Improving the assessment and outcome of free tissue transfer breast reconstruction

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Abstract

Introduction: Free tissue transfer using an abdominal tissue flap is a commonly used method of breast reconstruction. However, there are well recognised complications including venous congestion, fat necrosis and flap loss associated with the perfusion of these flaps. Post-operative aesthetic outcome assessment of such breast reconstructions have also proven to be difficult with current methods displaying poor inter-rater reliability and patient correlation. The aim of this research was to investigate potential improvements to the post-operative outcome of free abdominal tissue transfer breast reconstruction by assessing the effects of vascular augmentation interventions on flap perfusion and to assess the use of real-time digital video as a post-operative assessment tool.

Methods: An in-vivo pilot study carried out on 12 patients undergoing DIEP flap breast reconstruction assessed the effect on Zone IV perfusion, using LDI and ICG angiography, of vascular augmentation of the flap using the contralateral SIEA and SIEV. A further animal experimental study was carried out on 12 Sprague Dawley rats to assess the effects on main pedicle arterial blood flow and on Zone I and Zone IV perfusion of vascular augmentation of the abdominal flap using the contralateral vascular system. A separate post-operative assessment study was undertaken on 35 breast reconstruction patients who evaluated their own reconstructions via patient questionnaire and underwent photograph and real-time digital video capture of their reconstructions with subsequent panel assessment.

Results: Our results showed that combined vascular augmentation of DIEP flaps, using both the SIEA and SIEV together, led to an increase in Zone IV perfusion. Vascular augmentation of the rat abdominal flaps also led to a significant increase in Zone I/IV perfusion, but the augmentation procedure resulted in a decreased main pedicle arterial blood flow. Our post-operative assessment study revealed that real-time digital video footage led to greater inter-rater agreement with regards to cosmesis and shape than photography and also correlated more with patient self-assessment.

Conclusion: Vascular augmentation of abdominal free tissue flaps using the contralateral vascular system results in an increase to Zone IV perfusion, however this may lead to decreased main pedicle arterial blood flow. Real-time digital video is a valid post-operative aesthetic assessment method of breast reconstruction outcome and is superior to static photography when coupled with panel assessment.

Table of Contents

List of Tables

List of Figures

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Author's Declaration

I declare that this work has been written entirely by myself and is a record of research performed by myself. This work has not been submitted previously for a higher degree and was carried out under the under the supervision of Mr Iain R Mackay and Professor Paul Horgan.

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(Adam Gilmour)

Definitions/Abbreviations

ANOVA	Analysis of Variance
ALD	Autologous Latissimus Dorsi
ASA	American Society of Anaesthesiologists
CT	Computed Tomography
DIE	Deep Inferior Epigastric
DIEA	Deep Inferior Epigastric Artery
DIEP	Deep Inferior Epigastric Perforator
DIEV	Deep Inferior Epigastric Vein
DSE	Deep Superior Epigastric
DSEA	Deep Superior Epigastric Artery
DSEV	Deep Superior Epigastric Vein
IcG	Indocyanine Green
LDF	Laser Doppler Flowmetry
LDI	Laser Doppler Imaging
PROM	Patient Reported Outcome Measure
SIE	Superficial Inferior Epigastric
SIEA	Superficial Inferior Epigastric Artery
SIEV	Superficial Inferior Epigastric Vein
TD	Thoracodorsal
TRAM	Transverse Rectus Abdominus Myocutaneous

Awards, Presentations & Publications arising from work contributing to Thesis

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Publications

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- Gilmour A; Mackay IR. Bilateral Deep Inferior Epigastric Perforator Flaps based on unilateral perforators. J Plast Reconstr Aesthet Surg. 2013; 66: 848-850

Chapter 1: Introduction

1.1 Microvascular surgery and Free Tissue Transfer

The term "microvascular surgery" (microsurgery) was first devised in 1960 by Jules Jacobson when he published his laboratory based results of using the aid of an operative microscope and the techniques familiar to him as a vascular surgeon to anastomose small blood vessels with diameters less than 4mm[1]. Prior to his experimental work, the operating microscope was only routinely used by surgeons in the field of otolaryngology and neurosurgery to aid in middle ear and temporal bone surgery.

