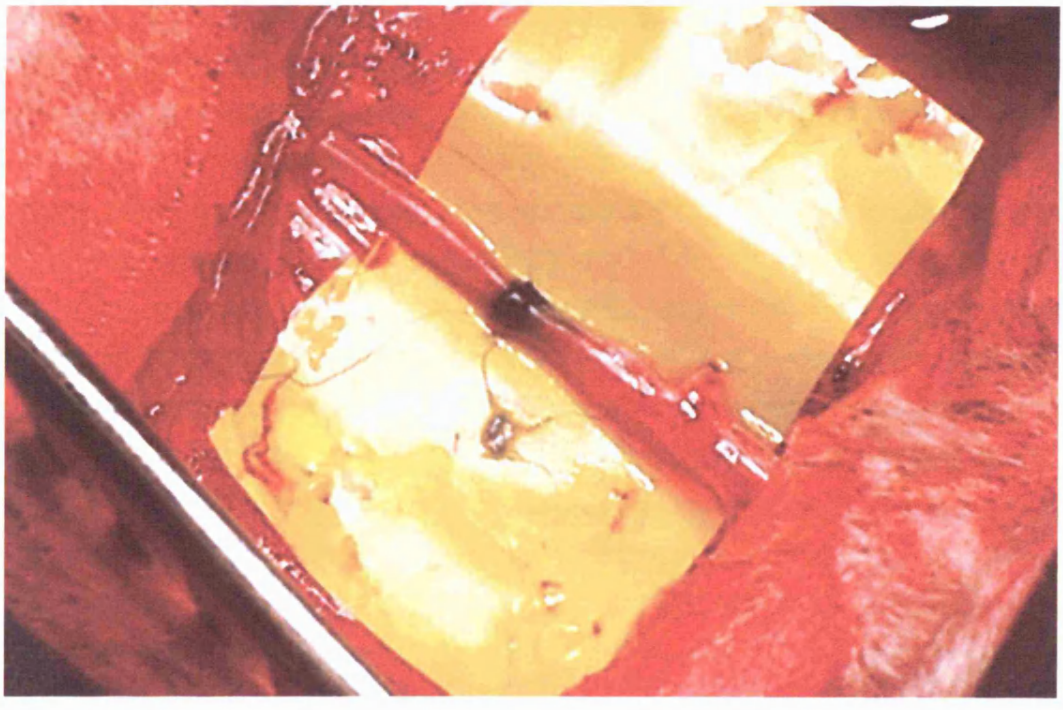


Fig 3.5: The completed anastomosis

3.3 Results

Solder re-absorption

The counts from the explanted vessels were corrected for radioactive decay and the counts translated into volume of adhesive remaining (Appendix B tables B2 & B3). This was then used to calculate the volume of adhesive left at the anastomotic site. The results of each group are summarised in figures 3.6 and 3.7 with the percentage of solder remaining shown in figure 3.8. The lines shown represent a 'best fit' function within Microcal Origin™. Statistical analysis showed no significant difference ($p>0.5$) between the two groups in terms of solder re-absorption and overall half-life. The half life of the solder in both cases was 10.1 days with gamma counting showing approximately 7% of the solder remaining after 60 days.

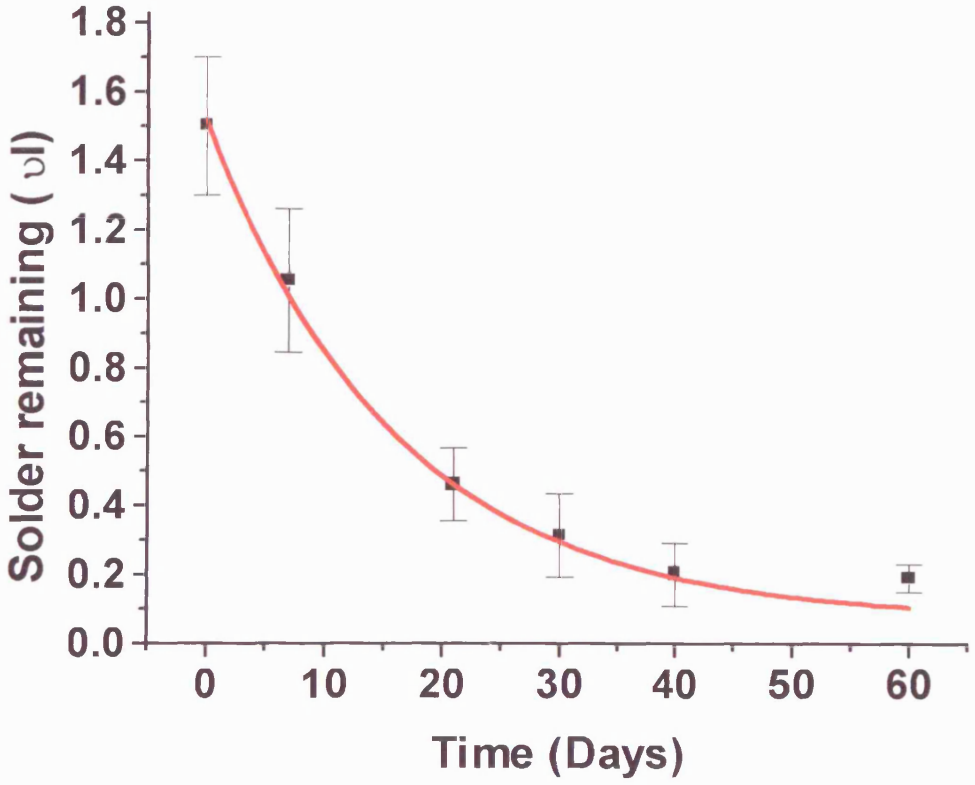


Figure 3.6: Solder re-absorption at high power (22.9 W cm^{-2})(+/-sem)

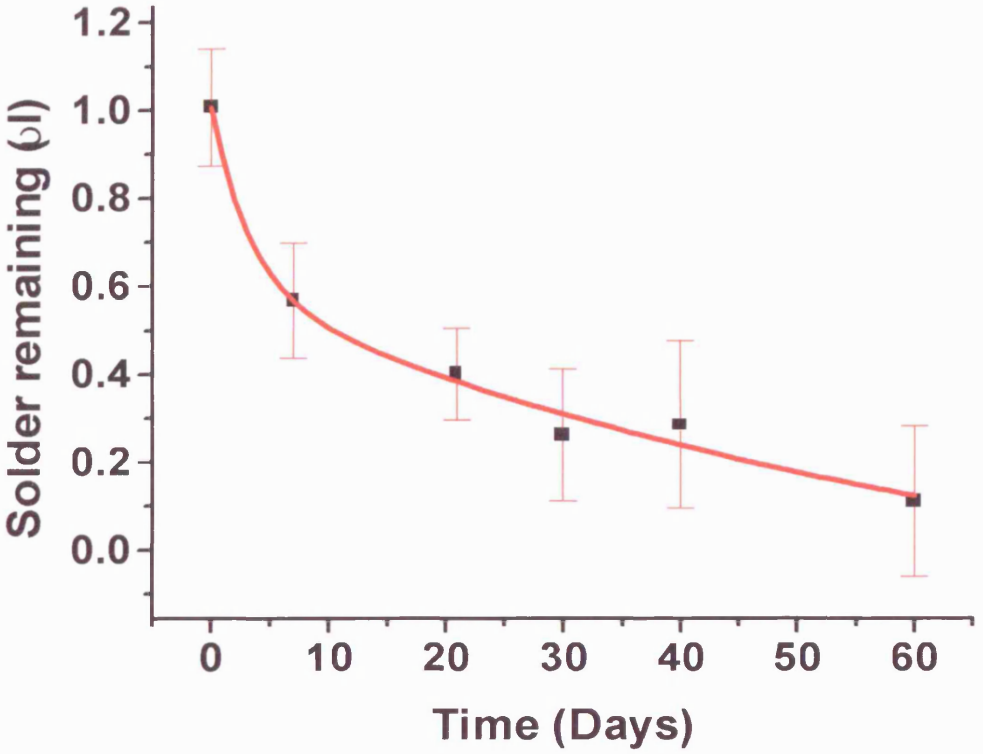


Figure 3.7: Solder re-absorption at low power (11.4W cm^{-2}) (+/-sem)

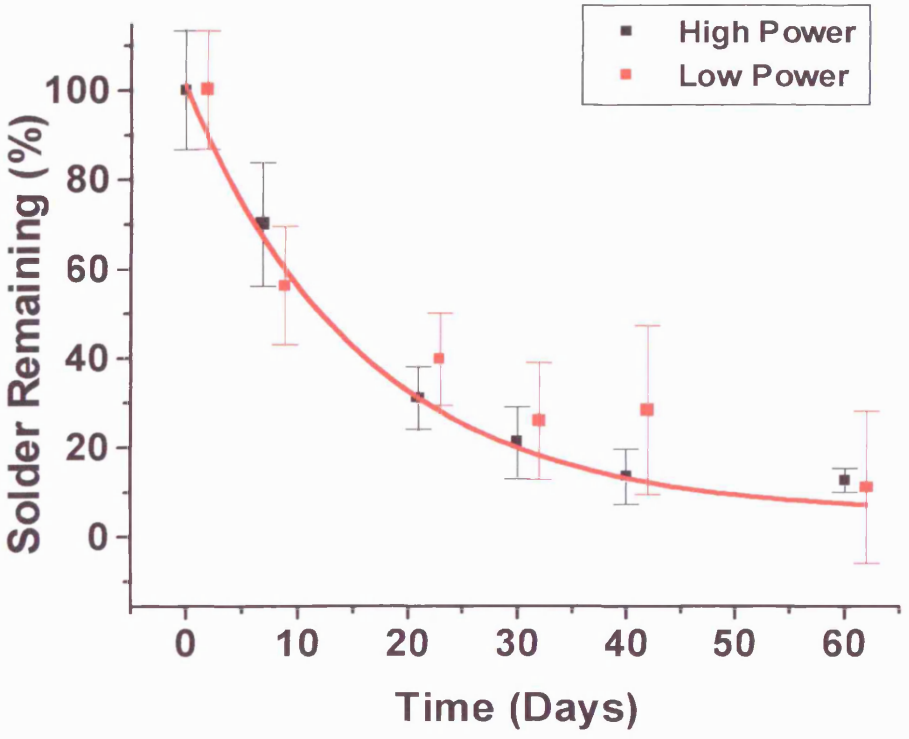


Figure 3.8: Solder re-absorption as a percentage (+/-sem)

Histology

There was a significant difference between the two groups in terms of patency and thrombosis as summarised in tables 3.1 and 3.2. The low power group showed a patency rate of 9.4%, as against 0% for the high power group ($p<0.001$). This is also reflected in the rate of thrombosis showing a 100% rate of thrombosis in the high power group as opposed to 6.7% in the low power group ($p<0.001$). High power microscopy (x200) showed the rate of endothelialisation was higher in the low power group at 100% from day 7 compared to 26.6% of those that could be assessed in the high power group ($p<0.001$). The incidence of intimal hyperplasia (IH) was higher in the low power group at 66.6% as against 20% in the high power group ($p<0.05$). No giant cells were evident in the low power group but were present in up to 40% of the high power group ($p<0.01$) excluding those found adjacent to stay sutures. Post operative constriction was found in none of those performed at low power as opposed to 13.4% in the high power group ($p=0.06$) and was not statistically significant. However intraoperative constriction was consistently seen in the high power group but this effect was no quantified. Neither group showed histological evidence of thermal injury, aneurysm formation or medial disruption, while neutrophil infiltration into the adhesive was ubiquitous throughout all specimens. The relevance of these comparisons is debatable since there was such a high rate of occlusion in the high power group, and meaningful comparisons of endothelialisation and IH cannot be made.

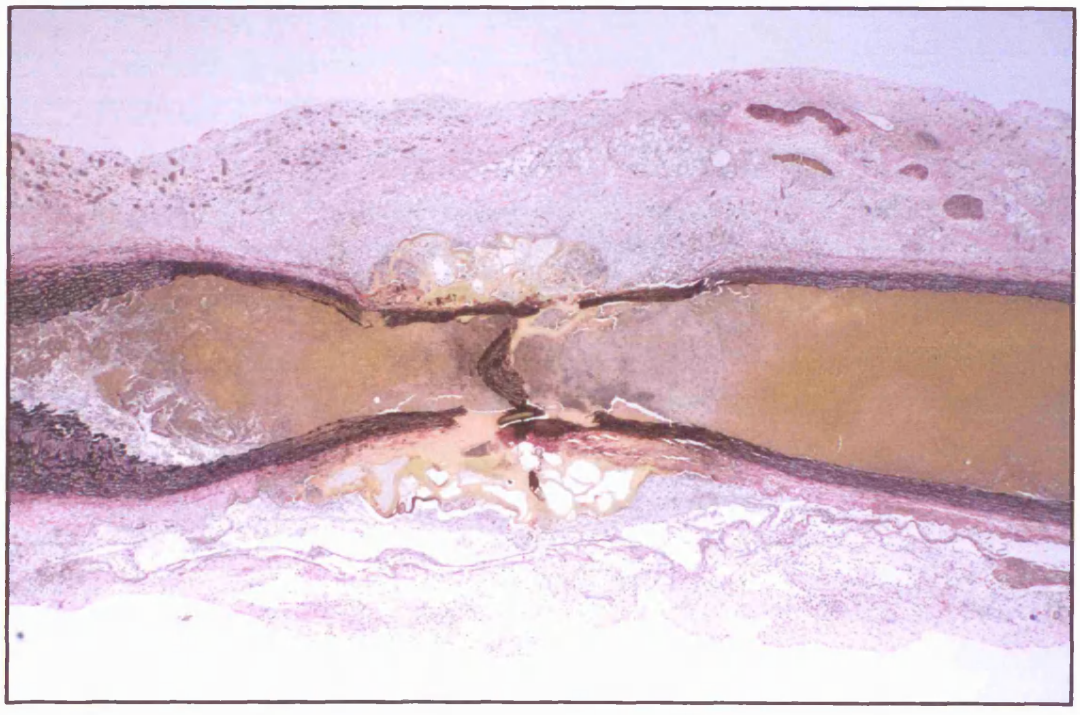


Figure 3.9 Longitudinal section of eight day vessel from high power group (EVG)

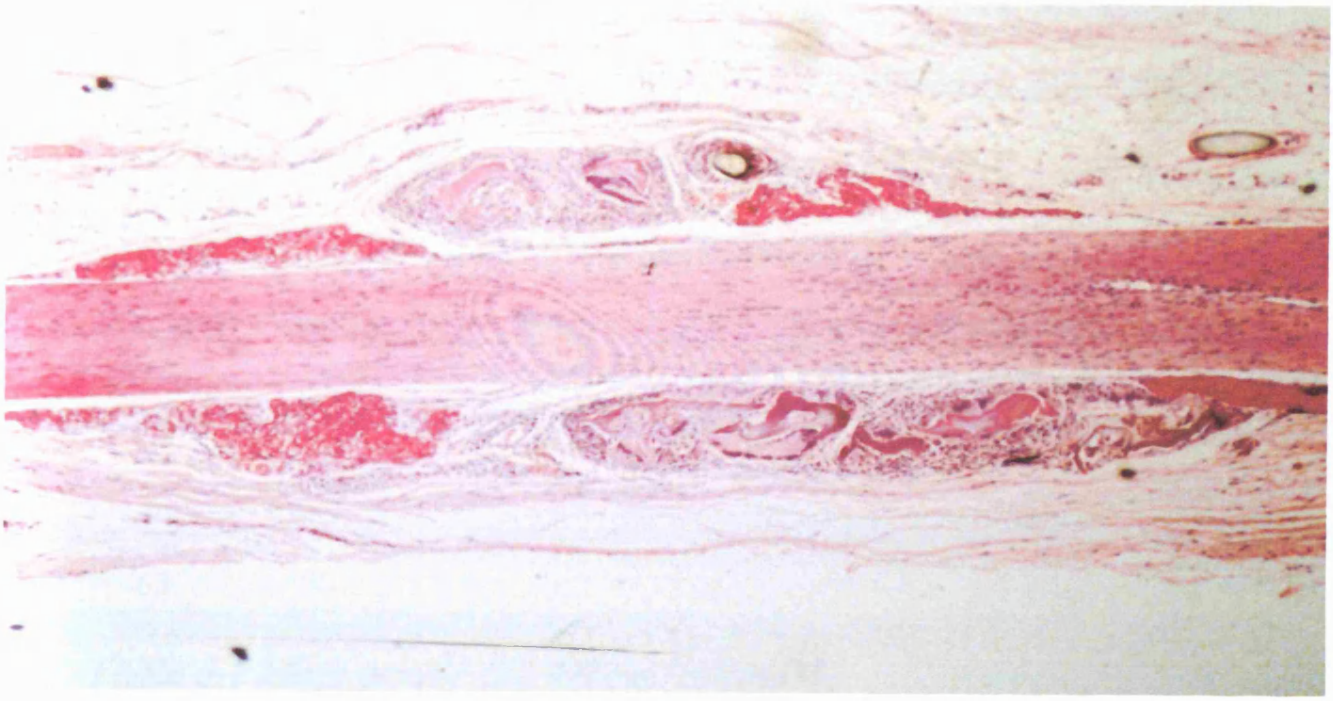


Figure 3.10: Sixty day sample from high power group (H&E)

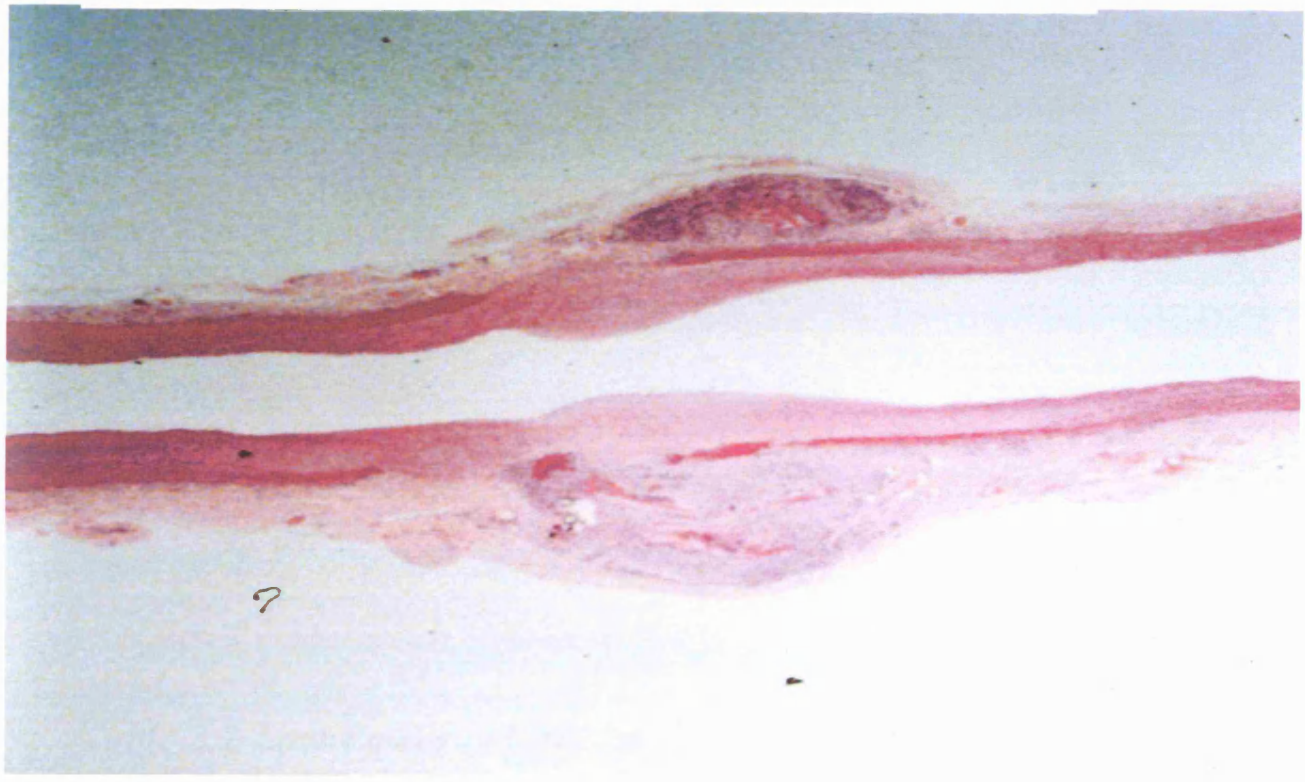


Figure 3.11 Sixty day sample from low power group (H&E)

Time period (Days)	Patent	Thrombosis	Endothelium	Giant Cells	IH	Thermal Damage
7	—	+	—	—	—	—
7	—	+	—	—	—	—
7	—	+	—	—	—	—
21	—	+	—	—	—	—
21	—	+	—	—	—	—
21	—	+	—	—	—	—
30	—	+	+	+	—	—
30	—	+	+	+	—	—
30	—	+	+	+	—	—
40	—	+	+	+	+	—
40	—	+	—	+	+	—
40	—	+	—	+	+	—
60	—	+	—	—	—	—
60	—	+	—	—	—	—
60	—	+	—	+	—	—

Table 3.1 High power (22.9Wcm^{-2}:180mW) **(+ = Present : — =Absent)**

Time period (Days)	Patent	Thrombosis	Endothelium	Giant Cells	IH	Thermal Damage
7	+	—	+	—	—	—
7	+	—	+	—	—	—
7	+	—	+	—	—	—
21	+	—	+	—	—	—
21	+	—	+	—	+	—
21	+	—	+	—	+	—
30	+	—	+	—	-	—
30	+	—	+	—	+	—
30	+	—	+	—	+	—
40	+	—	+	—	+	—
40	+	—	+	—	+	—
40	+	—	+	—	+	—
60	+	—	+	—	+	—
60	—	+	+	—	+	—
60	+	—	+	—	+	—

Table 3.2 Low Power (11.4W cm^{-2}:90mW) **(+ = Present : —— =Absent)**

3.4 Discussion

The re-absorption curves shown in the figures 3.6, 3.7 and 3.8 indicate that the solder has a half life of 10.1 days. It is of note that the longest running samples showed a plateau at around 10% of the solder remaining. This was true in both groups and indicates that there is a significant amount of the adhesive remaining at 60 days. However, histology of the samples does not seem to corroborate this showing very little of the solder left in these samples. This may be due to radio-iodine incorporation into the surrounding fibrous material or extra-cellular matrix. Alternatively the percentage error may be an artifact, low counts exaggerating the level of solder remaining in those samples. This is unlikely to be simply due to background radiation, which was 25-35 counts per minute for both groups and represents 0.05-0.3% of the counts obtained for the 60 day vessels.