The potential use of such techniques became apparent to surgeons worldwide and the field of microvascular research grew. A few years later in 1963, Kleinert and Kasdan published their success in re-vascularising a partially amputated finger using microvascular surgical techniques[2] and later in 1965 Komatsu and Tamai successfully replanted a completely amputated thumb, publishing their results in 1968[3]. During the same time period, Dr Harry Buncke began undertaking his own animal based research into microvascular surgery and the potential applications. He went on to perform the first successful ear re-implantation in a rabbit, using vessels as small as 1mm in 1964[4] and later the first toe to hand transplantation in a monkey in 1966[5]. His research and pioneering advances continued and in 1969, in collaboration with Captain Donald McLean, he went on to perform the first successful microvascular transplant in a human, transferring omentum to reconstruct a scalp defect; albeit not publishing the results until 1972[6]. Later in 1973 Taylor and Daniel, using a modification of the groin flap, described by McGregor the previous year[7]; raised it as a completely free variant, transplanting it through microsurgery to the leg of a trauma victim. They published their findings naming this type of composite tissue transfer a "free flap"; a term which has become synonymous with all types of microvascular free tissue transfer since[8].

The introduction of microvascular free tissue transfer (free flap) completely revolutionised the concept of reconstruction in plastic surgery. Prior to the advent of this type of procedure, plastic surgeons had to rely on donor sites in close proximity to the defect or upon multiple cumbersome delay or transfer type procedures. Through the concept of free tissue transfer virtually any part of the body with a suitable vascular supply could become a donor site leading to a huge potential selection in terms of the reconstructive surgeons' arsenal.

1.2 Skin and Fat Blood Supply

An in-depth understanding of the blood supply to the tissue being considered as a donor site is essential for any successful flap raising and transfer.

The skin and subcutaneous fat receives a rich blood supply from perforating cutaneous blood vessels. They can be either arterial or venous and vary in size, configuration and geometry but all can be traced back to supplying "source" vessels[9].

The route which these perforating vessels travel, prior to penetrating the deep fascia after originating from their source vessel to reach the subcutaneous fat and skin varies. As the popularity of basing flaps upon isolated perforating blood vessels has increased, there has been a number of conflicting descriptions of different types of perforators based upon their geometry published in the medical literature. In an effort to address this issue, and provide a simple acceptable classification system for the plastic surgery community to utilise, the "Gent" consensus was published in 2003[10]. This consensus suggested that perforators could be divided into the 5 following types based upon their surgical importance in terms of dissection:

1. Direct perforators which perforate the deep fascia only.

2. Indirect muscle perforators which predominantly supply the subcutaneous tissues.

3. Indirect muscle perforators which predominantly supply the muscle but have secondary branches to the subcutaneous tissues.

4. Indirect perimysial perforators which travel within the perimysium between muscle fibres before piercing the deep fascia.

5. Indirect septal perforators which travel through the intermuscular septum before piercing the deep fascia.

This is shown in Figure 1.1

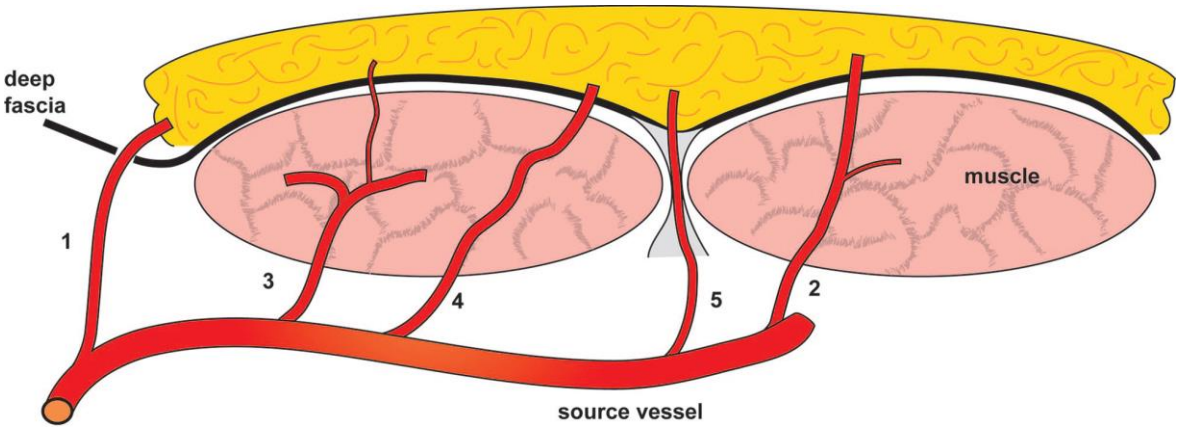

Figure 1.1: "Gent" Consensus on perforator terminology
1, Direct perforators perforate the deep fascia only; 2, indirect muscle perforators predominantly supply the subcutaneous tissues; 3, indirect muscle perforators predominantly supply the muscle but have secondary branches to the subcutaneous tissues; 4, indirect perimysial perforators travel within the perimysium between muscle fibers before piercing the deep fascia; 5, indirect septal perforators travel through the intermuscular septum before piercing the deep fascia. Image reproduced with permission from Wolters Kluwer Health, Inc. (Blondeel P, Van Landuyt K, Monstrey S, et al 2003)[10]

Perforator geometry has implications with regards to perforator flap raising as direct perforators and indirect septal perforators tend to be much easier to dissect in comparison to perforators which have an intramuscular course, which often require a tedious and technically demanding dissection through muscle to reach their vessel of origin.

Regardless of which type of perforator, upon piercing the deep fascia the perforating vessels will course through the superficial tissues often branching widely, forming interconnections with other perforating vessels especially as the skin is approached.

1.2.1 Cutaneous Territories, Angiosomes and Venosomes

Anatomical knowledge of cutaneous perforating blood vessels in terms of their origin, their course through the body and their vascular territory (surface area of tissue which they perfuse) is paramount to successful flap raising in plastic surgery. There have been a number of pioneers involved in furthering this knowledge over the past 125 years.

Carl Manchot, a German anatomist, published his cadaveric study on the cutaneous arterial supply to the body in 1889. In this study he made extremely detailed drawings of the arterial blood supply to the skin (albeit through mysterious methodology) linking perforating blood vessels back their source which remain accurate to this day[11].

Further advancements came in the 1930s when Michel Salmon (a French anatomist and surgeon) revisited the previous work of Manchot and with the aid of radio-opaque dye and

x-rays (not available to Manchot at the time) made even more detailed anatomical drawings of the various cutaneous territories of the body, whilst also including the territories of the hands, feet, head and neck. He also went on to describe the various interconnections between perforators to the skin and those occurring within muscles. The significance of these highly detailed and influential vascular maps, provided by Manchot and Salmon, were not however recognised at the time, perhaps because they were both only published in the author's native language of German and French respectively[12].

Coinciding with the emergence and growing popularity of free tissue transfer in the early to mid-seventies; an effort to map potential donor sites in the body was undertaken by Taylor and Palmer. They re-visited the works of Manchot and Salmon and undertook cadaveric injection, dissection and radiographic studies. Whilst the work of Manchot and Salmon was confined to the cutaneous vascular territory, Taylor and Palmer significantly advanced this knowledge by offering a three-dimensional model of tissue perfusion matching each source artery (and its accompanying vein) not only to the area of skin they perfused but also their perfusion of the underlying deeper tissues. These three-dimensional composite anatomical blocks of tissue were defined as angiosomes and the angiosomes of the body are shown in Figure 1.2[13].