Patency, thrombosis and endothelialisation

Histological study of the results showed that there was a dramatic difference in patency between the two groups and that the use of a power setting of 180mW (22.9W cm^{-2}) or above may result in thrombus formation at the site of the anastomosis. It is interesting to note that although thrombosis was seen to occur there was no evidence of thermal injury to elastic tissue as assessed using EVG and H&E stains. It is therefore possible that thrombosis may be due to mechanisms not involving thermal injury. Constriction at the anastomotic site was observed but not quantified at the time of anastomosis. This observation subsequently correlated with thrombosis at post-mortem. The operative constriction may occur as a result of crosslinking of the intrinsic proteins of the adventitia, or dehydration. Alternatively the effect may be artefactual, and simply the result of a restriction in expansion of the vessel from its resting unfilled state, to the active filled state. The current assay of thermal injury determines the amount of elastic tissue remaining at the site of the anastomosis, as well as the presence of charred tissue. In view of the findings here, it may be possible to injure tissues in a way that cannot be detected by current methods. In addition to these factors, an element of 'learning curve' may be involved. However, prior to this study up to 20 microvascular procedures were undertaken in this model. If operator error were responsible for a difference in the patency rate it would be expected that both groups be affected equally rather than a single group, such as is seen here in the high power specimens.

The inclusion of methylene blue in the formulation of the solder is intended to eliminate any thermal effects by virtue of its ability to fade. This has a twofold function, which serves firstly to warn the surgeon that sufficient lasering has occurred and secondly as an absorption switch, to stop the photochemical reaction taking place between the laser and the albumin. Thus, a combination of surgical judgement and an in-built switch may serve to reduce and possibly eliminate thermal injury. Although the absorption switch mechanism has not been quantified, a reduction in thermal injury, compared to lasered anastomoses (Quigley, 1986b), is immediately apparent even in the high power group, despite a high rate of thrombosis.

Examination of the degree of endothelialisation shows that there is significantly more endothelium present in the low power group than in the high power group, indicating that the regeneration of the endothelium may be inhibited by the overexposure of laser light. This may be caused by reduced endothelial regrowth, or a more extensive area of endothelial damage as a result of high laser power. Either way the precise cause may not be deduced form the current study.

Intimal Hyperplasia

An apparent paradox in the results indicates that the level of IH is greater in the low power group than in the high power group. A rational prediction might have stated that the group in which the greater amount of tissue and endothelial damage occurs will also correlate with the highest incidence of IH. However, the reverse was observed. This effect has been reported by Chow (Chow, 1983). In this study, the incidence of histological changes was examined in the rat femoral model. The paradoxical nature of the incidence of intimal hyperplasia was also remarked upon with the conclusion that it was a repair process of the adjacent intimal cells attempting to cover over the raw area of the anastomosis. Quigley et al (Quigley, 1986) compared the intimal hyperplasia in anastomoses formed in sutured and lasered micro-anastomoses in rat femoral arteries. Intimal hyperplasia was seen to occur more in the suture controls at 2 weeks, but by 6 weeks, there was no difference. This was thought to be due to the presence of medial damage in the laser anastomoses, inhibiting intimal proliferation. This inhibition was overcome by 6 weeks.

In the current study, the presence of endothelium at the anastomotic site will be protective but this assumes that the endothelial cover is normal and functional. The exposure to laser may cause changes in endothelial reproduction or surface proteins rendering it dysfunctional. This is turn may result in exposure to stimuli producing IH. However, the fact that the high power group was occluded

and thrombosed means that no firm conclusions can be drawn as to the influence of MB solder on IH stimulation.

The use of methylene blue, although a nitric oxide synthetase (NOS) inhibitor, appears to have little influence on the patency of the anastomoses. Nitric oxide, produced by the endothelium, is a vasodilator and prevents thrombosis. In a small vessel such as the rabbit carotid it is theoretically possible that NOS inhibition would result in thrombosis and vascular constriction. The fact that this is not seen is remarkable in itself, but indicates that the dose of methylene blue used does not result in a significant vasoconstrictor or prothrombotic effect.

Previous reports have described the rate of patency in sutured micro-arterial anastomoses as being 90-100% (Hayhurst, 1975; Acland, 1980; Acland, 1977; Chow, 1983). In these studies, a number of experimental conditions were used and a number of similar histological findings were seen. These included medial degeneration, intimal degeneration, and dehiscence of sutures in the short term, with the later appearance of intimal hyperplasia.

The incidence of medial necrosis and reactions was as high as 70% in one series (Acland, 1977) and 33% in another (Chow, 1983). Intimal damage was seen at some distance from the anastomosis, despite careful and non-traumatic handling of the tissues, up to 5-10 days with patchy regeneration thereafter (Acland, 1977). Descriptions of anastomotic constriction are rare in reports of sutured anastomoses but were quantified by Acland (Acland, 1977) as being 13% at 1 hour and 2 days increasing to 16% and 19% at 5 and 10 days, but reducing again to normal after 21 days. Constriction in this study was observed in the high power group and did not respond to papaverine. However, with the report that a 19% constriction at the anastomosis does not cause thrombosis it is unlikely that the thrombosis seen in the high power group is caused solely by constriction.

Further development work should be undertaken if the photofading properties of MB are to be used to their fullest potential as an absorption switch. This study demonstrates that the solder works to the extent that thermal damage is not seen but the cause of the thrombosis associated with high power laser exposure is more complicated than previously thought. It is possible that a reduction in the concentration of MB will result in a more sensitive solder but may compromise anastomotic strength. Other alternatives are the inclusion of reducing agents (e.g cystine, EDTA) to enhance MB fading and preventing re-oxidisation. The use of multiple, thinner layers is also a strategy that may will reduce the

absolute build up of temperature but would increase the time required to perform the anastomosis and require developments in solder application.

This comparison of two laser powers has shown a substantial difference in patency but no difference in solder re-absorption. From a re-absorption point of view, this immediately indicates that the re-absorption process is not dependant upon or affected by vessel patency.

Chapter 4

The conversion from laser to white light activation of methylene blue soldered microvascular anastomoses

The conversion from laser to polychromatic light activation of methylene blue soldered microvascular anastomoses

4.1 Introduction

The activation of solders has traditionally taken place with lasers of various wavelengths. The most commonly used have been the Argon ion laser, the milliwatt CO_2 laser and laser diodes. These were first applied to vascular and urological anastomoses.

Krueger et al (Krueger, 1985) used the Argon ion laser (488-514nm) to investigate the effect of blood coagulation at lasered anastomoses. This was technique was used by Chuck et al (Chuck, 1989) who combined blood with FITC. Conversely, Grubbs et al (Grubbs, 1988, Wang, 1988.) used the CO_2 laser to activate fibrin glue and produce blood bonding. Carbon Dioxide lasers were also used to anastomose nerves (Menovsky, 1994; Menovsky, 1996) and tendons (Kilkelly, 1997).

With the advent of laser diodes, these other lasers were replaced and the increased range of wavelengths available enabled other chromophores to be used. The predominant chromophore was ICG activated by means of a laser diode emitting at 808nm. This was used widely to perform vascular anastomoses (Oz, 1989; Oz, 1990, Oz, 1993) colonic anastomoses (Libutti, 1990), choledochotomy closure (Bass, 1991) and urinary tract reconstructions (Poppas, 1993a, Poppas, 1993b; Kirsch, 1995a; Kirsch, 1995b; Kirsch, 1995c). Indocyanine green changes to a yellow colour on activation and does not therefore allow the surgeon significant visual feedback, or any protection against over exposure.

The use of lasers in a clinical setting poses difficulties and exposes both the patient and the clinician to danger. Extensive safety precautions are therefore necessary including eye protection for theatre staff, the operator and any other observers, as well as the marking of doors and covering of reflective surfaces. These activities may take a considerable length of time, negating any time saving from using the laser system and distract theatre staff away from the operation.

In parallel with the safety considerations, there are financial considerations to the use of lasers. In the current climate of healthcare economics and rationing, the capital outlay of purchasing a medical grade laser may be prohibitive, particularly in an area where technology is developing and the life span of the laser is unknown.

To overcome the drawbacks associated with lasers, a white/polychromatic light system (WLS/PLS) was developed to activate solder. In the past, the lasers were simply required to provide photons of the correct wavelength and power to match with the chromophore used. The theory of

using filtered white light to provide the required energy was simple and would provide a more versatile source than previously possible, by changing filters. This would open the way for even more chromophores to be used for a variety of clinical applications.

The light source described in this chapter is based on a xenon arc lamp, similar to that used in endoscope lamps for other clinical applications. The light produced is heat filtered and wavelength filtered before being guided through a fibre-optic bundle to a hand piece. The final beam is focused to a point near the end of the pen. Lasers produce a beam that is both monochromatic, coherent and parallel; attributes that increase the danger associated with them. The WLS/PLS used here produced a divergent beam that is non-coherent and polychromatic, thus removing many of the dangers associated with lasers. The components used in making the PLS are all 'off the shelf' enabling a dramatic reduction in cost from a laser at estimated at £30K, to a provisional cost of £1-1.5K. This in itself brings the PLS and solder technology to within the grasp of hospital departments in the UK, even if the life span of the technology is less than 5 years.

4.2 Materials and methods

Solder preparation

See Chapter 2. The MB concentration was kept constant at 0.24% w/w.

Activating system

Activation *in vivo* was performed using either the laser diode system (Laser Module - HPM250/3139, Laser 2000, Ringstead, Northants, UK), or the white light system based on a xenon arc lamp emitting at 590-700nm with a variable power of 150-650mW (8.54 – 36.5 W cm^{-2}) with a 1.5mm diameter spot at 1.5mm from the end of the pen (Fig 4.1). The *in vitro* testing was performed using the PLS only.

Light source power was measured using a Coherent power meter (Model 210, Coherent, USA) (Appendix C).

In Vitro Tests

Anastomotic Technique

See Chapter 2.

Figure 4.1: The Tissuemed White Light Source

Burst pressure testing

The completed anastomosis was pressure tested using a syringe driver, pressure transducer (0-30psi) (RS Components, UK) and PC (Appendix C). The needle and vessel were mounted between the transducer and the syringe pump and the PC set to acquire data. The vessel was observed for signs of leakage and the maximum pressure was recorded and plotted. Side branches occasionally leaked, and these were occluded by ligation. This process was repeated 6 times for each concentration of chromophore and the results tabulated and plotted (Origin^{TM}, Microcal, UK).

In Vivo tests

Surgery

Twenty four New Zealand white rabbits (2.5-3.5 Kg) were divided into two groups, a) Laser 2000 activated solder anastomoses and b) PLS activated anastomoses. This resulted in three animals in each group. Since it was not expected that a large difference would be shown between the L2K and

PLS treated groups the number was kept low in accordance with the principles of reduction, refinement and replacement (Russell & Burch, 1959). In each instance the animal received a premedication dose of 0.3ml/Kg of Hypnorm (Fentanyl citrate 0.315mg/ml, Fluanisone 10mg/ml) 0.3mg/kg) IM 15-20mins prior to anaesthetic. The animal was anaesthetised using inhalational anaesthetic induction and maintenance (Halothane 5% reducing to 2% and 1.5 Ltrs O_2) and monitored with an oxygen saturation probe.

The animal was placed supine, the skin shaved and prepared with aqueous chlorhexidine and povidone iodine, and draped for surgery. A midline incision was made and the left carotid was exposed and prepared for anastomosis with haemorrhage controlled with bipolar diathermy. Heparin was administered (1000-1500 iu, IV). The vessel was clamped using an Acland 3V clamp, transected and stay sutures applied (8/0 Polyamide). Solder was applied to one face of the anastomosis at a time and activated using one of the two activating systems. The Laser 2000 (L2K) system was used at a power setting of 90 mW (11.9 W cm^{-2}), while the PLS was used initially at a power of 300mW (17.1W cm^{-2}), for approximately 5 seconds per spot.

Having performed one procedure, it was seen that excessive constriction occurred in a similar way to the constriction noted in the high power group in chapter 3 and the anastomosis was deemed as not being viable. The power was reduced to 200mW (11.3W cm^{-2}) for the second anastomosis and a similar effect was noted. The third anastomosis was performed at 150mW (8.5W cm^{-2}) and this was seen to hold securely while not exhibiting the constriction associated with anastomoses that will thrombose. It was therefore decided that the study should continue at a power setting of 150mW (8.5W cm^{-2}) with three animals in each group for four time periods (Table 4.1).

Activation Source	**Termination Time (Days)**				
	1	5	14	28	Total
Laser 2000	N=3	N=3	N=3	N=3	12
PLS	N=3	N=3	N=3	N=3	12
					24

Table 4.1 A Summary of the in vivo study

Typically, 4-5 spots of irradiation are required for each face. Two layers of adhesive were applied to each surface. The clamps were then removed and Doppler flow measurement taken of the

vessel. Once haemostasis was achieved, the wound was closed with 3/0 Vicryl.

The animals were kept for 1, 5, 14 or 28 days, after which time the animal was sacrificed, the vessel inspected for patency, thrombosis or disruption, and removed.

Histology

The vessels were fixed in 10% neutral formalin and then routinely processed. Sections of 4 microns were stained with Haematoxylin & Eosin for general morphology and with elastic Van Geison to assess the collagen and elastic elements of the tissue. The sections were examined in a blinded fashion, for thermal injury, thrombus formation, giant cells, endothelialisation, constriction, neutrophil infiltration, medial disruption and intimal hyperplasia.

Statistical Analysis

This was performed using Fishers' Exact Test calculated on Prism™ (Graphpad software Inc, UK) for nonparametric data. A p value of 0.05 was considered significant.

43 Results

In Vitro Test - Burst pressure

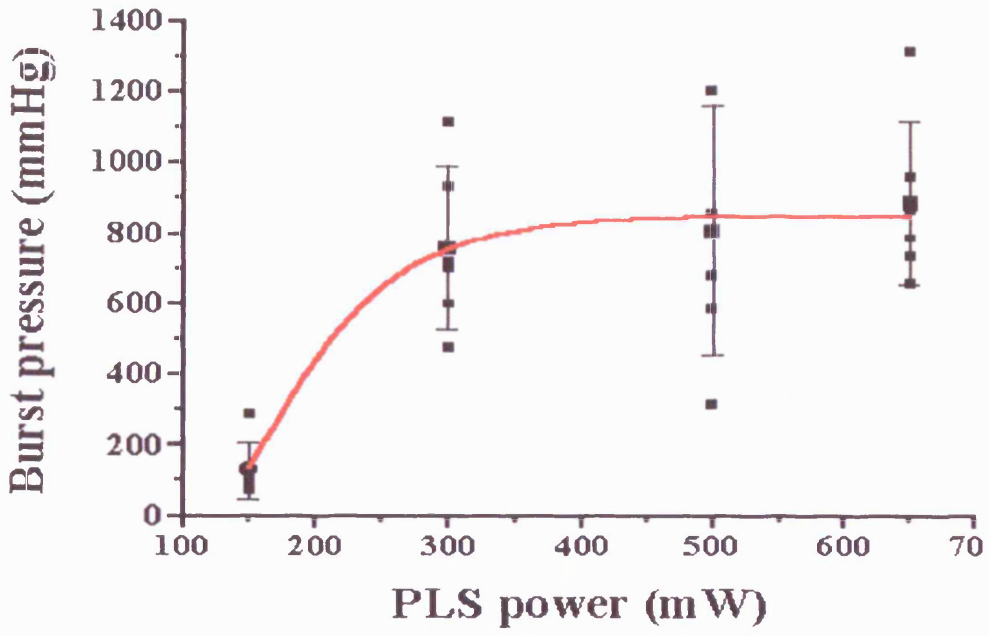


***Figure 4.2: PLS power against burst pressure strength** (+/-sem)*

As with the laser 2000 system tested in Chapter 2, the white light source is able to produce anastomoses capable of withstanding supraphysiological pressures. This is seen to occur in a dose response type pattern with a rapid increase seen to occur between 150mW (8.5 Wcm^{-2}) and 300mW (17.1Wcm^{-2}) of power. The plateau is seen to occur at 300 to 500mW (17.1 to 28.5 Wcm^{-2}) with no increase in strength from 500 to 650mW (28.5 to 36.7 Wcm^{-2}). The importance of this data lies in its equivalence with the data produced for the laser 2000 in varying methylene blue concentration and is therefore capable of producing anastomoses of similar quality to those formed using laser activation.

In Vivo tests - Histology

Both the anastomoses formed at 300mW (17.1Wcm^{-2}) and 200mW (11.3Wcm^{-2}) were removed at 1 day and were seen to be thrombosed. The final anastomosis formed at 150mW (8.5 Wcm^{-2}) was seen to be patent. These anastomoses were taken as a pilot study and not included in the

final results table. They are however extremely important results and will be discussed fully below.

Of the anastomoses performed after the pilot study, both groups showed a 100% patency rate at all time points with good evidence of flow at the anastomotic site at the time of explant. The results are summarised in tables 4.2 and 4.3. Comparison of the patency data shows no significant difference between the two treatment groups (p=1). From a macroscopic point of view there was no evidence of constriction or aneurysm formation, nor were there other complications such as unexpected deaths, haematomas or false aneurysms.

Histological examination also showed similar results. Although none of the anastomoses were thrombosed, thrombus was present in 50% of the L2K group, while the PLS group showed thrombus in 24% (p=0.2). Endothelialisation was seen in both groups in a single specimen at 5 days while it was seen to be complete by day 14 and day 28 (p=0.65).

Intimal hyperplasia (IH) was seen to be higher in the L2K group at 42% as against 25% in the high power group (p=0.09). No giant cells were evident in either group at any time point, while constriction and thermal injury were also absent with no difference between either group.

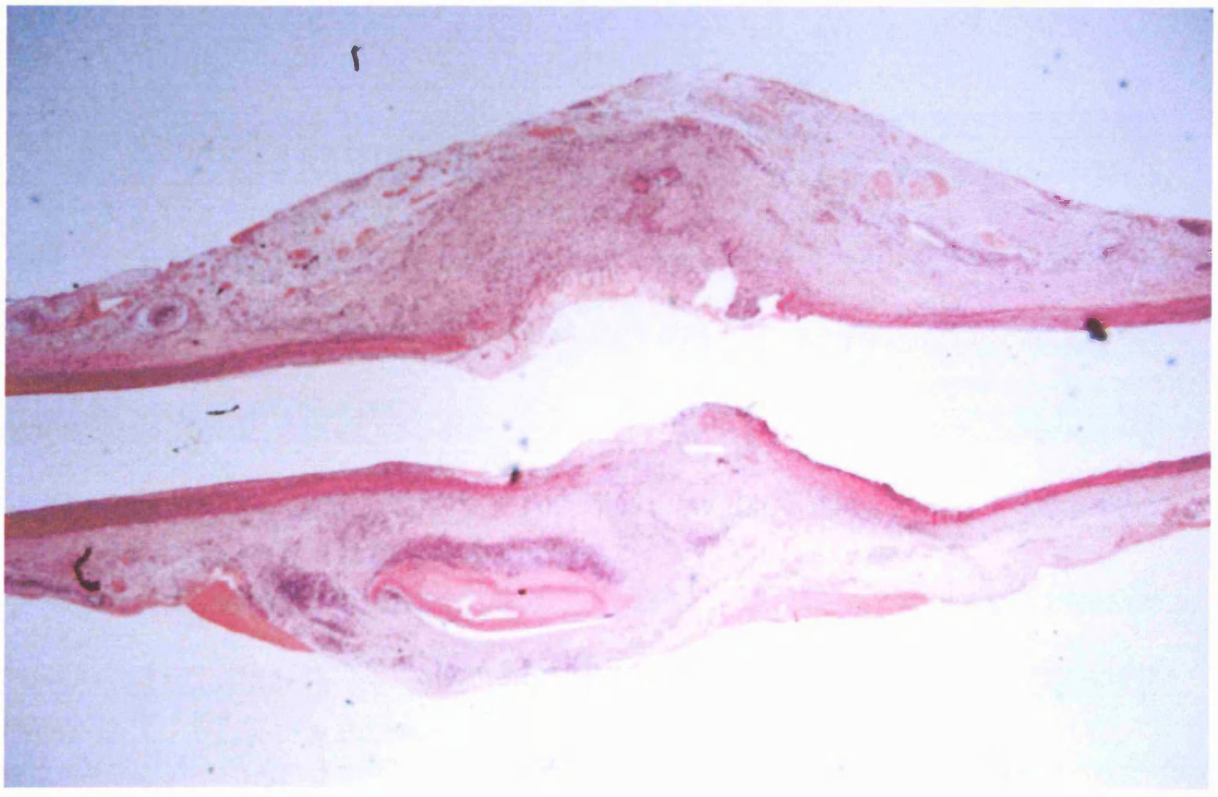


Figure 4.3: Day 28 PLS Anastomosis (H&E)

Time period (Days)	Patent	Thrombus	Endothelium	Giant Cells	IH	Thermal Damage
1	+	+	—	—	—	—
1	+	—	—	—	—	—
1	+	+	—	—	—	—
5	+	+	—	—	—	—
5	+	—	—	—	—	—
5	+	—	—	—	—	—
14	+	+	+	—	+	—
14	+	—	+	—	+	—
14	+	—	+	—	+	—
28	+	+	+	—	+	—
28	+	—	+	—	+	—
28	+	+	+	—	+	—

Table 4.2: Laser 2000 **(+ = Present : — =Absent)**

Time period (Days)	Patent	Thrombus	Endothelium	Giant Cells	IH	Thermal Damage
1	+	—	—	—	—	—
1	+	—	—	—	—	—
1	+	+	—	—	—	—
5	+	—	—	—	—	—
5	+	—	—	—	—	—
5	+	+	+	—	—	—
14	+	+	+	—	—	—
14	+	—	+	—	—	—
14	+	—	+	—	—	—
28	+	—	+	—	—	—
28	+	—	+	—	+	—
28	+	—	+	—	+	—

Table 4.3: PLS **(+ = Present : — =Absent)**

4.4 Discussion

In Vitro

The results indicate that there is a dose response relationship between PLS power and burst pressure strength. This is of an equivalent nature to the *in vitro* testing seen in Chapter 1 with the variation of methylene blue concentration. An initial rise gives way to a plateau with a point of saturation being seen. It is interesting to note that although the relationship between solder absorption and burst pressure strength gives a shallower slope, this data mirrors the relationship seen with methylene blue concentration. Since this is known to be an inaccurate representation of the true relationship between methylene blue and burst strength it is also possible that an effect is true for PLS power. At a power of 500mW (28.5W cm^{-2}), the burst strength is seen to maximise, followed by a plateau. This therefore represents the saturation point of the solder and a further increase in power does not affect anastomotic strength. It is therefore likely that this represents the maximum temperature to which the solder can rise after which no further increase in temperature can be produced. This may be the subject of future investigation and further work is required to show the relationship between incident power, chromophore concentration and solder temperature rise.