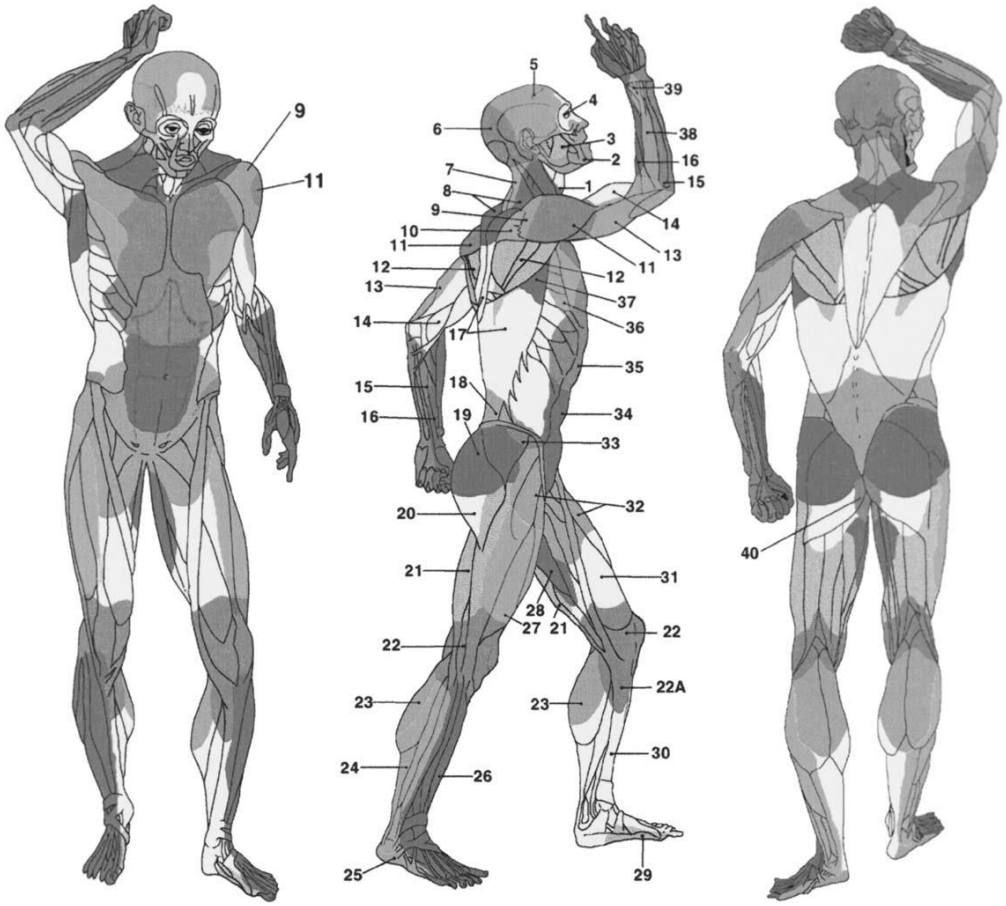

Figure 1.2: The angiosomes of the body
Image reproduced with permission from Elsevier Ltd. (Taylor and Palmer 1987)[13]

This work was initially only studying the arterial supply (arteriosomes) of the body, however, further research by the same team demonstrated that the venous supply (venosomes) closely matched the arterial supply, further corroborating the angiosome concept[14,15]. As such each angiosome can be considered as consisting of a paired arteriosome and venosome.

At the border of each angiosome they also identified a collateral arterial supply provided by smaller calibre anastomotic connections between perforating blood vessels which link adjacent angiosomes at all levels throughout the body. These connections were referred to as “true” or “choke” anastomotic vessels. These interconnections linked through the capillary bed mean that essentially every vessel in the body is connected in an unbroken network. A similar picture was found in the venous architecture to compliment these “choke” arterial anastomoses in the form of oscillating avalvular veins which connect the valved venous systems to each other and allow bi-directional flow. Upon raising a flap, the choke vessels linking the adjacent angiosome to the source vessel will dilate and it is usually feasible that the choke vessel increase will allow immediately adjacent angiosomes to be perfused safely, however, those out-with this area are subject to tissue necrosis[13,16]. A

similar process occurs in the venous architecture allowing additional venous draining of adjacent angiosomes through the linking avalvular oscillating veins. A schematic diagram showing closed and open choke vessels is shown in Figure 1.3.

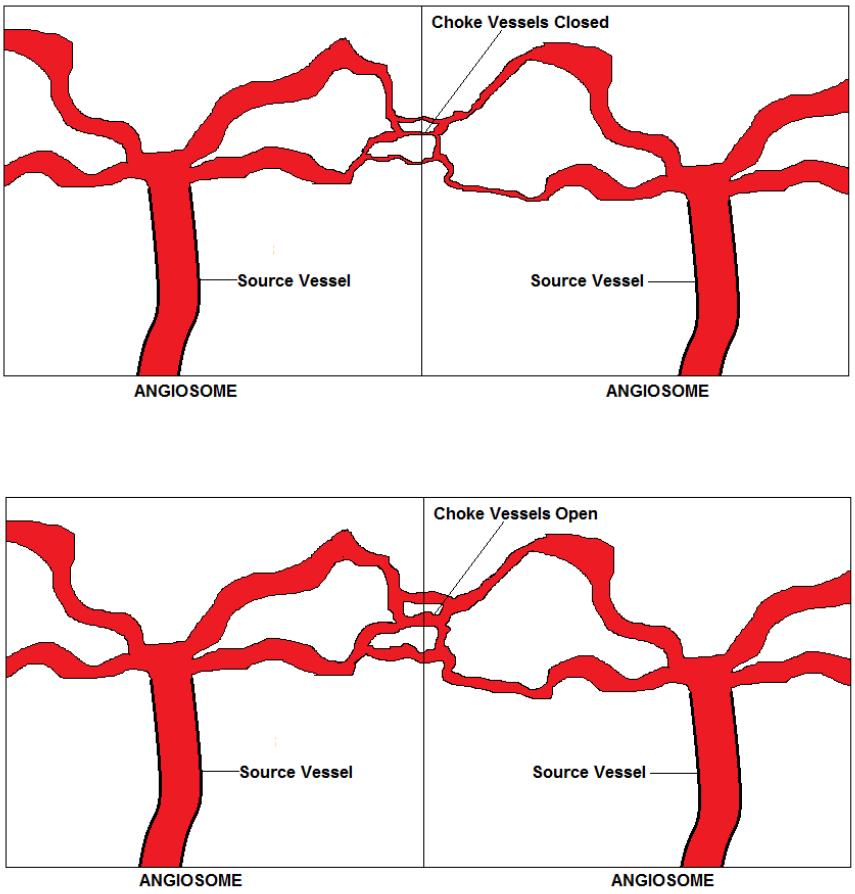

Figure 1.3: Diagrammatic representation of closed and open choke vessels

The maximum increase in choke vessel diameter is within the first 48-72 hours and is complete by day 7[17]. This increase in vessel diameter is permanent and irreversible[18]. The proposed trigger mechanism for change in choke vessel calibre is thought to be due to tissue hypoxia[12]. These findings essentially explain why the traditional delay type procedure (incising the borders of the proposed skin flap in a staged fashion rather than raising the flap in a single stage) worked; as by staging the flap raising it allowed the opening of adjacent choke vessels and improved perfusion to other areas of the flap.

The knowledge of three-dimensional vascular territories provided by Taylor and Palmer essentially offer the reconstructive surgeon a map through which they can plan the type of flap they require based upon reconstruction aims in terms of size, volume, constituents and pedicle length.

1.2.2 Perforasomes

Further advances in the understanding of tissue perfusion in perforator flaps have been that of the Perforasome concept. Saint-Cyr et al initially described the use of contrast enhanced CT angiography coupled with cadaveric dissection to aid in the identification of the vascular supply to perforator flaps[19]. Building upon this initial work, they went on to describe the concept of a perforasome as the three-dimensional area of tissue (vascular territory) perfused by a single arterial perforator, rather than the global area of tissue supplied by all the perforating vessels of a source vessel, as described by the angiosome theory[20]. (See Figure 1.4)

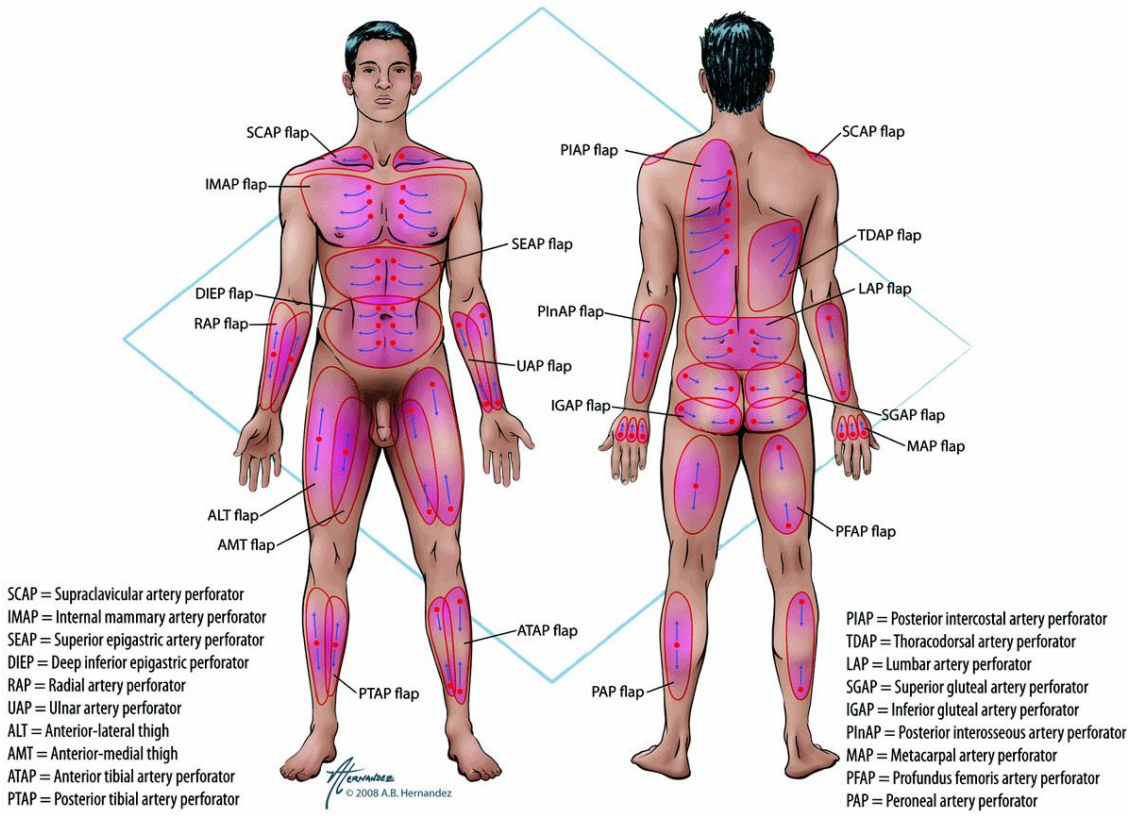

Figure 1.4: Common perforasomes of the body
Image reproduced with permission from Wolters Kluwer Health, Inc. (Saint-Cyr et al. 2009)[20]