In Vivo

Patency, thrombosis and endothelialisation

The initial pilot showed a 100% thrombosis rate for anastomoses formed at 200 and 300mW (11.3 & 17.1 W cm^{-2}). This reflects the findings seen in Chapter 3, in which anastomoses formed at high laser power (180mW/22.9W cm^{-2}) resulted in a 94% thrombosis rate. The current model shows that a similar state is true for the PLS and the use of powers as high as 200 and 300mW (11.3 W cm^{-2} and 17.1 W cm^{-2} respectively) result in thrombosis. These figures were chosen because of the level of burst pressure strength seen in the *in vitro* study.

It is interesting to note that the power density of the laser in Chapter 3 associated with a high patency rate was 11.3 W cm^{-2}, higher than the power density of the PLS at 200mW (11.3 W cm^{-2}) associated with thrombosis. The level of power finally settled on for the PLS (150mW: 8.54 W cm^{-2}) is lower than the level for the laser. The reasons for this are not immediately apparent as the power density levels should both be equivalent in terms of solder activation and heat generation. The results here imply that the PLS is delivering more photons than the laser despite the objective evidence that the power densities are equivalent. It is true that the PLS delivers across a wider spectral range than

laser, but this fact will be reflected in the power density. It is therefore possible that another factor is responsible for the fact that thromboses occur with the use of the PLS. In either case, the fact that thrombosis can be induced implies that the photofading nature of the solder is not having the desired protective effect. This is further discussed after the results of the formal in vivo study.

Both groups showed a high rate of patency with no occluded vessels (100% Vs 100%:p=1). There was however a numerical difference in the incidence of thrombus formation but this was not seen to be significant (p=0.2) indicating equivalence between the two groups and the two sources of solder activation. The degree of endothelialisation was also seen to be equivalent with 58% showing complete endothelialisation (p=0.65). Endothelialisation was seen as starting from day 5 in both groups being complete by day 14.

Intimal Hyperplasia

In parallel with the data in chapter 2, intimal hyperplasia was a prominent feature in both groups being seen in 17% of the PLS group, while present in 42% of the L2K anastomoses (p=0.09). The rate of IH in L2K anastomoses is similar to that seen in the anastomoses formed in Chapter 2 while those formed with the white light source seem to have a lower value (p=0.09). The situation seen in Chapter 3 showed that patent vessels gave a paradoxically high rate of intimal hyperplasia, agreeing with the findings of previous studies (Chow, 1983). This is thought to be due to a repair process in adjacent cells found only in patent anastomoses.

The absence of other complications also parallels the findings of previous studies. Although the data presented represents only short term healing, the lack of haematoma, aneurysm and false aneurysm formation compared to laser only anastomosis, is encouraging. It does however beg the question of the long term viability of these anastomoses. This will be the subject of a later chapter.

The fact that thermal injury is not seen is of consequence in light of the findings of the pilot study. Thrombosis was therefore induced without damage to the intrinsic proteins of the vessel wall. However damage of another kind was being induced either in the form of vascular constriction or endothelial damage. The former is likely to play a role and is seen with anastomoses formed at excessive power. This will result in changes in laminar flow and turbulence at the site of the anastomosis and encourage platelet and coagulation cascade activation. This is supported by the fact that a very small reduction in power will result in a reduction in the constriction seen and consequently the rate of thrombosis.

Constriction in the porcine splenic arteries was not seen at much higher power densities (upto

38W cm^{-2}). This implies that these vessels are much more able to resist the effects of solder heating. The splenic arteries used for *in vitro* testing had been stored in saline for several hours. It is possible that the vessels had taken on water, increasing the transfer of heat away from the site of the anastomosis and enabling them to withstand a higher heat generation. In the case of the rabbit carotids, the dimensions of the vessel are such that heat transfer is impeded by a vessel thickness of 70-100μm. While it is not anticipated that the solder will be primarily used for the minimal suture anastomosis of vessels of this dimension, it is indicative of the fact that further development of the solder is required to capitalise on the absorbtion switch mechanism.

Chapter 5

Long term '*in vivo*' investigation of methylene blue soldered microvascular anastomoses

Long term '*in vivo*' investigation of methylene blue soldered microvascular anastomoses

5.1 Introduction

Long term investigations of tissue solder are few and far between. Oz et al (Oz, 1990b), in one of the first studies to be published on ICG/Fibrinogen solders repaired arteriotomies and examined the results at 90 days. They reported complete fibrinogen re-absorbtion, no foreign body reaction and minimal fibrosis at the end of the 90-day period. More importantly, there were no anastomotic ruptures, thromboses or aneurysms at these time points. Other long term studies have focussed on solders in the clinical scenarios of urinary tract reconstruction and nerve repair. Kirsch et al (Kirsch, 1995b) used ICG/Albumin solder activated with an 808nm laser diode to perform such procedures as hypospadias and urethral stricture repair. The results are reported up to 9 months with a mean of 7.1 months. The results, interestingly, show a 20% suture disruption rate, although there were no complications up to that point, with no reports on the finer details of histology or wound appearance.

Nerve repairs were performed by Lauto et al (Lauto, 1997b) using protein bands, which were examined after 3 months. These showed no significant differences from sutured nerve repairs in terms of compound action potentials or histological appearance. There was no reported inflammatory cell in either group and the degree of myelination was similar.

The reasons for undertaking long term studies in vascular anastomoses are the findings reported for laser tissue welding. Quigley et al showed that there was a high rate of aneurysms associated with the use of the CO_2 laser in the rat femoral model (Quigley, 1986b). Overall, there was an 18.6% rate of aneurysms, with those in the long term group showing a rate of 29.8%. Histology of this group showed widespread necrosis of the media with loss of the elastic elements and the appearance of spindle shaped cells. Other investigators had reported a rate of aneurysm formation as high as 60-80% using the argon ion laser (Pribil, 1985). This study also used the rat femoral model and showed an increasing rate of aneurysm formation with time up to 1 month.

The incidence of intimal hyperplasia in laser welded anastomoses was not seen to be significantly higher in a later report by Quigley et al (Quigley, 1986a) with the sutured group initially showing an intimal height of 21.3 μm compared to 11.7μm (laser) at 2 weeks. However, by 6 weeks there was no significant difference between the two groups.

The initial studies performed by this group showed that there was a relationship between laser power and patency but that the rate of solder re-absorbtion was not significantly affected by vessel patency or incidental laser power. There were however significant differences in the rates of intimal hyperplasia between patent and thrombosed vessels, with the patent group paradoxically showing a much higher rate.

The main aim of this study was to continue on from the short term work and to specifically look for aneurysm formation seen in laser tissue welding as described by Quigley et al (Quigley, 1986b). It was also important to determine whether the absence sutures between the cut ends of a vessel in an anastomosis would lead to a higher complication rate.

5.2 Materials and methods

Solder preparation

See Chapter 2. The MB concentration of the solder was 0.24%w/v.

Activating system

Activation was performed using a Xenon Arc Lamp (XE003 Tissuemed, Leeds, UK) coupled to a silica optic fibre (50μm core diameter) at a wavelength of 590-700 nm, 150 mW (8.5 W cm^{-2}) power and a focused spot diameter of 1mm (+/- 100μm) at 1.4 mm. Lamp power was measured using a Coherent power meter (Model 210, Coherent, USA) (see appendix C).

Surgery

Twenty-four New Zealand white rabbits (2.5-3.5 Kg) were divided into two groups, a) Sutured anastomoses and b) Soldered anastomoses. In each instance the animal received a premedication dose of 0.3ml/Kg of Hypnorm (Fentanyl citrate 0.315mg/ml, Fluanisone 10mg/ml) 0.3mg/kg) IM 15-20mins prior to anaesthetic. The animal was anaesthetised using inhalational anaesthetic induction and maintenance (Halothane 5% reducing to 2% and 1.5 Ltrs O_2) and monitored with an oxygen saturation probe.

The animal was placed supine, the skin shaved and prepared with aqueous chlorhexidine and povidone iodine, and draped for surgery. A midline incision was made and the left carotid was exposed and prepared for anastomosis with haemorrhage controlled with bipolar diathermy. Heparin was

administered (1000-1500 iu, IV). The vessel was clamped using an Acland 3V clamp, transected and three stay sutures applied (8/0 Polyamide).

In the solder group, solder was applied to one face of the anastomosis at a time and activated using the Xenon Arc Lamp system at a power setting of 150mW (8.5 W cm^{-2}) for approximately 5 seconds per spot. Typically, 4-5 spots of irradiation are required for each face. Two layers of adhesive were applied to each surface. The clamps were then removed. Once haemostasis was achieved, the wound was closed with 3/0 Vicryl.

Those animals undergoing sutured anastomoses again had three stay sutures placed and the intervening gaps also sutured using 8/0 ethilon. Twelve to fifteen sutures were typically used.

The animals were kept for 3 or 6 months after which time the animal was terminated, the vessel inspected for patency, thrombosis aneurysm formation or disruption, and removed.

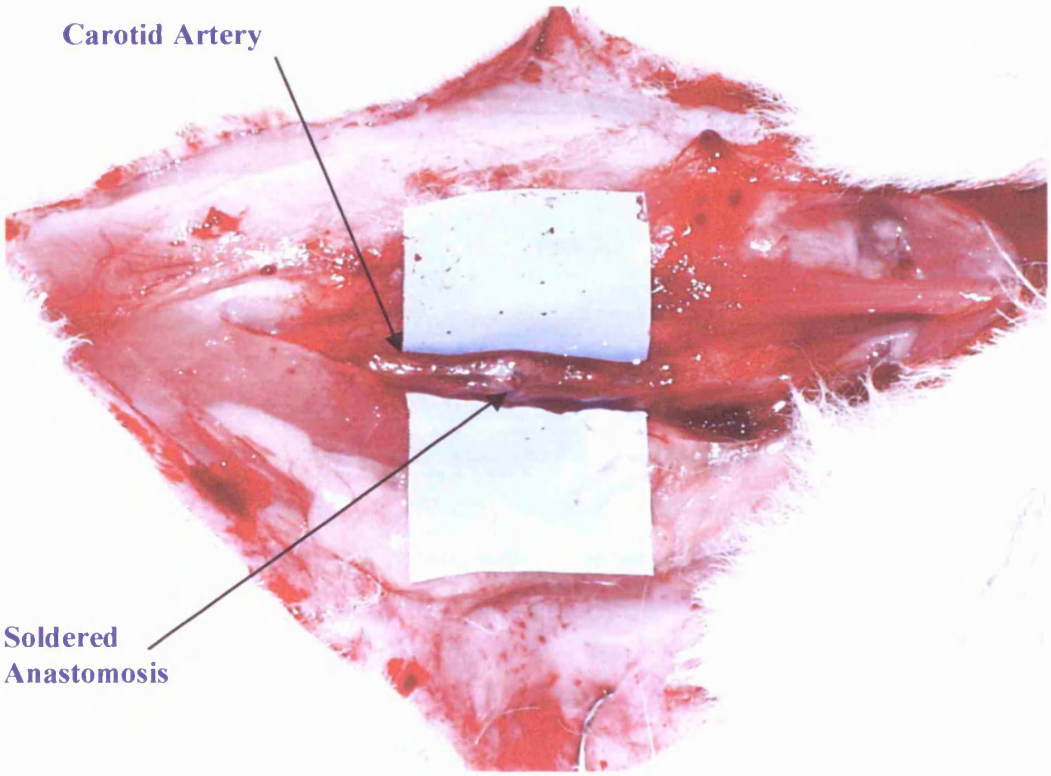


Figure 5.1 - 3-month soldered anastomosis at explantation

Histology

The vessels were fixed in 10% neutral formalin and then routinely processed. Sections of four microns were stained with Haematoxylin & Eosin. The sections were assessed for thrombus, acute inflammation, chronic inflammation, tissue reaction, intimal fibrosis, external fibrosis and eosinophilia.

To improve objective analysis of the histological samples, a grading system of 0 to 4 was used for each feature, except thrombus, as seen in Table 5.1

Thrombus was graded using a scale of 0 - 5 as seen in Table 5.2

0	**absent,**
1	**minimal,**
2	**mild,**
3	**moderate**
4	**severe.**

Table 5.1: Histological grading

0	**absent,**
1	**up to 10% arterial occlusion,**
2	**10-30% occlusion,**
3	**30-50% occlusion,**
4	**50-80% occlusion and**
5	**total occlusion.**

Table 5.2: Thrombus grading scale

Sections in which there was severe distortion due to the fixing process were counted as unscored. The headings for the histological sections are labeled in the results (Table 5.2 &5.3) as Anastomosis type (Anast), Thrombosis (Thromb), Acute inflammatory cells (Acute inflam), Chronic inflammatory cells (Chronic Inflam), Fibrosis within the vessel wall (Fibros Int), Fibrosis on the adventitia (Fibros Ext) and the presence of eosinophils (Eosino).

Statistical Analysis

Statistical analysis was performed using Fishers exact probability test via StatXact v.4 (Cytel Software Corporation, 675 Massachusetts Av, Cambridge, MA 02139 USA) as performed by Mr P Stibbons. A p value of 0.05 was considered significant.

5.3 Results

The results at explanation are summarised in tables 5.3 and 5.4.

Animal No	Anast	Thromb	Acute Inflam	Chronic Inflam	Fibros Int	Fibros Ext	Eosino
JB 12	Suture	0	0	0	0	0	0
JB 15	Suture	0	0	0	0	0	0
JB 16	Suture	0	2	1	2	0	0
JB 19	Suture	0	0	0	2	1	0
JB 20	Suture	0	0	0	1	1	0
JB21	Suture	0	0	1	1	1	0
JB 14	Solder	0	3	4	2	1	3
JB 17	Solder	**UN**	**SC**	**O**	**R**	**E**	**D**
JB 18	Solder	0	0	0	2	1	0
JB 22	Solder	0	0	0	1	1	0
JB 23	Solder	**UN**	**SC**	**O**	**R**	**E**	**D**
JB 24	Solder	**UN**	**SC**	**O**	**R**	**E**	**D**

(+ patent; — -occluded)

Table 5.3: 3 month histological data

Animal No	Anast	Thromb	Acute Inflam	Chronic Inflam	Fibros Int	Fibros Ext	Eosino
JB 01	Suture	0	0	1	1	1	0
JB 02	Suture	0	0	1	1	1	0
JB 03	Suture	0	0	0	1	0	0
JB 08	Suture	0	0	0	1	0	0
JB 10	Suture	0	0	1	1	0	0
JB 11	Suture	0	1	1	0	0	0
JB 04	Solder	**NO**	**ANAST**	**SEEN**			
JB 05	Solder	0	0	0	1	0	0
JB 06	Solder	0	0	1	1	0	0
JB 07	Solder	0	0	0	1	0	0
JB 09	Solder	0	0	2	1	1	0
JB 13	Solder	2	0	0	2	0	0

(+ patent; — -occluded)

Table 5.4: 6 month histological data

The results outlined above showed a number of interesting points. Firstly, on macroscopic inspection there was 100% patency in all vessels at all time periods and none of the vessels from any of the four groups showed evidence of aneurysm formation. In addition, there was no evidence that the anastomoses had ruptured or caused haematoma formation.

Comparison of the patency of the vessels showed no significant difference between any of the groups for either solder or suture, at 3 or 6 months ($p>0.25$).

Microscopic evaluation of the slides showed that the three-month vessels JB17, 23 and 24 were significantly distorted and could not be assessed. In spite of this statistical comparison showed no significant difference between the two treatment groups at either time point ($p>0.25$).

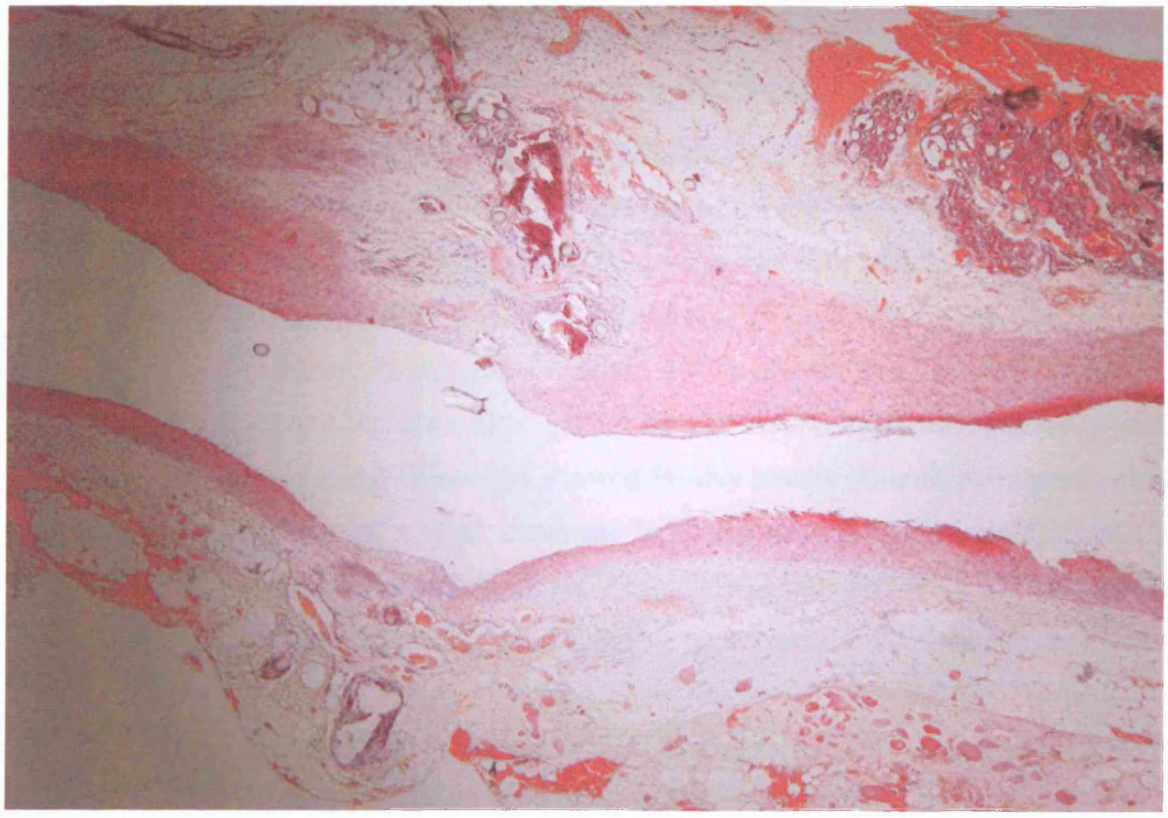


Fig 5.4: Microscopy of 3 month sutured anastomosis

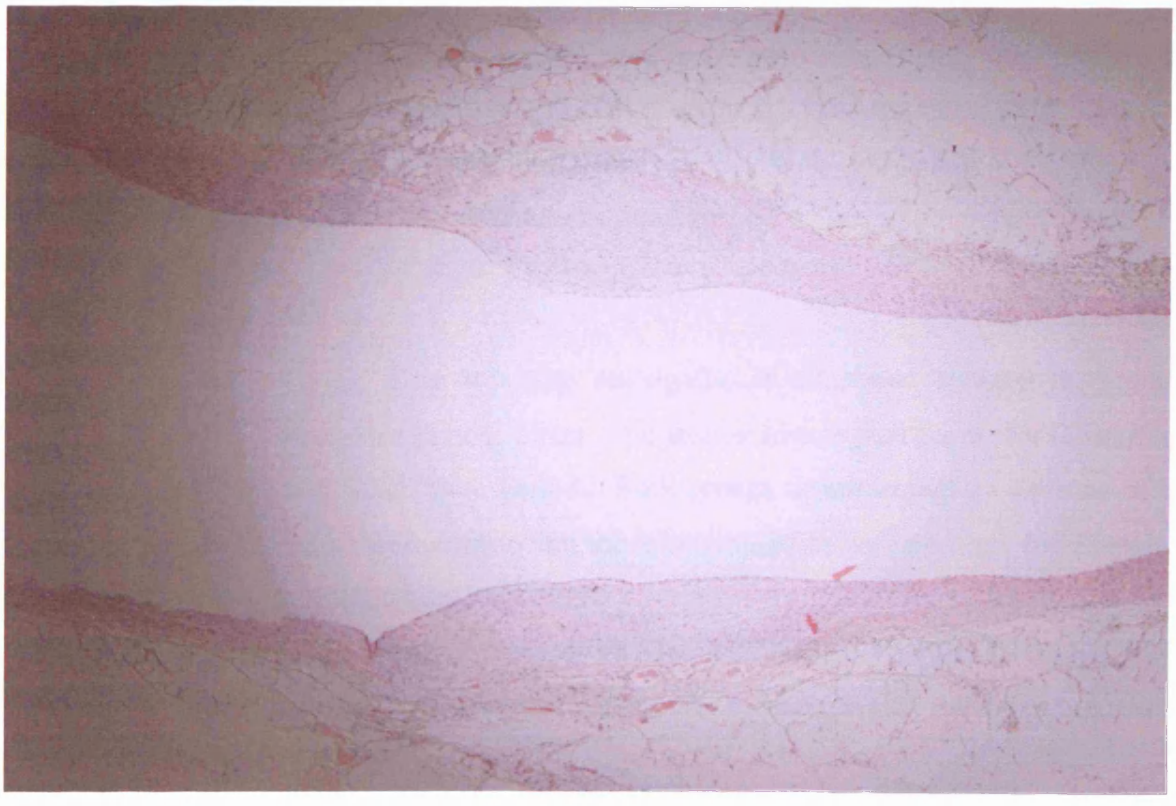


Fig 5.5: Microscopy of 6 month soldered anastomosis

5.4 Conclusions

Macroscopic

The long term data shows a number of interesting points. In the first instance, a macroscopic examination of the explanted vessels shows no significant difference between the two treatment groups of solder and suture at either of the time periods. These samples were seen to have the same patency rate as each other as well as the same rate of complications. The patency rate in this study (98%) is comparable with the patency of sutured (Acland, 1977) and lasered (Cirkrit, 1998) vascular anastomoses described in the literature although no similar reports exist for soldered anastomoses. Reports by Oz (Oz, 1990a) using fibrinogen showed 90-day results without aneurysm formation or anastomotic disruption. The high rate of aneurysm formation described by Quigley et al (Quigley, 1986a) has not been seen in soldered anastomoses at any time period. The fibrin soldered anastomoses described by Oz (Oz, 1990a) reported that the anastomoses were seen to be in tact and free of aneursyms, similar to the anastomoses described in the earlier chapters of this thesis. These were explanted at 60 days to assess the degree of solder resorbtion, as well as preliminary histological markers in early anastomoses. This study showed a power/patency relationship explicable by damage caused to the vessel when using solders of high chromophore concentration. In addition to the report by Oz (Oz, 1990a) these are the only soldered comparisons with which to compare the long term soldered vessels reported in this study. In sutured vessels there is a long history of patent vessels free of disruption or aneurysm formation (Hayhurst, 1975; Acland, 1980; Acland, 1977). These rates of patency are seen in the sutured controls performed in this study indicating a similar quality of anastomoses between these and the reported sutured anastomoses.

Microscopy

On a microscopic level, there was also no significant difference between the sutured and soldered anastomoses at either time period. There were similar histological scores for the anastomoses in both treatment groups and at all time periods. Both groups demonstrated an absence of chronic inflammatory reaction in the tissues surrounding the anastomosis, as well as a low incidence of acute inflammation, fibrosis or Intimal hyperplasia.

Soldered anastomoses have been reported by Kirsch (Kirsch, 1995a) and Lauto (Lauto, 1997a) in bladder and nerve repairs respectively. Again, these showed no evidence of significant

complications such as disruption or chronic inflammation, with histological appearances similar to those of the sutured controls.

The longer term soldered anastomoses at 6 months showed no significant difference from those at 3 months or those performed using sutures. These facts indicate that over a longer period of time (6 months) there are none of the previously described problems associated with soldered or lasered anastomoses. The incidence of micro-aneurysm formation was previously described at up to 30% (Quigley, 1986a), while soldered anastomoses had a tendency to intimal hyperplasia (Oz, 1990a). This has been seen in studies described earlier, with an intimal hyperplasia rate of 66% in vessels examined up to 60 days. Intimal hyperplasia although present was not a significant feature of the histological sections examined in this study. This contrasts with anastomoses explanted at 60 days in which intimal hyperplasia was a significant feature in those anastomoses that were patent. The anastomoses performed at high power were seen to show a very low rate of intimal hyperplasia as previously described by Chow (Chow, 1983). This was explained by the absence of an intimal stimulus from adherent platelets within a flowing blood stream. In the study by Chow, this was thought to be due to the absence of a repair process in adjacent normal intimal cells. Quigley (Quigley, 1986a) also commented on the rate of intimal hyperplasia seen in lasered and sutured anastomoses observing that sutured controls had a higher rate of intimal hyperplasia, reducing to no difference by 6 weeks. The conclusion in this case was the presence of medial damage in the laser anastomosis inhibiting intimal proliferation.

Overall, it is encouraging to see the comparison of the soldered and sutured anastomoses at the two time periods with little to distinguish them in terms of macroscopic or microscopic appearance. From a statistical point of view, the numbers used in this study would only have shown a large difference between the two groups giving rise to the possibility that a type 2 error could be responsible for the results seen. However, with the ongoing development of the solder it is likely that further studies will be undertaken to look at the behaviour of solders in more detail. Suffice it to say that currently, it can be stated that there is a laser power to patency relationship and that there is little difference between soldered and sutured anastomoses. Most importantly, the problems of aneurysm formation have been overcome although the incidence of intimal hyperplasia may prove to be problematic.

Chapter 6

The immunological response to porcine serum albumin based soldered microvascular anastomoses

6.1 Introduction

The protein content of tissue solders acts as a structural element to provide strength for the anastomosis but introduces potentially antigenic material into the animal. Early studies used human fibrinogen as a readily available structural protein. On-going concerns over prion transmission (Collinge, 1999, Will, 1996) as well as difficulty in purifying autologous fibrin have restricted its application. Later groups used human albumin and reported its use in urethral reconstruction in humans (Kirsch, 1995). Regulatory restrictions and public confidence toward human albumin in the UK (Anon, 1998), has lead this group to seek an alternative source of protein for use in solder. Fears over HIV, BSE (Diringer, 1995) and the advent of New Variant CJD (Collinge, 1999, Will, 1996) precluded the use of human or bovine albumins.

Porcine albumin has not been shown to have any of the risks associated with other sources of protein and being readily available, was chosen as a suitable protein for incorporation into a solder. Studies have shown that the solder is capable of producing anastomoses that can withstand high pressures in vitro (Birch, 2000). In vivo studies have shown that given the correct laser power, micro-anastomoses can be produced with a high degree of patency and similar histological appearances to sutured anastomoses (Birch, 2002).

In order to proceed from an animal model to clinical trials and satisfy LRQA, it was necessary to determine any immune response to the presence of porcine albumin in the solder as no such studies have been reported to date. The data obtained in this study was submitted as part of an application to obtain a CE mark for the solder.

With this in mind we set out to determine the local and systemic reactions in the rabbit carotid model. Firstly, we looked at the humoral response to the solder, measuring anti-Porcine albumin IgG in both naïve and pre-sensitised animals. Secondly immunohistochemical staining of kidneys samples was preformed to look at immune complex and C3 deposition.

The study includes the presence of both a negative control group, receiving no treatment, and a comparative control group that was treated with human albumin based solder. A further group was included that had been pre-sensitised to porcine albumin prior to treatment with the porcine solder.

The primary immune response is known to be detectable after 5 days and declines by day 20-21 (Janeway, 1996). Therefore, days 7 and 21 were chosen for sacrifice to determine the early and late

response to a single dose of solder. The presensitisation group was designed to give maximal antibody titres, with memory cell generated initially and again at day 14 (Hudson, 1989). Therefore, immunization was performed 35, 21, and 7 days prior to solder application. The last immunization 7 days prior to surgery was designed to give maximal antibody titres (tertiary response) to solder to simulate a worst case scenario.

Additional kidney samples were taken from a parallel study in which PSA solder was used, to augment the results for immune complex deposition. The animals in this arm of the study were males and therefore possibly less immunologically sensitive than females.

6.2 Materials and methods

Study Design

Twenty four female New Zealand white rabbits (2.5-3.5 Kg) were divided into four groups (n=6). Six animals acted as negative controls (group 1), receiving no treatment. These were venesected and kidney biopsies removed for ELISA and immunohistochemistry respectively. Of the remaining animals, those in group 2 (n=6) were venesected at day 0 and subjected to the surgical procedure using porcine albumin immediately. Samples of blood and terminations were performed on day 7 (n=3) and day 21 (n=3). The animals in group 3 (n=6) were treated in a similar manner, with the inclusion of human albumin based solder rather than porcine albumin.

The animals of group 4 (n=6) each received a subcutaneous dose of 2ml serum (0.25mg PSA in 2ml Freuds incomplete Adjuvant – Sigma Aldrich Chemicals, USA) divided into four sites 35 days prior to surgery. Twenty one days prior to surgery, the animals received a second identical presensitisation dose. At 7 days prior to surgery the animals were venesected and a third presensitisation dose was given as before. All of the animals underwent a unilateral end-to-end anastomosis using laser activated tissue solder (PSA based). The animals were also venesected on the day of surgery. Following surgery the animals underwent termination at day 6 (n=3) and 21 (n=3) with venesection.

In addition to these animals kidney biopsies were taken from animals in a parallel study (Histology) that were terminated at 21, 30, 40 and 60 days (n=3 at each time point). These animals had undergone a unilateral end-to-end micro-anastomosis using PSA based solder. The kidney samples from these subjects were treated in an identical fashion to the original specimens.

Group Nº	Presensitisation	Surgery	Termination
Group 1		Controls only	
Group 2 (Porcine Albumin)	n/a	Day 0	Day 7 & 21 (n=3)
Group 3 (Human Albumin)	n/a	Day 0	Day 7 & 21 (n=3)
Group 4 (Porcine Albumin)	Day –35,-21,-7	Day 0	Day 7 & 21 (n=3)
Supplementary Group	n/a	Day 0	Day 21,30,40 and 60 (n=3)

Table 6.1: A Summary of the treatment groups

Surgery

In each instance the animal received a pre-medication dose of 0.3ml/Kg of Hypnorm (Fentanyl citrate 0.315mg/ml, Fluanisone 10mg/ml) 0.3mg/kg) IM 15-20mins prior to anaesthetic. The animal was anaesthetised using inhalational anaesthetic induction and maintenance (Halothane 5% reducing to 2% and 1.5 Ltrs O_2) and monitored with an oxygen saturation probe.

The animal was placed supine, the skin shaved and prepared with aqueous chlorhexidine and povidone iodine, and draped for surgery. A midline incision was made and the left carotid was exposed and prepared for anastomosis with haemorrhage controlled with bipolar diathermy. Heparin was administered (1000-1500 iu, IV). The vessel was clamped using an Acland 3V clamp, transected and stay sutures applied (8/0 Polyamide). Solder was applied to one face of the anastomosis at a time and activated using the Laser 2000 system at a power setting of either 90 or 180 mW for approximately 5 seconds per spot. Typically 4-5 spots of irradiation were required for each face. Two layers of adhesive were applied to each surface. The clamps were then removed and doppler flow measurement taken of the vessel. Once haemostasis was achieved the wound was closed with 3/0 Vicryl.

At termination the vessel was inspected for patency, thrombosis or disruption, and removed. The vessel was stored in 10% formalin, sectioned and stained using Haematoxylin & Eosin stain and Van Geison stain. The sections were mounted and the vessels was examined for immunohistology. Kidney samples were also removed and examined for evidence of immune complex deposition by immunohistology.

Solder preparation

See chapter 2. The final albumin concentration was kept constant at 41% w/w, while the MB concentration of the solder was 0.24%w/w.

Activating system

See Chapter 3. Laser power was constant at 90W (11.3 W cm^{-2})

Treatment of blood samples

The blood was collected into plain sterile Perspex tubes and allowed to clot at 37°C for 1 hour. The serum was then separated from the clot by centrifugation and the serum stored at –20°C until analysed.

Serum IgG ELISA

Flat bottomed 96 well plates (Nunc; High affinity) were coated with human or porcine albumin (0.2ml; 1μg/ml) in coating buffer (Sodium carbonate; 0.1M;pH 9.2) at 4°C overnight. The wells were washed 3 times for 30 minutes with 0.2ml wash buffer (phosphate buffered saline (PBS) containing 1% (w/v) Triton X100 and 2% Marvel.

The sera were allowed to thaw and reach room temperature slowly on the bench. Two fold dilutions of the test sera and control sera (non-immune rabbit; rabbit anti-human albumin – Sigma: Rabbit anti-porcine serum – Sigma) were prepared in PBS (Oxoid). Duplicate samples (0.2ml) of the test sera and control sera dilutions were added to the appropriate coated plates, which were then incubated for 2 hours at room temperature.

The plates were washed in wash buffer for 10 minutes 3 times (0.2ml per well). Sheep-anti rabbit IgG (1/500 dilution in PBS – Sigma) was added to all wells (0.2mls) and the plates incubated for 2 hours at room temperature.

The plates were washed in wash buffer for 10 minutes 3 times (0.2ml per well). Sheep-anti rabbit IgG (1/500 dilution in PBS – Sigma) was added to all wells (0.2mls) and the plates incubated for 2 hours at room temperature.

The plates were washed in wash buffer for 10 minutes 3 times (0.2ml per well). Next p-Nitrophenyl-phosphate (1mg/ml) in 10% (v/v) diethanolamine buffer (0.2ml) was added to each well

and the plates incubated for a further 30 minutes. The reaction was then stopped by adding 50ml of 3M NaOH. The optical density of the wells was then determined at 420nm using ELISA plate reader (Dynatech MR700).

Kidney Staining

The kidney biopsies obtained from the post mortem were flash frozen in liquid nitrogen for transportation. Subsequently they were treated and stained according to techniques previously described (Hsu, 1981).

Kidney biopsies taken from all four groups were stained for rabbit IgG at dilutions of 1/20,000 and 1/40,000 and anti-C3 at dilutions of 1/15,000 and 1/30,000. The sections were also stained for anti-porcine albumin antibodies at a range of dilutions. Each kidney section was analysed for non-specific staining (no antibody control) staining of glomeruli, blood vessels and tubules for IgG, C3 and porcine albumin.

The staining was graded according to the degree of intensity:

0 No staining throughout section, over and above the negative control

1 Possible very small degree of positivity or occasional positive glomerulus

2 Positive, of low intensity

3 Positive, of medium intensity

4 Positive, of high intensity

5 Positive, of extremely high intensity

Statistical Analysis

The results of the ELISA titres (reciprocal log 2) were calculated using a one-way analysis of variance with a +/-95% confidence limit (StatXact v.4 - Cytel Software Corporation, 675 Massachusetts Av, Cambridge, MA 02139 USA) as performed by Prof E Ingham.

6.4 Results

Antibody Titres

The mean optical density for each dilution of each test serum was determined and the highest dilution giving an optical density of 3 SD above the negative control serum was taken as the anti-body titre.

The reciprocal log 2 of the titres was calculated and the means +/- 95% confidence limits determined for each group of animals at each time point. The data was analysed by one-way analysis of variance (ANOVA) for each group. This analysis revealed that, for group 3 (Fig 6.2) treated with porcine albumin tissue solder, there was a significant increase ($p<0.01$) in the anti-porcine albumin levels at day 21 following the surgical procedure. The same result was obtained in group 2 (Fig 6.1) following treatment with human albumin based tissue solder ($p<0.01$). The group 4 (Fig 6.3) animals showed a dramatic rise in their anti-porcine albumin antibody levels ($p<0.001$) after just one immunisation (day – 8 prior to surgery).

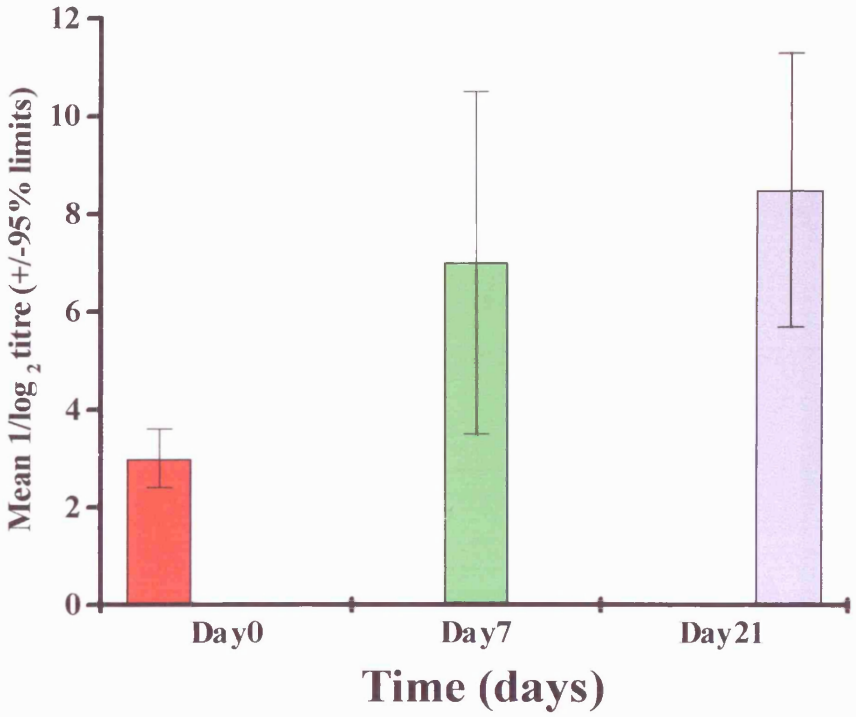


Figure 6.1: IgG From Group 2 (PSA)

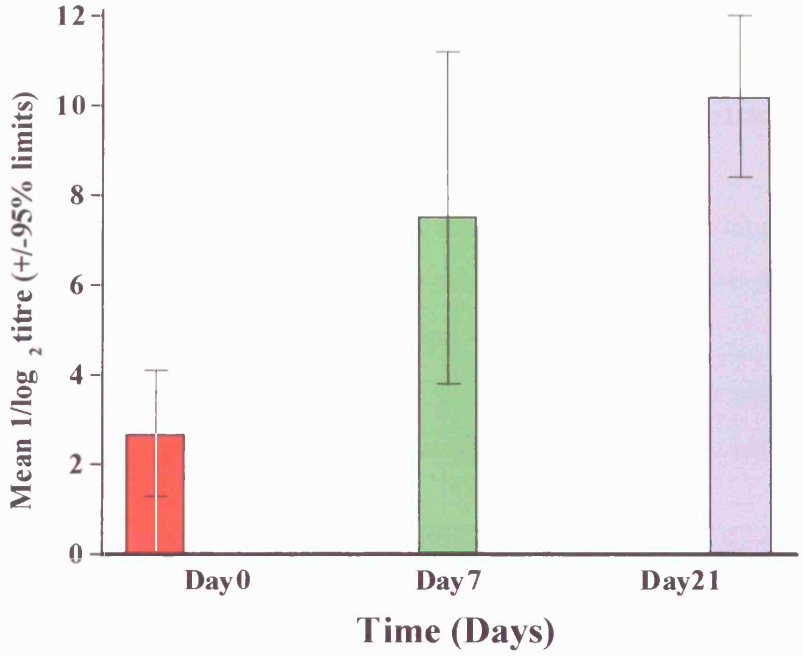


Figure 6.2: IgG From Group 3 (HSA)

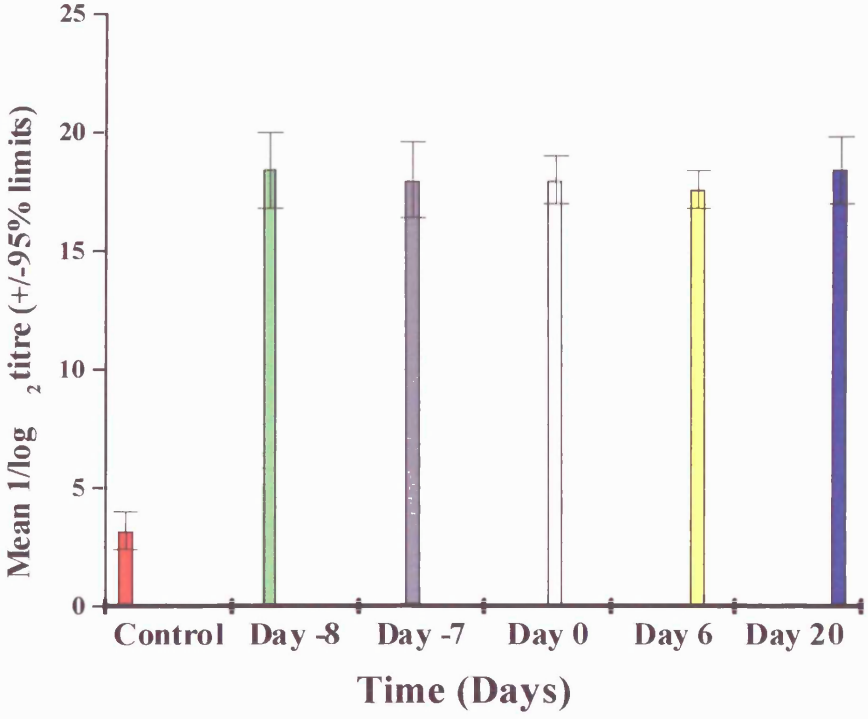


Figure 6.3: IgG From Group 4 (Pre-sensitised)

Immunohistochemistry

None of the kidneys samples showed positive staining for porcine (or Human) albumin.