They also identified that a perforasome was linked to each adjacent perforasome via direct linking vessels or indirect linking vessels; in a similar fashion to that of true and choke vessels as described by Taylor et al[13,16]. The direct linking vessels are large vessels which anastomose directly between perforators and can allow for capturing of multiple adjacent perforasomes providing the flow/filling pressure through the original perforator is high. The indirect linking vessels link adjacent perforasomes via their supply to the subdermal plexus and recurrent flow[20]. (See Figure 1.5)

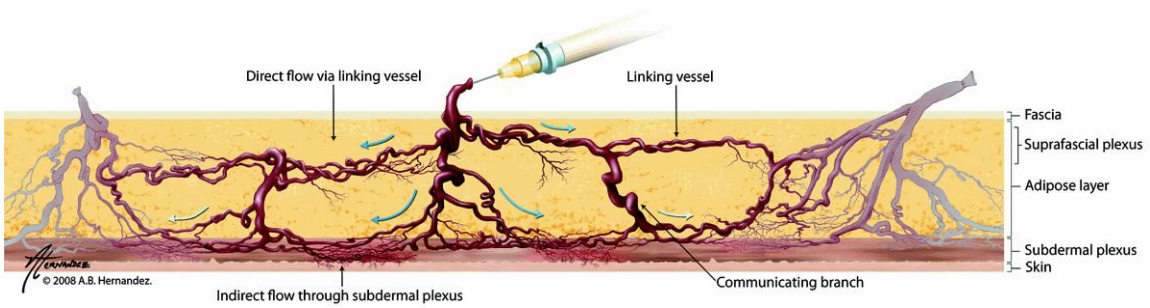

Figure 1.5: Linking of adjacent perforasomes via direct and indirect vessels
Image reproduced with permission from Wolters Kluwer Health, Inc. (Saint-Cyr et al. 2009)[20]

Taylor accepted the concept of the perforasome and believed that each perforasome territorial unit would link together to form the same overall angiosome territory from the source artery of the perforators[21]. (See Figure 1.6)