The staining for IgG at 1/20,000 dilution gave a high degree of background staining and so the 1/40,000 dilution was used to interpret the data. Staining for C3 was weak at 1/30,000 so the dilution of 1/15,000 was used to interpret the data.

The most important data relates to the extent of IgG and C3 deposition in the kidney glomeruli, since this is the site of immune complex deposition. Attempts were therefore made to compare the data for the glomerular staining between groups.

Since data collection was subjective it was not possible to use statistical methods (eg mean score on this data). The method adopted was to assign a score for each group of animals. This was calculated as follows:

The grades for the staining of the 6 or 8 glomeruli (2 kidneys, 3 animals per group) for 1gG (1/40,000) and C3 (1/15,000) were summed. A total score was assigned out of a maximum of 30 (maximum grade of 5 or 6 kidneys) for each group (Fig 6.4)

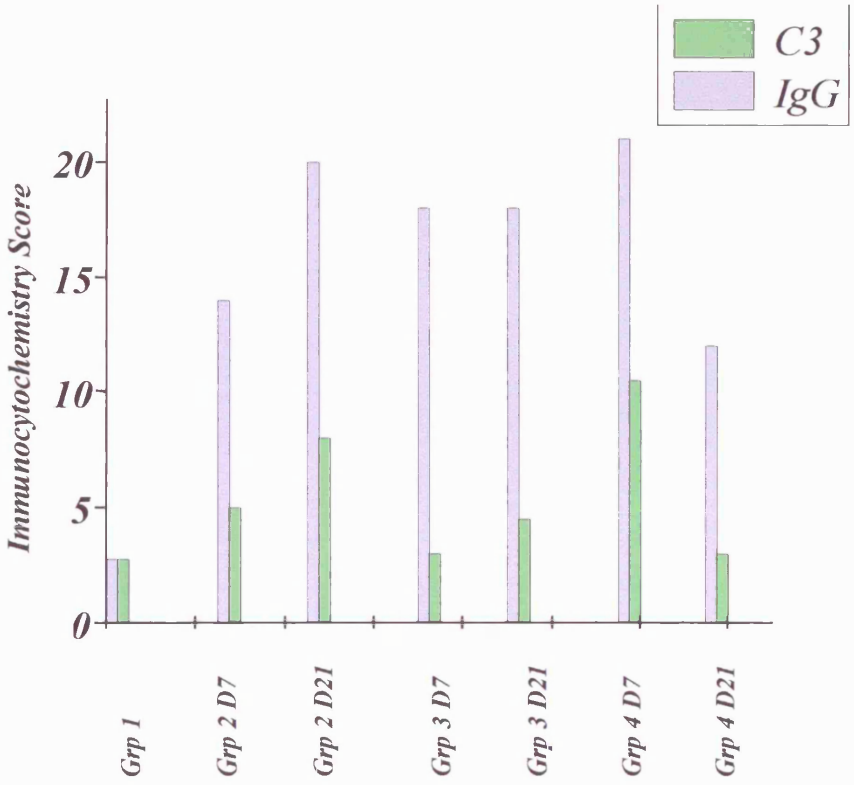


Figure 6.4: The immunocytochemical scoring of kidneys from group 3 (Presensitised)

Group 1 (Controls)

The controls gave very little evidence of IgG or C3 deposition in the glomeruli with no kidney having a grade above 1.

Group 2 (Porcine Albumin)

These samples had extensive IgG deposition in the glomeruli at 7 and 21 days. There was very little C3 in these samples.

Group 3 (Human Albumin)

The samples from Day 7 post op had a moderate IgG deposition and very little evidence of C3 deposition in the glomeruli. At 21 days, the group 2 animals had extensive IgG deposition and one animal had moderate C3 deposition in the glomeruli.

Group 4 (Pre-sensitised)

These samples showed extensive IgG deposition at 7 days together with moderate C3 deposition in the glomeruli. At 21 days these samples had moderate IgG deposition with little or no evidence of C3 deposition in the glomeruli.

Supplementary Group

This group showed little or no evidence of IgG deposition in the glomeruli at 21 days. This increased to moderate deposition of IgG and C3 in the glomeruli at 30 and 40 days. By 60 days there was little or no evidence of either IgG or C3 deposition in the glomeruli.

6.5 Discussion

Immunology

There are two observations to be drawn from the results of this study. Firstly the tissue glue procedure introduced sufficient protein into the rabbits to significantly increase levels of antibodies from a titre of 1/4 (1/8) in naive animals to 1/256 (1/8192) following treatment with the porcine or human albumin respectively. The human albumin appeared to be more immunogenic than porcine

albumin in the rabbit, since the levels of antibodies generated were an order of magnitude higher. The levels of antibodies following the procedure were much lower than those levels generated in group 3 by immunisation.

Secondly the levels of antibodies to porcine albumin in the pre-sensitised animals (group 3) at the time of the surgical procedure were extremely high, over 1/65,136. Thus the aim of the experiment, to perform the procedure in pre-sensitised animals was fulfilled. It is also clear that the levels of antibodies did not rise further as a result of the surgical procedure and no systemic side effects were noted.

It is important to note that the controls in this study did not undergo any surgical procedure. While it is not thought that this is significant, a refinement to future studies could be the inclusion of sutured controls without solder to confirm that the presence of IgG either circulating or fixed is solely due to an immune response to the tissue solder and not as a result of non-specific processes.

Immunohistochemistry

This study has shown that there was deposition of IgG in the glomeruli following surgical treatment with the tissue glue. For the animals treated with PSA solder, the extent of IgG deposition increased between 7 and 21 days and at 21 days was accompanied by evidence of C3 deposition in at least one animal. For the animals treated with the human albumin the deposition appeared to be to the same extent at 7 and 21 days, but with little evidence of C3.

These results indicated that a single dose of tissue glue delivered to a vessel wall was sufficient to cause IgG deposition in the kidney glomeruli. Since there was no evidence of the deposition of PSA in group 2, it cannot be stated that the IgG was anti-albumin. However the fact that this was not seen in the control animals and the fact that the tissue glue treatment has been shown to sensitise the animals to produce anti-albumin antibodies, provides strong evidence that this was the case.

There was no evidence of pathological change in any of the kidney sections from the animals in group 2 or 3. The important question is whether the IgG deposited would have lead to pathology or whether the deposition was transient and would have been cleared with no detrimental effect on kidney function. This is discussed later in the consideration of the supplementary group.

It has been shown that the administration of large doses of bovine serum albumin to rabbits will result in 'serum sickness' within 6 to 12 days accompanied by the deposition of immune complexes

containing antigen in the glomeruli (Peters, 1975). The deposition of as little as 20μg was sufficient to induce acute nephritis. The immune complexes were however cleared with a half-life of approximately 12 days. This acute 'one shot' serum sickness caused by foreign protein is a self-limiting condition with rapid resolution of the lesions after immune complex elimination. In order to provoke a disorder resembling chronic nephritis, it is necessary to administer small doses of protein antigen over a period of many months.

Thus it may be concluded that the small dose of albumin given to the rabbits in groups 2 and 3 was sufficient to induce immune complex deposition in the kidney glomeruli, but that immune complexes did not induce pathology and would be cleared as the antigenic stimulus was removed. The fact that IgG was evident at 21 days may be due to the continued presence of albumin at the anastomosis. This tissue solder study differed from the 'one shot' experiments in that the tissue glue would have been continually present to stimulate antibody production until it was completely absorbed. If this were the case, it would be expected that the IgG deposits in the glomeruli would decrease at around the same time point as the albumin in the solder at the anastomosis site was absorbed.

In group 4, high titres of anti-porcine albumin antibodies were seen in the serum at the time of the procedure and corresponded with extensive deposits in the kidney at 7 days following the procedure. The IgG was accompanied by strong evidence of C3 deposition with no evidence of albumin in the glomeruli. It is of interest that the deposition of IgG and C3 had decreased by 21 days, indicating that in this group of animals, the presence of high titres of anti-albumin antibodies led to an earlier clearance of the immune complexes from the kidneys. What cannot be deduced from the current data is whether the immune complexes deposited at 7 days were the result of solder reabsoption, or of solder that remained in the vessel after the procedure. A refinement to the protocol may have been to include three extra animals in group 4 that were terminated after 8-10 hours. In this instance the immune complexes would have formed and been deposited only if the solder had entered the vascular tree after the procedure.

It has been suggested that animals with a strong antibody response can eliminate antigen quickly so that immune complexes do not persist and are less likely to localise to the kidney glomeruli. The results obtained with the group 4 animals were in keeping with this and is encouraging since it indicates that the prior sensitisation of humans to heterologous albumin should not render them at

increased risk from treatment with the tissue solder.

The supplementary group showed slightly different results. These animals showed low levels of IgG deposition at 21 days after treatment that increased at 30 and 40 days and then reduced to control levels at 60 days. These results agreed with the results for the group 2 animals, in that the procedure was sufficient to stimulate the deposition of IgG in the glomeruli. However the time course appeared to be delayed. The group 2 animals had moderate deposits at 7 days that increased at 21 days. Since there was no further data for the group 2 animals it was not known whether the levels would have increased further at 30 or 40 days. The extent of IgG deposition at 21 days in the group 2 and supplementary animals was clearly different. This could have been due to several factors including the age and sex of the animals, the degree of prior sensitisation and the application of the solder. The encouraging results from the supplementary group animals are that there is a loss of IgG deposition in the glomeruli at 60 days, which indicates that any immune complexes which had been deposited had been cleared in 2 months following the procedure. In addition it is important to note that no pathological changes were seen in the kidney samples of the supplementary groups at any of the time points.

Conclusions

In conclusion the current study has demonstrated that the presence of the tissue solder in a microvascular procedure can induce the presence of circulating IgG and immune complex deposition. In addition the pre-existing presence of anti albumin IgG does not prejudice the animal at a local or systemic level if the tissue solder is subsequently used. Immune complex deposition was seen extensively in groups using PSA but there was no evidence of pathological changes and the immune complexes were seen to resolve by 60 days. These results give a measure of confidence that the use of an albumin based tissue solder in a clinical scenario would not cause immune related pathology.

Chapter 7

The compliance of soldered vascular anastomoses

The compliance of soldered vascular anastomoses

7.1 Introduction

The importance of vascular compliance has become apparent over the past few years since it was first reported by Baird et al (Baird, 1977). A reduction in vascular compliance is seen to be associated with disease states such as hypertension, as well as an increased predisposition to atherosclerosis and neo-intimal hyperplasia (Giannattasio, 1996). This is a particular problem at anastomotic sites, which are areas of greatly reduced compliance and therefore become a focus of damage and thrombosis and intimal hyperplasia (Ballyk, 1998).

Many indices have been derived to describe and quantify the physical behaviour of vessels occurring in response to an intra-luminal force. This has led to debate amongst investigators as to the most appropriate index of change in arterial distension with respect to intra-luminal pressure. In 1960, Petersen and co-workers defined the elastic modulus E_p (Peterson, 1960), an index of arterial stiffness, which describes the relationship of strain to intra-luminal pressure in an open-ended vessel. The original description referred to the change in vessel volume, but as the arterial lumen is generally circular in cross-section, the equation has been modified to:

$$\mathbf{E_p = Ps - Pd / strain}$$

Where strain is defined as the fractional pulsatile diameter change that occurs in an artery exposed to a given change in intra-luminal pressure i.e:

$$\mathbf{Strain = (Ds - Dd) / Dd}$$

Where D and P are diameter and pressure, and d and s denote diastole and systole respectively. The inverse of Petersen's elastic modulus is known as cross sectional, or diametrical compliance C and is given by:

$$\mathbf{C\ (\%\ mmHg \times 10^{-2}) = (Ds - Dd / Dd) \times 10^4 / Ps - Pd}$$

Both E_p and C are useful indices of functional vascular distensibility in the presence of a change in blood pressure and describe the relative diameter change of a vessel for a given pressure change.

Various strategies have been adopted to combat compliance mis-match, focussing mainly on the use and application of compliant graft material. This has lead to the development and evaluation of graft materials such as 'compliant polyurethane' in an attempt to reduce graft failure. Tai et al (Tai, 2000) compared the compliance characteristics of ePTFE, compliant poly (carbonate) polyurethane (CPU), and Dacron grafts to traditional allografts such as saphenous vein and muscular arteries. This study showed that CPU gave similar compliance values to muscular arteries, while Dacron and ePTFE were markedly reduced in compliance.

However, despite this the surgeon is still reliant on suturing for the formation of anastomoses. Soldering not only offers the surgeon a technique with which to perform fast anastomoses in inaccessible areas, but may also show improved compliance at the anastomosis. In order to demonstrate the efficacy of this technique it was proposed to look at the physical characteristics of soldered anastomoses and compare them to standard continuous sutured anastomoses. The compliance of three types of end-to-end vascular anastomosis was compared in the porcine carotid artery model: Standard Continuous Suture (SCS) anastomosis, laser activated soldered vascular anastomosis and solder re-inforced SCS.

7.2 Materials and methods

Solder preparation

See Chapter 2. The MB concentration of the solder was kept constant at 0.24% w/w.

Activating system

See Chapter 2. Laser power was constant at 90mW (11.3Wcm^{-2}).

Surgery

Nine Large White Landrace pigs (25± 4 kg) were used for this study. Following an overnight fast, the animals were pre-medicated with Azaperone (Stresnil™, Janssen Pharmaceutical Ltd. UK) 0.1 ml/kg IM. After induction of anaesthesia using Ketamine hydrochloride (Ketaset™, Willows Francis Veterinary, UK) 5mg/kg IV, the animals were intubated and mechanically ventilated. Anaesthesia was maintained using Halothane (May and Baker Ltd, Dagerham, UK), nitrous oxide and oxygen via a

standard anaesthetic circuit. The animal's temperature was maintained at 36-38 °C using an electronic heating mat. A pulse oximeter probe (Ohmeda Biox 3740-pulse oximeter, Ohmeda Co., Louisville, USA) was used for continuous monitoring of arterial oxygen saturation (SaO_2) and heart rate (HR).

The common carotid artery was exposed, clamped and cut for re-anastomosis using either 1) Standard Continuous Suture, 2) Solder reinforced continuous suture or 3) solder alone. Heparin was administered prior to clamping at a dose of 100-150u/kg IV, and 1% papaverine injected into the loose adventitial tissue surrounding the artery to prevent arterial spasm. The common carotid artery (CCA) blood flow was measured using a transonic Medical Flowmeter system (HT207, Transonic Medical System Inc, USA) with perivascular flow probes of 4mm in diameter. A pressure catheter was inserted into the CCA to monitor the blood pressure which was stabilised with a mean diastolic pressure of 50-80mmHg.

After the procedure, the animals were kept for 28 days with compliance measurements being taken at 5, 10, 21 and 28 days, as percutaneous compliance measurement (PCM).

	Day 0	**Day 5**	**Day 10**	**Day 21**	**Day 28**
SCS anastomoses	PCM n=3	PCM n=3	PCM n=3	PCM n=3	Sacrifice n=3
Reinforced anastomoses	PCM n=3	PCM n=3	PCM n=3	PCM n=3	Sacrifice n=3
Soldered anastomoses	PCM n=3	PCM n=3	PCM n=3	PCM n=3	Sacrifice n=3

Table 7.1: The scanning schedule for SCS, reinforced and soldered anastomoses

Measuring Procedures

Change in vessel wall diameter with respect to each cardiac cycle was measured at discrete sites along the anastomosis artery, with measurements taken in the sagittal plane at 90 degrees to the long axis of the vessel. Segments of the artery were imaged using a specially adapted duplex colour flow ultrasound system (Pie 350, Pie Medical Systems, Maastricht, Netherlands) with signal output to a high-resolution echo-locked wall tracking system (Wall Track, Pie Medical Systems, Maastricht, Netherlands). This system, with the manufacturer-stated tracking accuracy of 8μm, allowed measurement of vessel wall movement over time by automatically tracking assigned points of the

induced radio-frequency signal deemed to be representative of anterior and posterior arterial wall. Real time M- and B-Mode images of the arterial wall obtained via a 7.5MHz linear array probe were acquired (Figure 7.1) (Baguneid, 2001).

With the M-Mode cursor positioned perpendicular to the long axis of the vessel, the change in induced radio-frequency (RF) signal received from the vessel walls was sampled. The data were transferred to a personal computer for real time display of the displacement waveforms of the anterior and posterior artery walls. This is displayed in Figure 7.2 in which the continuous display of data received is displayed as a waveform. End-diastolic and end-systolic intraluminal diameters were automatically determined for each cardiac cycle.

Vessel wall motion was recorded at 11 mm proximal to the anastomosis, at 3 mm intervals and at the anastomosis. Four registrations of vessel wall movement, each lasting 5 seconds, were made at each site. Carotid artery pressure was recorded simultaneously with each registration using a pressure tip catheter.

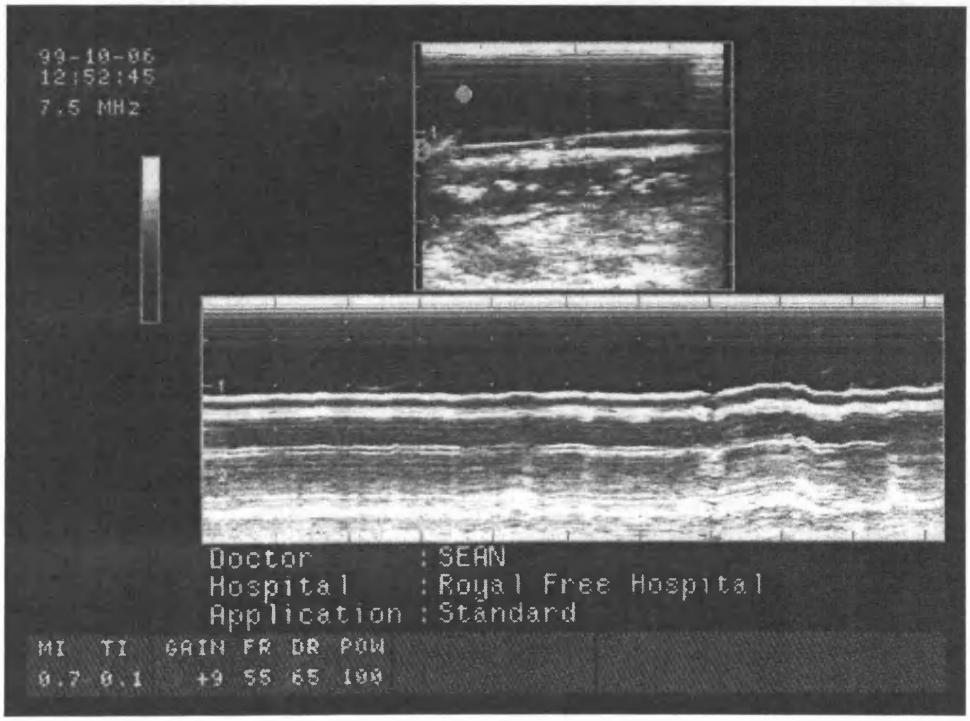


Fig 7.1: Wall tracking using M- and B-mode ultrasound

Data analysis and statistical methods

Vessel wall movement (over the four cardiac cycles in each registration) was averaged, and compliance and stiffness index calculated according to the blood pressure measured at the time of registration. The values are expressed as mean (±SD). For statistical analysis, a Student's t-test was used. A $p<0.05$ was considered statistically significant.

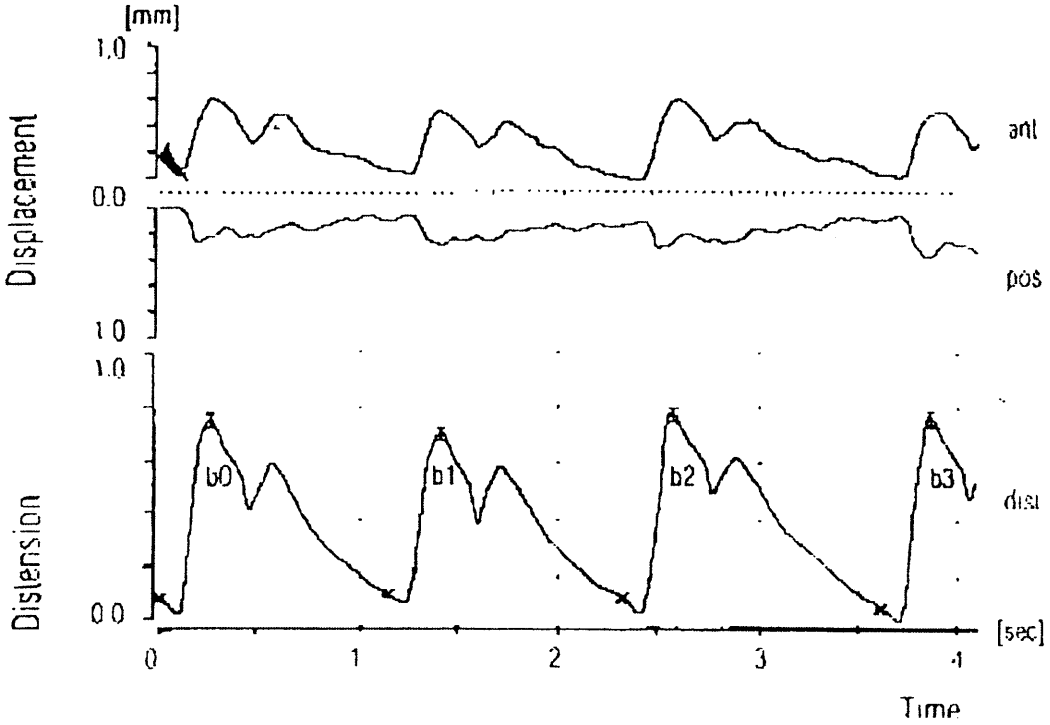


Fig 7.2: Wall Displacement and Distension

7.3 Results

Figures 7.3, 7.4 and 7.5 show the immediate compliance profile of SCS, reinforced SCS and laser soldered anastomosis vs distance along the common carotid artery blood vessel (Raw data Appendix D). The anastomosis is at 0 mm. The positive distance values are towards the head. As it shown, there was significant reduction in compliance at the anastomosis in all three techniques ($p<0.05$). In addition, the reduction in vessel elasticity, due to the anastomosis, generated a para-anastomotic hypercompliant zone (PHZ) just pre and post anastomosis. However, the compliance reduction was less in soldered compared to standard suture (SCS) technique ($p<0.05$) and due to this, the compliance of PHZ in the soldered anastomoses was not significantly different compared with

compliance of the vessel at -11 and +11 mm respectively. There was no significant difference ($p<0.56$) between standard suture and suture plus reinforcement compliance at the anastomosis. There was significant difference ($p<0.001$) between soldered and standard suture compliance and the anastomosis. The mean blood flow post anastomoses were 141, 137 and 127 ml/min in-group 1,2 and 3 respectively. There was no significant postoperative difference in blood flow between three groups.

Duplex ultrasound scanning of the animals was undertaken at day 5 and 10 post-operatively. Five day scanning showed no flow in the operated carotid artery. This was confirmed both using B-mode and colour doppler. As a consequence of this the animals were sacrificed at day 10 and no long term compliance measurements were taken.

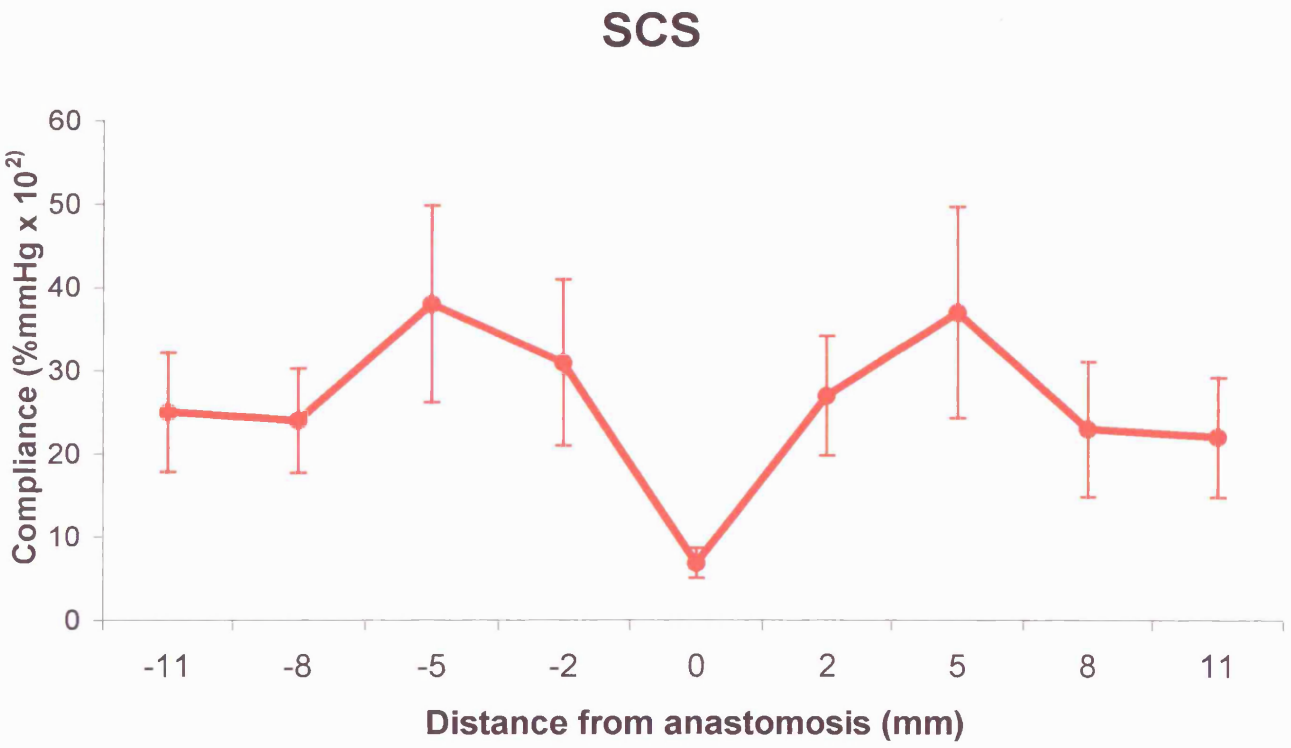


Fig 7.3: Continuous Sutured anastomotic compliance

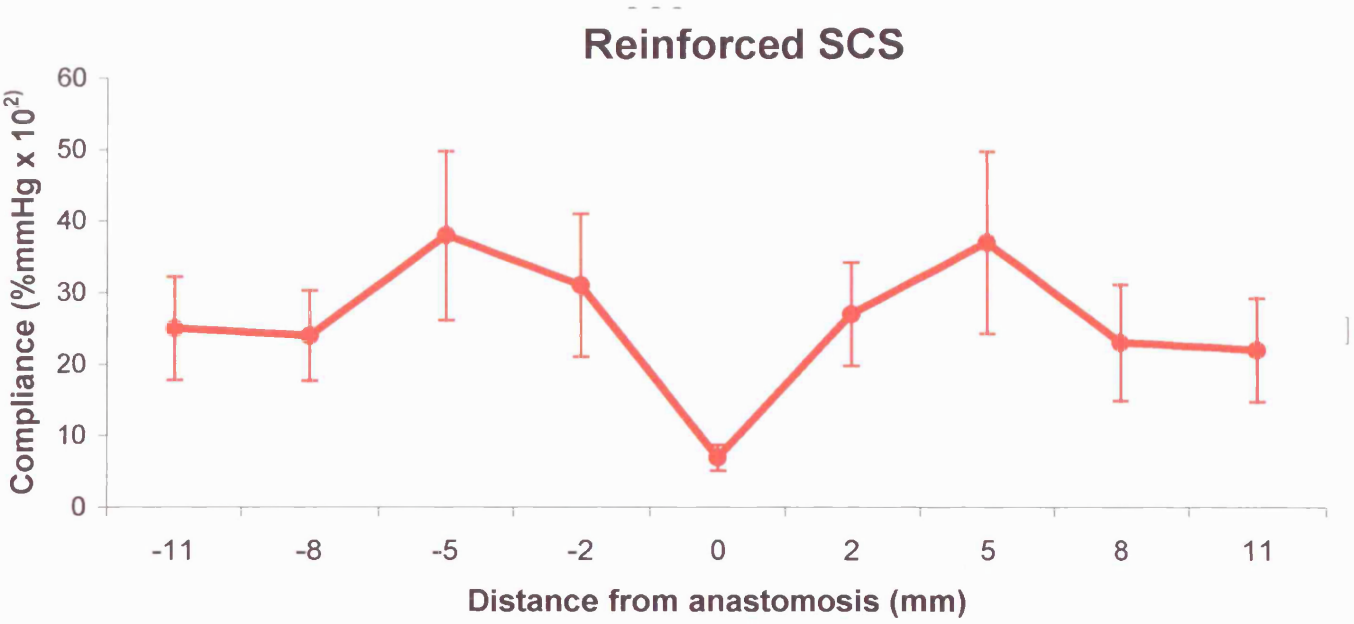


Fig 7.4: Reinforced continuous sutured anastomotic compliance

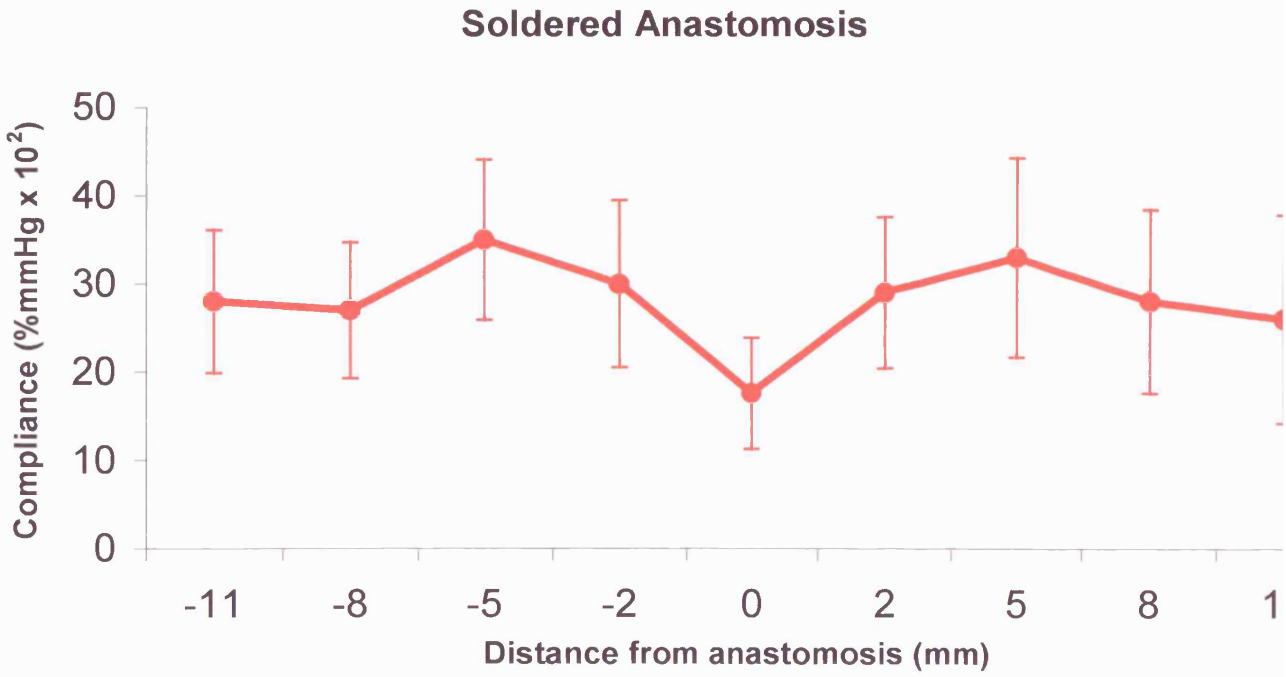


Fig 7.5: Soldered anastomotic compliance

7.4 Discussion

The importance of anastomotic compliance was first highlighted by Baird et al (Baird, 1977). Since that time a considerable amount of work has concentrated on arterial wall compliance particularly from the point of view of producing compliant vascular grafts.

Work on anastomotic compliance has shown that anastomotic technique is of considerable importance. Klein et al (Klein, 1982) reported this in 1982, showing that both a continuous and interrupted suture technique will significantly reduce anastomotic compliance. The interrupted technique was seen to result in a significantly lower reduction in compliance than continuous sutured anastomoses. The point is also made that other factors influence the patency of anastomoses, namely those of internal diameter, internal surface characteristics and flow.

Similar work was performed by Hasson et al (Hasson, 1986) who looked in detail at the para-anastomotic hypercompliant zones of anastomoses formed in interrupted and continuously sutured anastomoses. The results of this study again showed that there was a significant reduction in compliance using both techniques, whereas there was less of a reduction using interrupted sutures. In addition, it was seen that the PHZ was seen in only 50% of the interrupted sutured anastomoses, while the continuous sutured anastomoses showed PHZ in 86% of cases. Peak compliance was seen at 3.6 mm from the anastomosis and was independent of suture technique. The results of this study show that peak anastomotic compliance is found at 6mm for the SCS and soldered groups while it is seen at 6 and 8mm for the proximal and distal PHZ of the reinforced group. The differences from the findings seen in the literature may be attributed to differences in arterial dimensions between the canine and porcine carotid arteries. In addition, the sutures in this study used were 6/0 prolene as were those used by Hasson, whereas Klein used 7/0 prolene.

The differences in PHZ between the groups in this study, is of note. It might have been expected that the reinforced and soldered anastomoses would show a peak PHZ further away from the anastomosis than the SCS anastomoses. This is due to the observation that soldered anastomoses may be wider than sutured anastomoses. This is seen as solder activated adjacent to the anastomosis and would act to splint vessel movement. The fact that the soldered anastomoses, seen here, have the same PHZ peak as the SCS group would indicate that, although solder may be activated, it is not having a functional effect on the movement of the vessel wall. In this context, is not surprising to find that the

addition of solder to a sutured anastomosis, while not changing the amplitude of vessel compliance, may influence the area of its effects with the restriction caused by both the sutures and solder acting in concert.

The interrupted anastomoses in Hasson's study (Hasson, 1986) show compliance values of 5.8-7.2 $\%mmHg^{-2}$ while the continuous sutured anastomoses gave a range of 5.7-6.2 $\%mmHg^{-2}$. Both of these values are comparable with the results seen in this study, with SCS and reinforced anastomoses showing values of 5.3 and 6.9 respectively. Compared to these, the values for soldered anastomoses at 17.6 $\%mmHg^{-2}$, are significantly higher than either sutured or reinforced anastomoses ($p<0.05$). The inference from this is that the final solder substance has an elastic component and that this is able to flex with changes in arterial pressure improving compliance. This in turn is presumed to improve the flow characteristics of the anastomosis and reduce the incidence of thrombus formation and long term intimal hyperplasia. The benefits of this are quite clearly not sufficient to prevent thrombosis as all three groups showed a 100% thrombosis rate. The reasons for this are unlikely to be related to the use of solder since the sutured controls showed the same response. The use of heparin intra-operatively would have prevented clotting cascade activation but would not have prevented platelet activation. Since this is also more likely in a high flow situation the addition of an antiplatelet agent such aspirin or clopidogrel, may have prevented the observed late thrombosis and allowed longer term measurements of compliance to have taken place. In addition to these variables operator experience and operative technique may have been a factor in the high rate of thrombosis. The use of an interrupted suture technique may have improved the outcome, but since this is related to an increase in compliance, the soldered anastomoses should have shown a lower thrombosis rate. Further work is required to investigate this phenomenon and produce a large animal model of long term vascular compliance.

The passive compliance of laser anastomoses has previously been reported in vitro. Dalsing et al (Dalsing, 1992) performed bilateral carotid anastomoses in a canine model, with one side undergoing laser anastomoses while the other was formed with interrupted sutures. These were then removed and tested in vitro for static compliance using a photo-electric force transducer. Recordings of passive and active stress were taken and used to compare the two techniques over time (1 and 8 weeks). The control and laser repaired vessels showed similar compliance while it was claimed that the sutured repairs had a greater compliance than the controls (arterial tissue taken adjacent to the

anastomosis). The same was found after eight weeks with the sutured anastomoses showing a greater compliance than the lasered anastomoses. These anastomoses were found to be patent at 1 and 8 weeks without the use of antiplatelet agents.

Although these anastomoses were investigated in vitro, it is interesting to note that the sutured anastomoses were found to have a greater compliance than either the laser anastomoses or the tissue immediately adjacent to the anastomosis. The thickness of the rings taken was 1mm at the anastomosis and at a distance of 1cm from the anastomosis excluding artifact from the para-anastomotic hypercompliant zone. The results of Dalsing's work goes against the findings of both Hasson (Hasson, 1986), Klein (Klein, 1982) and this study, although interrupted sutures were not used. This could be due to the technique of measurement and the reliance on passive in vitro data, rather than in vivo measurements.

The finding of improved initial compliance in soldered end to end arterial anastomoses as compared to SCS is encouraging. There still remain significant questions from this study, namely the cause of the thromboses seen in all groups and what if any changes take place with time in soldered anastomotic compliance. In addition, since most anastomoses formed are end to side anastomoses it will be important to assess the compliance and performance of solders in this configuration.

Chapter 8

The haemostatic effects of Laser tissue solder as a re-inforcement to anastomoses with ePTFE grafts

The haemostatic effects of Laser tissue solder as a re-inforcement to anastomoses with ePTFE grafts

8.1 Introduction

Expanded PTFE vascular grafts are commonly used in clinical practice particularly relating to peripheral vascular reconstruction or vascular access surgery. In these instances, conventional surgical techniques applied to ePTFE will result in excessive bleeding at the site of the anastomosis. Suture materials commonly used such as polypropylene or polyamide leave holes in such prostheses. To compound the problem patients are often anticoagulated improving the chances of graft survival or having dysfunctional platelets. These factors increase bleeding time, the time required to achieve haemostasis and also the post operative complications related to bleeding such as haematoma formation.

It was therefore intended to apply the techniques of soldered vascular anastomoses to such a scenario, by reinforcing the anastomotic suture line of grafts placed in an animal model, with MB based solder. The bleeding times, overall operating times and postoperative complications were then analysed and compared to sutured controls.

In view of the disappointing results found in the previous studies, it was determined to perform these anastomoses in a different species. Goats have been used in many studies (Baguneid, 2000) and are know to have a readily accessible vascular tree. In addition to reduce animal numbers, bilateral procedures were undertaken.

8.2 Materials and Methods

Animals and operative procedures

Eleven ePTFE grafts were inserted into 8 goats (30-60 kg). Following an overnight fast, the animals were pre-medicated with Azaperone (Stresnil™, Janssen Pharmaceutical Ltd. UK) 0.1 ml/kg intramuscularly. After induction of anaesthesia using Ketamine hydrochloride (Ketaset™, Willows Francis Veterinary, UK) 5mg/kg IV, the animals were intubated and mechanically ventilated. Anaesthesia was maintained using Halothane (May and Baker Ltd, Dagerham, UK), nitrous oxide and

oxygen via a standard anaesthetic circuit. The animal's temperature was maintained at 36-38°C using an electronic heating mat. A pulse oximeter probe (Ohmeda Biox 3740-pulse oximeter, Ohmeda Co., Louisville, USA) was used for continuous monitoring of arterial oxygen saturation (SaO_2) and heart rate (HR).

The common carotid artery was exposed and prepared for anastomosis with the ePTFE graft. Heparin was administered at a dose of 100-150iu/kg intravenously prior to clamping and 1% papaverine injected into the loose adventitial tissue surrounding the artery to prevent spasm. The blood flow (ml/min) was measured prior to clamping using a transonic Medical Flowmeter system (HT207, Transonic Medical System Inc, USA). The vessel was clamped and a 5cm segment of the artery removed. Using continuous suture technique (6/0 prolene – Ethicon) a 5cm segment of ePTFE graft (6mm Gelsoft- Sulzer-Vascutek, Inchinin, Scotland, UK) was inserted with the aid of loupe magnification. Following graft insertion the sutureline was either reinforced with activated protein solder (2 layers) or left in its native state.

Treatment groups

It was initially intended that all of the procedures be performed bilaterally, with the initial data relating to the effects of the solder on haemostasis, with the animals continuing on to give histology and patency data. Unfortunately the animals receiving bilateral procedures were seen to succumb to early bilateral occlusion regardless of the treatment group. With this in mind the protocol was modified to include unilateral procedures in which the anastomosis at one end was reinforced while the other was left in its native state. Having performed 3 bilateral procedures the remaining animals underwent unilateral procedures with the data in the first three used to compare pre and post operative blood flow.

For bilateral procedures the left carotid was reinforced with solder while the right remained in its native state.

In order to measure blood loss, the area was thoroughly swabbed prior to the release of the clamps and all excess fluid removed and extrinsic bleeding controlled with monopolar diathermy. On releasing the clamps preweighed swabs were placed around the anastomosis and pressure applied as necessary. The swabs were removed when bleeding had stopped or to inspect the anastomosis for bleeding. The swabs were collected, weighed and the volume of blood in them was calculated. Once this was complete on one side the other side was operated on in the same manner.

For the unilateral procedures the graft was sutured in place, with the proximal end reinforced with solder and the distal end left untreated. Again swabs were placed around the anastomoses and the clamps released. With careful application of swabs and pressure the bleeding from each end was kept separate from the other. As with the bilateral procedures the time taken for haemostasis was recorded and the swabs weighed.

The total operating times were recorded, along with the bleeding times and the difference in time to achieve haemostasis. The total volume of blood lost from each anastomosis was also calculated.

The animals were kept for 7 days after which the grafts were imaged using duplex ultrasound duplex colour flow ultrasound system (Pie 350, Pie Medical Systems, Maastricht, Netherlands). Patent vessels were to continue while animals with occluded grafts were terminated.

Solder preparation

See Chapter 2. Solder MB concentration was kept at 0.24%w/v.

Activating system.

See Chapter 3. Laser power was kept constant at 180mW (22.9W cm^{-2}).