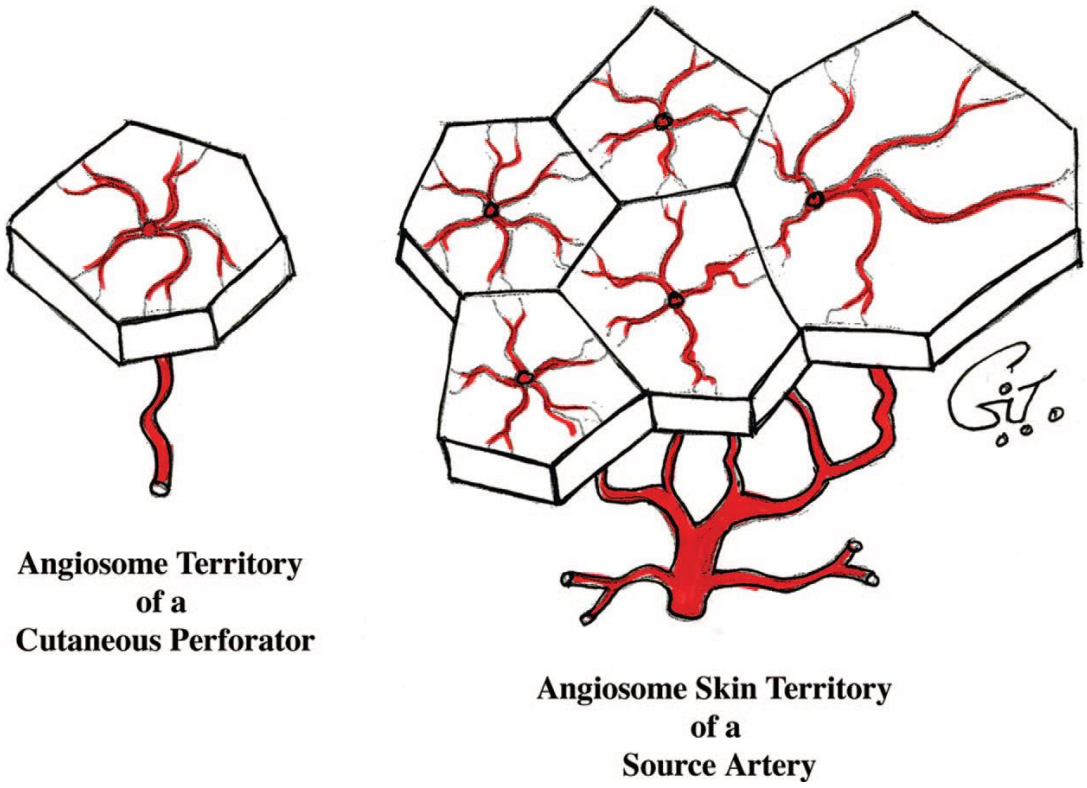

Figure 1.6: Schematic diagram showing territory of a solitary perforator (left) and perforators from a single source artery combined to show overall cutaneous territory (right)
Image reproduced with permission from Wolters Kluwer Health, Inc. (Taylor et al. 2011) [21]

Taylors' original work on angiosomes proved revolutionary to reconstructive surgeons in identifying potential donor sites to be used for free tissue transfer. With the current trend towards performing perforator based flaps; the perforasome concept may prove similar in allowing the identification of suitable donor site areas for perforator based flaps.

1.2.3 Perforator flap perfusion physiology

Perfusion refers to the blood flow within specific organs or tissues, such as the skin and fat of perforator based flaps. In normal physiological circumstances this should be a dynamic process with the events following a continuous circuit.

Within a healthy perforator flap blood flow is characterised by high pressure input/supply via arterial perforating vessels, subsequent microcirculation within the capillary bed and low pressure output/drainage via the perforating veins.

In order to understand the perfusion within perforator flaps the basic physiological principles of flow most be comprehended.

In its most simplistic form flow can be calculated using the following equation:

$$Flow\ Rate\ (mL/min)\ =\ \frac{Effective\ Perfusion\ Pressure\ (mmHg)}{Resistance\ (mmHg\ \text{x}\ (\text{min/mL}))}$$

Or

$$Q = \frac{\Delta P}{R}$$

Where the effective perfusion pressure (ΔP) is the mean intraluminal pressure at the arterial end minus the mean pressure at the venous end (i.e. pressure difference) of the vascular circuit[22].

Resistance to blood flow through vessels; however is a complex process dependent upon multiple factors. Blood vessels are essentially cylindrical in nature and Poiseuille described various factors known to influence the resistance to flow through a cylindrical tube. These factors include the radius within the cylinder, the length of the cylinder; viscosity of the fluid within the cylinder and the inherent resistance to the flow.

Incorporating these factors resistance (R) within blood vessels can be calculated using the following equation:

$$R = \frac{8L\eta}{\pi r^4}$$

where L = vessel length, η = viscosity of blood, and *r* = radius of the vessel lumen.

By combining both equations into a single expression (commonly known as the Hagen-Poiseuille Equation) it allows the calculation of blood flow:

$$Q = \frac{\Delta P \pi r^4}{8L\eta}$$

Where Q = Flow; ΔP = effective perfusion pressure; L = vessel length, η = viscosity of blood, and *r* = radius of the vessel lumen.

In order to ensure adequate perfusion and thus perforator flap survivability each component of the above equation has to be addressed. Effective perfusion pressure is crucial and the successful completion of the microcirculatory process (delivering of oxygen/nutrients to the tissues and removal of waste products) within the capillary bed is dependent upon the existence of the high pressure (at arterial end) to low pressure (at venous end) gradient and is essential for tissue survival. Failure of this process will lead to tissue ischaemia and ultimately necrosis. Inadequate arterial supply will lead to tissue hypoxia and ischaemia whereas inadequate venous drainage in the presence of an appropriate arterial supply will result in an increase in venous resistance, loss of the pressure gradient and failure of the microcirculatory process.

In general patients undergoing perforator flap surgery should be optimised peri and post operatively ensuring that their blood pressure is adequate (to maintain a good effective perfusion pressure) and they are kept well hydrated (to prevent an unwanted increase in blood viscosity). With regards to the vasculature supplying perforator flaps it is apparent that the radius of the supplying blood vessels is incredibly important and changes in this factor will have huge influences on overall perfusion (i.e an approximate increase in vessel radius of 19% will double blood flow). As such efforts must be taken during perforator flap surgery to ensure that flaps are based on vessels of adequate calibre and that these vessels are free from spasm or external compression.