Statistical analysis

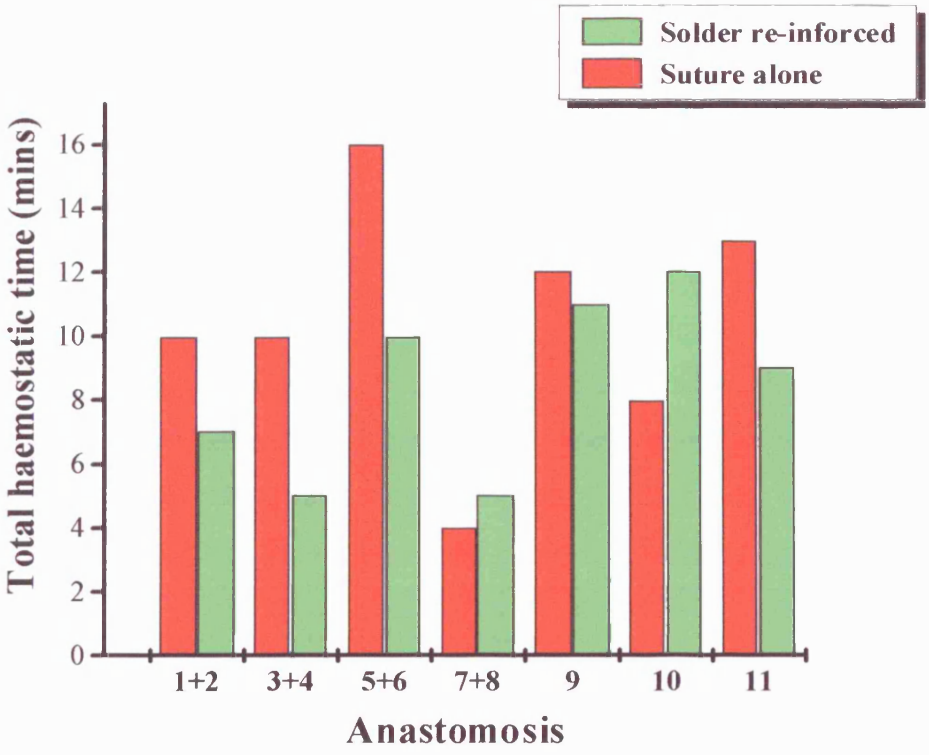
The results were analysed using paired and unpaired t-tests where appropriate calculated on Prism™ (Graphpad software Inc, UK) for parametric data. A p value of <0.05 was considered significant.

8.3 Results

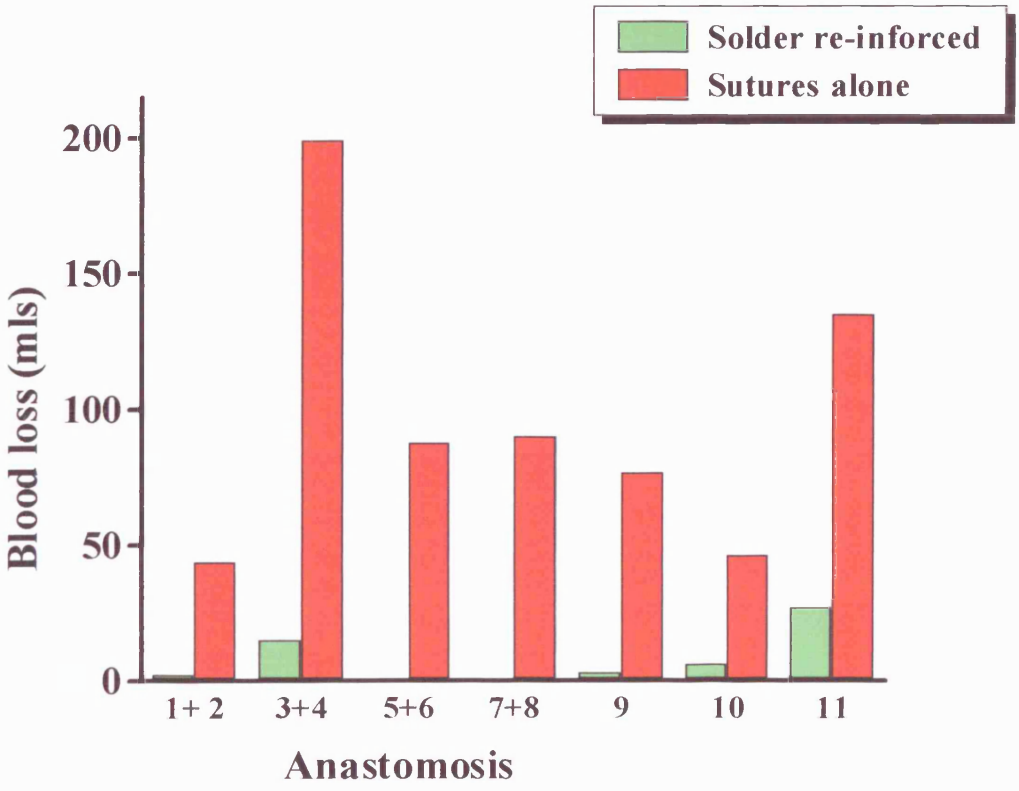
The results are summarised in tables 8.1-8.3 and graphs 8.1, 8.2 and 8.3. From table 8.1 it can be seen that there was a significant reduction in bleeding time (*$p<0.05$) and total blood loss ($p<0.02$) (table 8.3 & graph 8.1 & graph 8.3), with no significant decrease in total anastomotic time ($p=0.186$) or suturing time. Overall haemostatic time, which allowed for the extra time taken to apply the solder, did not quite achieve significance between the two groups (†$p=0.065$). This was due to the additional time required to re-suture the single laser damaged anastomosis (‡). This was related to the misapplication of the solder and laser exposure to an uncovered suture.

REINFORCED ANASTOMOSES											
Graft No	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Suture time (mins)	20	20	14	14	15	16	17	17	17.5	14	18
Bleeding time (mins)*	2	2	0	0	5	5	1	1	7	0	3
Laser time (mins)	5	5	4	5	4	5	7	4	4	12‡	6
Total anastomotic Time (mins)	27	27	18	19	24	26	25	23	28.5	26	27
Haemostatic time(mins)†	**7**	**7**	**4**	**5**	**9**	**10**	**8**	**5**	**11**	**12**	**9**
SUTURED ANASTOMOSES											
Graft No	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Suture time (mins)	20	20	18	18	14	14	15	16	17.5	14	18
Bleeding time (mins)*	10	10	10	10	16	16	4	4	12	8	13
Laser time (mins)	-	-	-	-	-	-	-	-	-	-	-
Total anastomotic Time (mins)	30	30	28	28	30	30	19	19	29.5	22	31
Haemostatic time (mins)†	**10**	**10**	**10**	**10**	**16**	**16**	**4**	**4**	**12**	**8**	**13**

Table 8.1 Comparison of sutured and re-inforced anastomoses (Bleeding time - *p<0.05: Haemostatic time - †p=0.065)



Graph 8.1: Total Haemostatic time



Graph 8.2: Total Bleeding time

The blood flow in each group is summarised in tables 8.2 and 8.3. Comparison of the pre and postoperative blood flow in the re-inforced group showed that there was a significant reduction in blood flow from 365ml/min to 290ml/min (*$p<0.05$). The same was seen in the soldered group with a reduction from 412ml/min to 390ml/min (†$p<0.05$). Comparison between the two groups showed that there was no significant difference in the reduction in blood flow pre and post operatively ($p>0.22$)

The animals undergoing bilateral procedures inevitably resulted in bilateral occlusion of the grafts with no differences seen between those having solder and not. Two animals were affected acutely while the third survived 14 days prior to planned termination. This animal was seen to have a haematoma on the suture alone side. Animals undergoing unilateral procedures were also seen to occlude the grafts with no detrimental clinical effects. These animals were also sacrificed at 14 days. No other post operative complications were observed.

No	TREATMENT	FLOW Pre-op (ml/min)	FLOW Post-op (ml/min)	CHANGE IN FLOW (mls/min)
1	Reinforced	330	210	-120
3	Reinforced	380	340	-40
5	Reinforced	330	310	-20
8	Reinforced	420	300	-120
	Mean blood flow	**365 ml/min**	**290 ml/min**	**-75ml/min***

Table 8.2: Mean blood flow in re-inforced anastomoses (*$p<0.05$)

No	TREATMENT	FLOW Pre-op (ml/min)	FLOW Post-op (ml/min)	CHANGE IN FLOW (mls/min)
2	Sutured	520	400	-120
4	Sutured	420	430	+10
6	Sutured	340	370	+30
7	Sutured	450	280	-170
	Mean blood flow	**412ml/min**	**330ml/min**	**-82ml/min†**

Table 8.3: Mean blood flow in sutured anastomoses (†$p<0.05$)

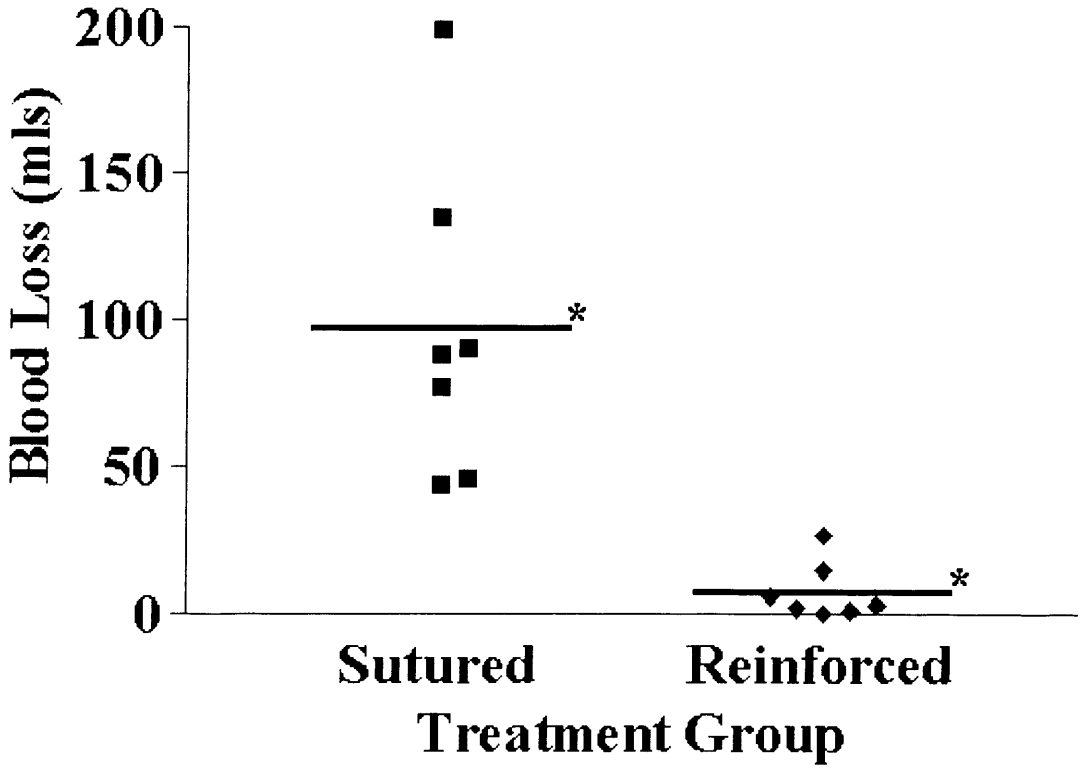


Figure 8.3 Blood loss: Sutured Vs reinforced anastomoses (*p<0.001)

8.4 Discussion

The application of solders to suture lines as a reinforcement is not a new idea. The suture lines in rat urethra anastomoses had been reinforced by Poppas et al (Poppas, 1988) who used egg albumin without chromophore to reinforce the suture line. A higher rate of patency was achieved in those reinforced with solder. Similarly Kirsch et al (Kirsch, 1995) reported the first clinical use of a 42% human albumin solder with ICG to reinforce the suture line in correction of congenital urological abnormalities such as hypospadias. An improved initial burst pressure was reported but suture disruption was also noted related to the exposure of suture material to the laser. This mirrored the findings by the same group into the effects of laser and solders on sutures commonly used in urological procedures (Kirsch, 1996).

Other applications of solder to reinforce suturelines were performed by Libutti et al (Libutti, 1990) in canine colonic anastomoses. Human fibrinogen solder containing ICG was applied and

activated with an 808nm diode laser. In this instance a threefold increase in the initial burst pressure was seen with no evidence of thermal injury. No longer term results were published to describe the benefits to healing or the effect on morbidity.

Vascular suture reinforcement was performed by Cikrit et al (Cirkrit, 1998) using a fibrin/ICG solder to reinforce jugular vein anastomoses. The solder was activated using a milliwatt CO_2 laser and showed a reduced patency of the reinforced anastomoses over sutured controls (82% Vs 93%). In addition medial necrosis was seen in the soldered group negating any advantage of using a suture and going someway to explaining the reduced patency.

In this study methylene blue based (porcine) albumin solder was used to reinforce a vascular anastomosis that already been established using sutures. In conventional surgery, haemostasis is achieved using compression, with sometimes excessive loss of blood and requiring additional theatre time. The original aim of this study was to look at the haemostatic effects of the solder and compare patency in the two groups. However, the rate of graft occlusion was such that a comparison could not be made and further work is required to develop this animals model. Despite the use of heparin anticoagulation graft occlusion was 100% by 7 days. The initial flow data showed an overall reduction in flow post operatively despite a good size match between the host vessel and the graft. This implies that thrombosis may have commenced early after the clamps were released. Previous reports of anastomoses in porcine carotid arteries, of similar dimensions to those in the goat, have reported higher patency rates with lower doses of heparin and no anti-platelet therapy (Angelini, 1999). However the use of antiplatelet agents may improve patency without deviating from established clinical practice.

The data pertaining to the time of anastomosis shows that there was however a significant reduction in blood loss ($p<0.02$) and bleeding time ($p<0.02$) between the two groups. There was no reduction in the operating time required to perform the anastomosis. In breaking these times down it becomes apparent that the time taken in applying and activating the solder is similar to the time required to apply compression to the sutureline. The fact that there was no overall saving in time may be a reflection of the experience of the surgeon in applying the solder. On one occasion 12 minutes of lasering was undertaken. Had this single event taken the average time for lasering the haemostatic times would have been significantly different ($p<0.05$). In addition the practice of applying two layers of solder had been developed from previous experience in forming minimal suture microvascular

anastomoses. In this case, the practice was continued adding time but a single layer would have been likely to have had a similar effect.

The only haematoma seen was found in the untreated group. The predisposition of untreated anastomoses to haematoma can be explained in their reliance on thrombus formation to plug suture holes. By 7 days the fibrinolytic system has removed much of the initial thrombus formed in the suture holes allowing secondary haemorrhage to occur. Recent studies by this group have established that the half life of the MB/PSA solder is 10-13 days, by which time endothelial and medial regeneration will be more advanced within the vessel and the fibrotic reaction will be maximal outside the graft.

Suture damage was seen in one anastomosis mirroring the findings of Kirsch et al (Kirsch, 1996) in sutures used in urological procedures. The polypropylene sutures commonly used in vascular anastomoses contain copper thalocyanate which absorbs at a similar wavelength to the methylene blue solder and laser diode emission spectrum. Consequently, the sutures themselves will generate heat which may be sufficient to melt the suture material. It is significant that the heat generated by the photothermal reaction of the laser and MB solder had no effect on the sutures and was seen to protect the sutures from the effects of the laser.

Methylene blue based porcine albumin solder has been successfully used to reinforce the suturelines of vascular anastomoses formed with ePTFE. A reduction in the bleeding time and blood loss was seen despite no significant differences being seen in overall anastomotic time or haemostatic time. There was a reduction in flow post operatively in all groups. No patency data was obtainable from this study and further work is required to produce a large animal model of vascular anastomoses using solder.

Chapter 9

Conclusions and prospects for future work

Formulation -Optimal MB concentration

Initial work on the MB based solder showed that there was a good correlation between chromophore concentration and anastomotic strength. This relationship showed an initial sigmoid dose response type curve rising to a maximum at 0.24% w/w of MB. This then forms a plateau with little change up to 0.6% w/w MB. The strength of the anastomoses was seen to rise to a maximum of 1188 mmHg at 0.24% w/w with little change over the remaining increase in chromophore concentration. It was noted that the relationship between the concentration of MB and solder absorbance was non-linear. A negative deviation from the Lambert-Beer law was seen and attributed to the fact that as the concentration of MB increases there is an increase in the formation of dimers and trimeric isomers of MB. As these isomers have different absorption characteristics the absorption profile of the solder changes with the concentration of MB.

Short term Histology & Reabsorbtion

The labelling of porcine albumin to determine the re-absorption of solder with time was performed using radio-labelled iodine. This was done in conjunction with a study looking at the effect of laser power on the re-absorption of the solder and on the histology of the vessels repaired. This showed primarily that the use of high laser power results in a low patency rate and thrombosis early in the healing process. This is however caused by a mechanism separate from thermal injury, which was previously identified as the mechanism of injury in the formation of aneurysms, where lasered micro-anastomosis was used. In this case, the injury caused to the media resulted in weakness, which resulted in a 30% aneurysm rate at 6 months. The mechanism of thrombosis in this case may have been related to endothelial injury or to constriction at the site of the anastomosis. Certainly when a lower power was used the rate of thrombosis dropped to a significantly lower level. However, with the drop in thrombosis there came a consequent increase in intimal hyperplasia.

From the point of view of solder re-absorption there was no significant difference between the two groups and both showed a half life of the solder of 10.1 days.

Laser alternatives

Lasers had been used to activate solders since their introduction in 1989. In these instances, the lasers used were of the Argon ion or milliwatt CO_2 types. Later with the introduction of

laser diodes much work centred around the use of the 810nm laser diode in conjunction with the chromophore ICG.

The use of lasers has a number of implications in the application of the techniques of soldering to the clinical scenario. In terms of activation of the solder, the lasers used are ideal providing the user with an activation device that works at a distance, is specific to the absorbtion profile of the solder and activates a small and controllable area of the solder preventing excessive irradiation of tissue. However, the use of lasers also has negative aspects, including those of safety, utility and cost. The safety aspects of laser use relate to potential damage to the retina that can be caused by lasers and the need for goggles in the clinical setting. This is also accompanied by other safety mechanisms such as theatre security and rigorous checks on the activation of the laser. The activation of the solder by laser while occurring over a controllable area is limited in size and as larger areas require activation, the time saving in using a solder method becomes diluted. As such, a mechanism that can handle larger areas is required without the need for multiple laser units. This later point will also increase the already high cost of using a laser activating source.

As part of the development of the solder anastomosis system, a Xenon Arc lamp was developed to activate the solder. This is based on a 300mW xenon arc bulb which when heat sunk and filtered is able to deliver upto 700mW of light over the spectrum of 590-700nm.

This system was found to be effective in activating the solder and showed a similar profile of power to anastomotic strength that was seen with the previously used laser 2000 device. In addition, the use of the arc lamp in vivo was seen to result in a high rate of patent anastomoses.

The disadvantages of the arc lamp are that the emergent beam has a 60^{o} angle of divergence with a focal point at 1.4mm from the end of the focusing lens. This means that the activation of solder involves all but contact with the solder prior to activation. With successive generations of arc lamp it is hoped that, not only will it be possible to work at a distance, but also work over a larger area and at a considerably reduced cost to the health service purchaser.

Immunology

The decision to use non-human albumin was arrived at in the midst of the controversy over British beef, the rise in incidence of New Variant CJD and against the background of HIV and Hepatitis associated with human products. As a consequence, the use of albumin from both human and bovine sources, while being both readily available and cheap, presented infection problems and were

rejected in favour of porcine albumin. Porcine products are used in a variety of other healthcare products including porcine insulin for injection.

As a consequence of the use of porcine albumin, the question arose as to whether such an antigen presented in this way might result in an immune reaction either on first, or more importantly, on subsequent exposures to the solder. To answer this question anastomoses were formed in both naive and pre-sensitised animals, with circulating and fixed immunoglobulins being assessed. The results showed that the use of the solder did result in an increase in circulating and fixed IgG in naïve animals. In addition, the pre-sensitisation of animals with porcine albumin also saw a rise in circulating and fixed IgG. However there was no additional increase in these factors with the use of the solder in either pre-sensitised or naïve animals, with no evidence of either systemic immune reactions, or local kidney pathology.

Compliance

The site of an anastomosis is known to be an area of reduced compliance and consequently increases the risk of early thrombosis and late intimal hyperplasia. In order to compare soldering with conventional techniques, end-to-end anastomoses were formed using either continuous suturing, re-inforced continuous suturing, or solder alone. These anastomoses were then studied using M-mode ultrasound and wall tracking software. The compliance was calculated for each case and showed that the use of solder as a reinforcement made no difference to the compliance of the anastomosis compared to continuously sutured anastomoses, but that the formation of solder only anastomoses showed a significant increase in anastomotic compliance.

Haemostasis

The first clinical use of the solder was proposed to be as a sealant for anastomoses in areas of risk of bleeding such as those formed in ePTFE. As a result, the solder was assessed in sealing anastomoses formed using ePTFE grafts secured using a standard suturing technique. These anastomoses were compared with anastomoses formed using sutures alone and were assessed for bleeding time, haemostatic time, blood loss and complications. It was clearly seen that the use of the solder as an anastomotic sealant reduced both the bleeding time and the volume of blood lost during the procedure, while the total haemostatic time was not significantly different. This latter was true because of suture damage occurring as a result of laser exposure requiring re-suturing. If this event had

been avoided then there would have been a significant improvement in haemostatic time. The complication rate was low in both groups, with only a single haematoma formed in the suture only group. There was a 100% rate of graft occlusion in all groups.

Future work

The scope and versatility of tissue soldering lends itself to a wide variety of applications in clinical surgery of many specialities. The simplicity of the mechanism of application and solder activation also allow greater flexibility in applying the solder not only to procedures already in existence but also to areas of the body previously inaccessible to suturing.

As well as exploring the practical applications of tissue solder, much remains to be determined regarding the theoretical nature of the bonds formed by and the nature of the process, of tissue soldering.

The bonds formed by the activation of tissue solder are presumed to be disulphide bridges, but the use of mercapto-ethanol results in incomplete dissolution of the activated mass that is left. In addition, there is a significant dehydration element to the process of solder activation with a reduction in mass by 50-60% in activating the adhesive. In parallel with this, it has been found that simple dehydration of the solder on glass can result in bonding of considerable strength equivalent to 10 Newtons per microlitre (personal communication Mandley, 1999). This is remarkable in itself as there can be no disulphide bridges formed with simple dehydration. However, as has been reported previously the bonds formed in this way or with laser activation are highly susceptible to re-hydration and may break down altogether. For this reason a more precise understanding of the bond nature is required. The issue of re-hydration strength has been addressed to a degree with the work by Chan et al (Chan, 1998) into the uses of a micro-jet device to prevent re-hydration. In the areas of urological surgery, this would be of particular importance.

The temperature to which the solder rises in the course of solder activation is an issue of some controversy and debate. Many groups have attempted to control the finite rise in temperature associated with chromophore photon absorbtion. However, the techniques used to measure temperature have not taken into account the small area over which the solder is generally activated and produce inaccurate readings. Since the area of activation is less than $1mm^2$, a device capable of sampling such a small area should be used, although these instruments are not widely available.

The clinical applications of the solder in its current liquid form are potentially enormous. The current plan is to apply the solder to vascular surgery as a reinforcement to anastomosis prior to other applications in vascular surgery. These may include minimal suture or sutureless anastomoses in either medium or small vessels. Moving on from simple procedures, tissue solder will lend itself to the anastomosis of vessels via laparoscopic instrumentation. This may lead the way to minimally invasive vascular techniques and raises the possibility of minimally invasive coronary revascularisation.

Non vascular applications may include scar reduction surgery with the application of solder to the dermis or epidermis in skin closure. Preliminary work into this application has shown an improvement in the appearance of the scars. The application of solder to burns may improve the restoration of a normal epithelial surface and could act as a delivery device for fibroblasts or keratinocytes.

Other applications may include the anastomosis of ureters, vas deferens or tubal reconstruction, while orthopaedic applications may include tendon repair or meniscal repair, again via minimally invasive endoscopic instruments. The anastomosis of bowel has been attempted in experimental models using a laser alone, and tissue solder would again be a potential application as either a re-inforcement to sutured anastomosis in the contaminated patient, or as a minimal suture anastomosis.

The use of tissue solder although not yet in the clinical arena offers the surgeon of the future a simple versatile tool with which to appose tissue. As it encourages the intrinsic activities of neutrophils and fibroblasts it is also a substance that will enhance and promote the rapid restoration of form and function within the tissue to which it has been applied. The versatility of its application will also enable surgeons to perform procedures not previously possible and further improve the morbidity and mortality associated with major surgical intervention.

Appendix A

Histological Methods

This section gives a brief account of the histological methods used for staining paraffin embedded tissue prior to light microscopy. The staining and sectioning was carried out by Mr A Posteyalko, Covance, Harrogate and are referred to in chapters 3 and 5.

Haematoxylin and Eosin (H&E)

Haematoxylin and Eosin refers to the staining of nuclei by oxidised haematoxylin through chelate bonds of metals such as aluminium, followed by counterstaining by the xanthine dye eosin, which colours cytoplasm and other background fibres in varying different shades (Bancroft , 1984).

The slides were stained on an automated stainer (Leica Autostainer, XL. DDR).

The Cycle for the Autostainer is shown below along with the reagents used in the procedure.

Staining Schedule for Leica Autostainer

1.0 Wash with Xylene for 2.5 mins
1.1 Rinse in IMS for 90 seconds and 70% IMS for 45 seconds
1.2 Rinse with de-ionised water
1.3 Stain with haematoxylin for 9 mins
1.4 Rinse with de-ionised water for 1 min 15 seconds
1.5 Wash in blueing solution for 45 seconds and 95% IMS for 10 seconds
1.6 Stain with Eosin for 6 mins 10 seconds
1.7 Rinse with IMS for 45 seconds and Xylene for 2.5 mins

Stains for use with the Leica Autostainer

1.1 Varistain haematoxylin

De-ionised water	730ml
Ethan-1,2-diol	250ml
Glacial Acetic Acid	20ml
Haematoxylin Cl 75290	3g
Sodium iodate	0.2g
Aluminium Sulphate	35g

Mix for 1 hour and filter into storage container after having discarded the old haematoxylin and rinsing the container with de-ionised water. The mixture is stored at room temperature.

1.2 Varistain eosin

95% industrial methylated spirits	1000ml
Eosin Y Cl 45380	10g

Add the eosin to the industrial methylated spirits, stirring continuously for 2 hours. The mixture is filtered and stored at room temperature.

2.0 Reagents

2.1 Blueing Solution

De-ionised water	2000ml
Industrial methylated spirits	8000ml
Conc Ammonia solution (35% w/v NH_3 0.880)	200ml

2.2 95% IMS

De-ionised water	500ml
Industrial methylated	9500ml

2.3 70% IMS

De-ionised water	3000ml
Industrial methylated	7000ml

Elastic/Van Geison

Elastic fibers are composed of the protein elastin and are found predominantly in the skin, respiratory tract and circulatory systems. It is seen as single fibres, as in the dermis, or as a membranous structure, as in large arteries. Elastic fibres are highly eosinophilic and may be seen in H&E stained sections, but special techniques are required for demonstration of very fine fibres

Verhoeff's method for elastic fibre staining call for careful differentiation of the stain to achieve consistent results.

1.0 Procedure

1.1 Treat with 1% acidified potassium permanganate for 5 mins and rinse in de-ionised water

1.2 Bleach in 1% oxalic acid and rinse in de-ionised water

1.3 Stain in Verhoeff's' stain and rinse in de-ionised water

1.4 Differentiate in 2% iron III Chloride until only elastic fibres are stained black. Rinse in de-ionised water and 95% ethanol

1.5 Stain in Van Gieson and blot dry

1.6 Dehydrate rapidly through alcohols and clear with xylene

1.7 Mount in synthetic resin

This results in elastic fibres stained black, Muscle stained yellow and collagen stained red.

2.0 Reagents

2.1 Lugol's iodine

De-ionised water	100ml
Potassium iodide	2g
Iodine	1g

The potassium iodide is dissolved in the de-ionised water, and mixed with the iodine until dissolved. This is stored at room temperature.

2.2 Verhoeff's stock solution

Ethanol	40ml
Haematoxylin Cl 75290	2g
De-ionised water	16ml
Iron III Chloride	1.6g

Dissolve the haematoxylin in the ethanol and the Iron III Chloride in the de-ionised water. Mix the two solutions.

2.4 Verhoeff's stain

Verhoeff's stock solution	56mls
Lugol's iodine	16mls

Appendix B

Radio-iodination of Porcine Albumin

Materials:

Porcine Albumin(Fraction V) (Sigma)

Sodium Iodide (I^{125}) solution (IMS 30) 3.7 GBq per ml on 19 Oct 1998 (Amersham);

1,3,4,6-tetrachloro-3,6-diphenylglycouril(Iodogen) (Sigma)

Dichloromethane (Fisons)

Water for Injection (Baxter)

Method:

A solution of iodogen (40 mg/ml) in dichloromethane was prepared. 300 [ti aliquots were dispensed into 1 ml conical-based polypropylene tubes (Sarstedt) and evaporated to dryness under nitrogen.

A solution of porcine albumin in water was prepared at a concentration of 1 mg/ml . A 0. 5 ml aliquot was added to an iodogen tube together with approximately 30 MBq (10µl) of IMS 30. After 1 5 minutes incubation at room temperature, the reaction mixture was passed through a Sephadex G25 PD10 column, (Pharmacia LKB) eluting with water and collecting the eluate in 0.5 ml aliquots.

Activity in each aliquot was measured using a Vinten Instruments Isocal 11 ionisation chamber. Results are shown in table 1. Most of the activity was detected in fractions 7 and 8 A 30 µl aliquot of fraction 8 was added to a sample of the adhesive to give a final product containing 1.375 kBq 1-125 activity per 5µl (see calculation below)

Analysis

Samples were analysed by electrophoresis on Sepraphore-111 medium with high resolution buffer (Product No. 51104, Gelman Sciences). Separation was performed for 30 minutes at 300 volts, after which the media strip was divided lengthwise. One half of the strip was developed using a stain of Ponceau S in 7.5% trichloroacetic acid, which

showed the presence of albumin as a single pink band. The position of this band was traced on to the corresponding unstained portion of the strip. Using the trace as a guide, the strip was divided into three zones - anode end, stained peak and cathode end, and the sections assayed for radioactivity in a scintillation counter (LKB Compugamma). Results are shown in table 1.

Labelling efficiency was high, being of the order of 93 % as determined by the activity remaining on the gel column. Approximately 2% of the activity in the final product was present as free iodide as demonstrated by the counts at the anode end of the electrophoresis strip. Approx. 10% of the activity in the final product was associated with a molecular fraction of larger molecular weight than the albumin, possibly indicative of some degree of aggregation or polymerisation of the protein.

Calculation of activity added to adhesive

Fraction 8 contains 13.02 MBq 1-125 activity in a volume of 0.5 ml (see table 1)

30 μl of this solution corresponds to 781 kBq 1-125 activity

Mass of adhesiveused=3.1064g. Density=1.107g/cm^3

Therefore volume of adhesive = 2.806 ml.
Total volume (including 30 μl fraction 8) = 2.836 ml

5μl of the radiolabelled adhesive therefore contains (781/2836)*5 = 1.377 kBq 1-125 activity

Fraction Number	Activity (MBq)
Residue in Reaction Vessel	0.82
Fraction 1	0
Fraction 2	0
Fraction 3	0
Fraction 4	0
Fraction 5	0.19
Fraction 6	0.29
Fraction 7	11.26
Fraction 8	13.2
Fraction 9	2.1
Fraction 10	0.43
Column	1.89
Total recovered	30.00
Labelling efficiency	93.07 %

Table B1: **Activity in successive samples from PD10 gel filtration**

Raw data from reabsorption study

	Low Power anastomoses (11.4Wcm^{-2})				*High Power anastomoses (22.9 Wcm^{-2})*			
Counts (x1000)	*1*	*2*	*3*	*Mean*	*1*	*2*	*3*	*Mean*
D0 Counts (cpm)	-	-	-	-	-	-	-	-
D7 counts (cpm)	*6.85*	*5.55*	*4.38*	*5.46*	*16.57*	*14.62*	*12.57*	*14.62*
D21 counts (cpm)	*2.30*	*2.81*	*4.34*	*4.62*	*10.23*	*8.66*	*7.01*	*8.66*
D30 counts (cpm)	*1.89*	*1.26*	*2.45*	*1.82*	*4.06*	*2.59*	*3.22*	*3.22*
D 40 Counts (cpm)	*2.28*	*2.10*	*0.66*	*1.74*	*1.86*	*2.88*	*0.9*	*1.86*
D60 counts (cpm)	*0.27*	*0.59*	*1.33*	*0.59*	*0.828*	*1.01*	*0.64*	*0.82*

Table B2: Counts obtained from explanted vessels

	Low Power (11.4Wcm^{-2})				*High Power (22.9 Wcm^{-2})*			
	1	*2*	*3*	*Mean*	*1*	*2*	*3*	*Mean*
D0 volume (µl)	*1.12*	*1.04*	*0.85*	*1.00*	*1.70*	*1.50*	*1.29*	*1.50*
D7 volume (µl)	*0.70*	*0.57*	*0.45*	*0.56*	*1.24*	*1.05*	*0.85*	*1.05*
D21 volume (µl)	*0.29*	*0.34*	*0.53*	*0.44*	*0.58*	*0.37*	*0.46*	*0.46*
D30 volume (µl)	*0.27*	*0.18*	*0.35*	*0.26*	*0.55*	*0.28*	*0.46*	*0.46*
D 40 volume (µl)	*0.38*	*0.35*	*0.11*	*0.29*	*0.31*	*0.48*	*0.15*	*0.31*
D60 volume (µl)	*0.06*	*0.13*	*0.29*	*0.13*	*0.18*	*0.22*	*0.14*	*0.18*

Table B3: Volume of solder remaining (µl)

Appendix C

Porcine Splenic Artery Burst Pressure Testing Equipment

The standard test for the strength testing of the soldered anastomoses in vitro is the burst pressure test. The equipment used to perform these tests is described and illustrated below. The components include :

1) A syringe driver (Fig B.1)
2) Fenestrated needle (Fig B.2)
3) A PC used for data acquisition (Fig B.3)
4) Digital manometer (Fig B.4)
5) The components arranged (Fig B.5)
6) A typical burst pressure profile for a splenic artery (Fig B.6)

Burst Pressure Testing (BPT) procedure

The anastomosis was pressure tested using a syringe driver, pressure transducer (0-30psi) (RS Components, UK) and PC. The needle and vessel were mounted between the transducer and the syringe pump, and the PC set to acquire data. A digital manometer was also used to corroborate the results. The vessel was observed for signs of leakage and the maximum pressure was recorded and plotted. Side branches occasionally leaked, and these were occluded by ligation.

Fig B.1 Syringe driver

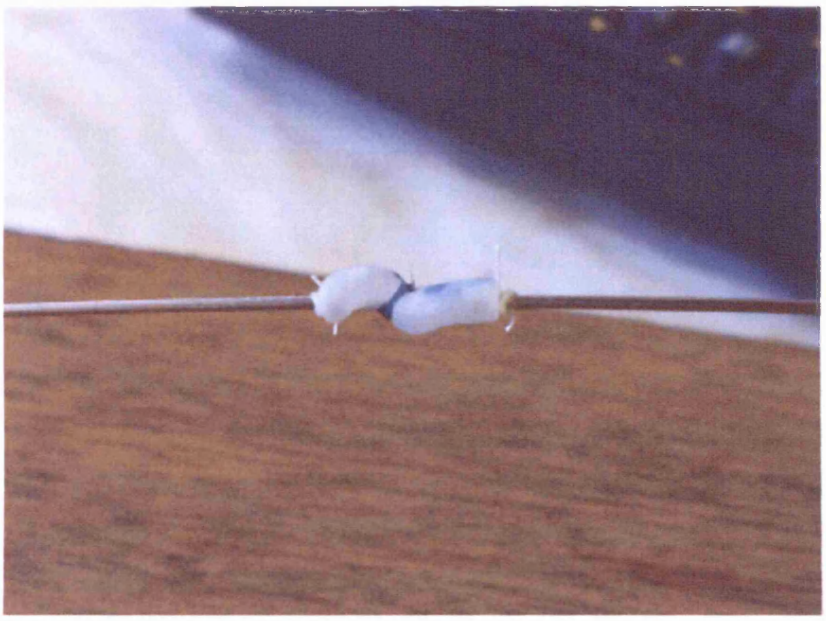


Fig B.2 Fenestrated needle and splenic artery

Fig B.3 A PC used for Data acquisition

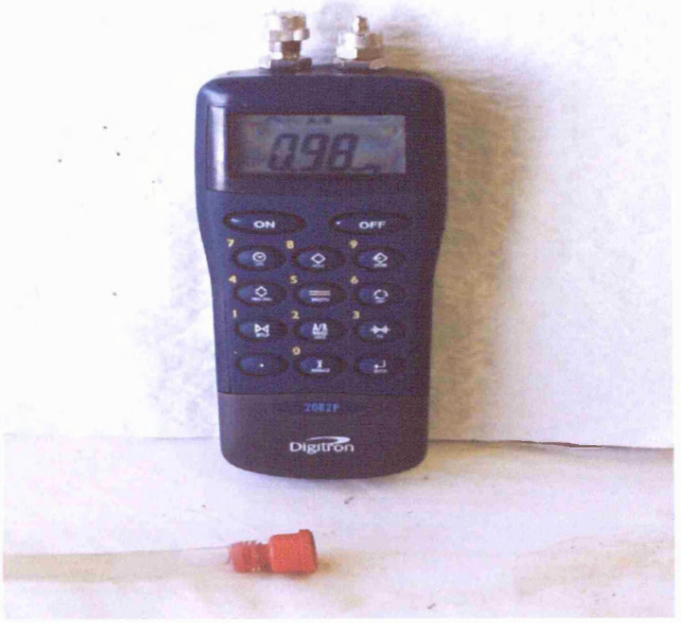


Fig B.4 Digital Manometer

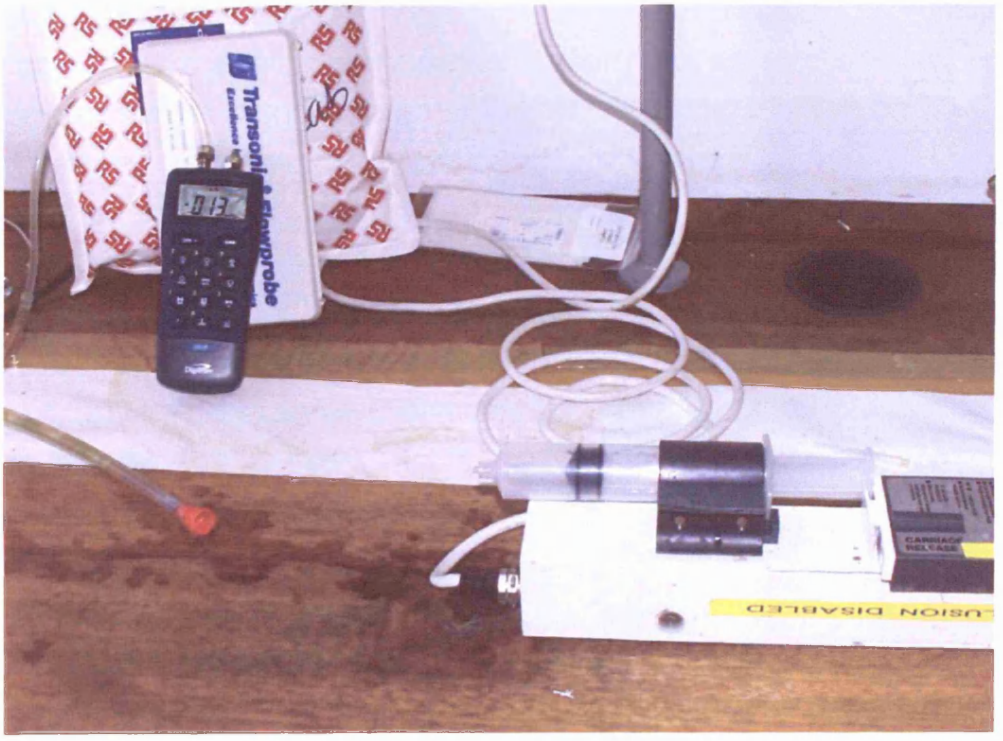


Fig B.5 The components arranged

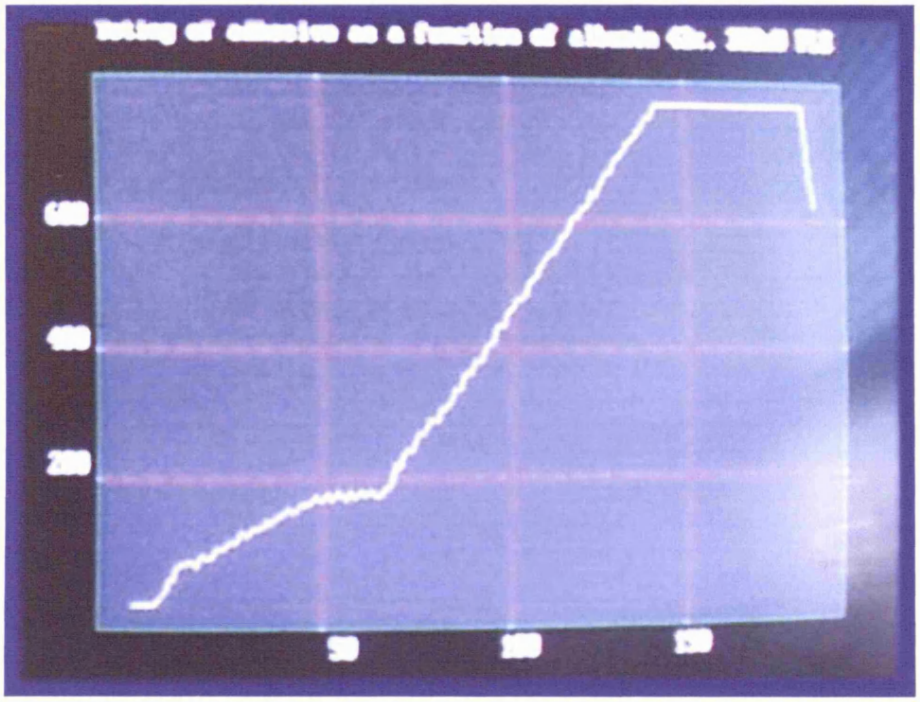


Fig B.6 A typical burst pressure profile for a splenic artery

FigB.7 Coherent power meter

Appendix D
Compliance data

Distance from anastomosis (mm)	SCS	SCS
-11	25	7.2
-8	24	6.3
-5	38	11.8
-2	31	10
0	6.9	1.8
2	27	7.2
5	37	12.7
8	23	8.1
11	22	7.2

Table D1; Compliance data (+/- sem) for SCS

Distance from anastomosis (mm)	Solder	Solder
-11	28	8.1
-8	27	7.7
-5	35	9.1
-2	30	9.5
0	17.6	6.3
2	29	8.6
5	33	11.3
8	28	10.4
11	26	11.8

Table D2; Compliance data (+/- sem) for soldered anastomosis

Distance from anastomosis (mm)	SCS+Solder	SCS+Solder
-11	24	5.4
-8	25	4.5
-5	41	11.3
-2	38	10.4
0	5.3	0.9
2	19	5.9
5	31	9
8	36	9
11	20	8.1

Table D3; Compliance data (+/- sem) for SCS + solder

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