1.3 Breast Reconstruction

The ideal goal of breast reconstruction surgery is to replace the excised breast tissue with something similar in terms of size, shape and texture which can best achieve symmetry with the contralateral breast or act as a substitute to that lost in the case of bilateral mastectomies[23].

It has been shown that breast reconstruction after mastectomy leads to an improvement in patients' self-esteem, quality of life and sexuality[24-28].

The types of surgical reconstruction available consist of implant based reconstruction or autologous based reconstruction. Autologous based reconstructions involve using the patients' own tissue to reconstruct the breast. The current options for autologous reconstruction involve regional tissue transfer or free tissue transfer. A relatively new method of autologous reconstruction in the form of free fat transfer (lipomodelling) has also been described as an option to carry out total reconstructions, but is much less utilised in comparison to the other available methods[29]. Instead this technique is often used when the defect is only small; such as after wide local excision or to further augment/revise the other reconstructive techniques by adding more volume or addressing contour irregularities, usually at a later stage[30].

When it is not possible to recreate a symmetrical replacement to the contralateral breast (usually due to volume of implant or tissue required) secondary subsequent procedures, such as symmetrising breast reduction, may be considered on the normal breast.

Where bilateral mastectomies are required, the aim of the reconstruction is to provide bilateral substitutes of similar size and shape to achieve an aesthetically acceptable result for the patient.

1.3.1 Choice of Reconstruction

As with any type of reconstructive procedure the reconstruction should be tailored to each individual patient, encompassing their reconstructive requirements and also their expectations and wishes. As such, each of the various types of reconstruction may have significant indications in one patient, but significant contraindications in another patient.

Dispute, however, remains amongst the plastic surgical forum as to which reconstructive technique provides the best results for patients and some surgeons advocate certain types of reconstruction over others.

Recent evidence amongst larger studies would suggest that patients undergoing autologous tissue breast reconstruction have higher satisfaction rates than those undergoing implant based techniques[24-26,31,32]. Furthermore it may be that those patients undergoing free abdominal tissue breast reconstruction have the highest satisfaction[33] and that these patients are more satisfied with their breast from an aesthetic point of view in the long-term in comparison to those undergoing implant based techniques[24,26].

This thesis will concentrate on autologous free tissue transfer and in particular the Deep Inferior Epigastric Perforator (DIEP) Flap.

1.4 Free Autologous Tissue Breast Reconstruction

1.4.1.1 Donor Site Selection

In terms of donor site selection for reconstructing a defect; the ideal donor site would offer completely the same mix of tissue to that which has been removed, whilst leaving a suitable secondary defect which can be closed primarily or reconstructed, without significant further deformity or insult to the patient. With regards to breast reconstruction, the human female breast consists of primarily adipose (fat) tissue, glandular tissue and skin. The proportion of breast glandular tissue varies throughout a woman's lifetime and undergoes significant atrophy upon aging. It would not be appropriate, in reconstructive terms, to replace the removed glandular tissue with further breast glandular tissue. As such, dependent upon the amount of skin removed at time of mastectomy, the donor site for breast reconstruction would have to supply a suitable amount of soft tissue to replace the removed glandular and adipose tissue with the option of a skin paddle if required.

Adipose tissue is the ideal replacement of composite breast tissue given its consistency, rich vascular supply, texture and abundance within the human body. As with any type of surgery, patient selection for each type of procedure is key and different patients will have different volumes of adipose tissue in different areas of the body. There are a variety of flap donor sites which can be utilised for breast reconstruction, ranging from regional flap transfer options in the abdomen and back; to free tissue transfer options from the abdomen, back, buttocks and thighs which in general offer good volumes of adipose tissue, option of

a skin paddle and a suitable blood supply for breast reconstruction. In some of the sites muscle tissue also has to be taken to ensure blood supply or increase volume of tissue available. Out of all the sites, in the majority of patients, the lower abdomen will offer a good option for free tissue transfer, due to the abundance of skin, subcutaneous fat, reliable blood supply and a defect that can generally be closed easily with acceptable scarring to the patient.

1.5 Abdominal Blood Supply

The abdominal skin and fat receives a rich vascular supply from various sources. These include perforating vessels from the intercostal muscles, the epigastric arcades (superficial / deep superior and inferior epigastric vessels), the superficial (and possibly deep circumflex vessels) and to a smaller degree the superficial pudendal vessels as shown in Figure 1.7.

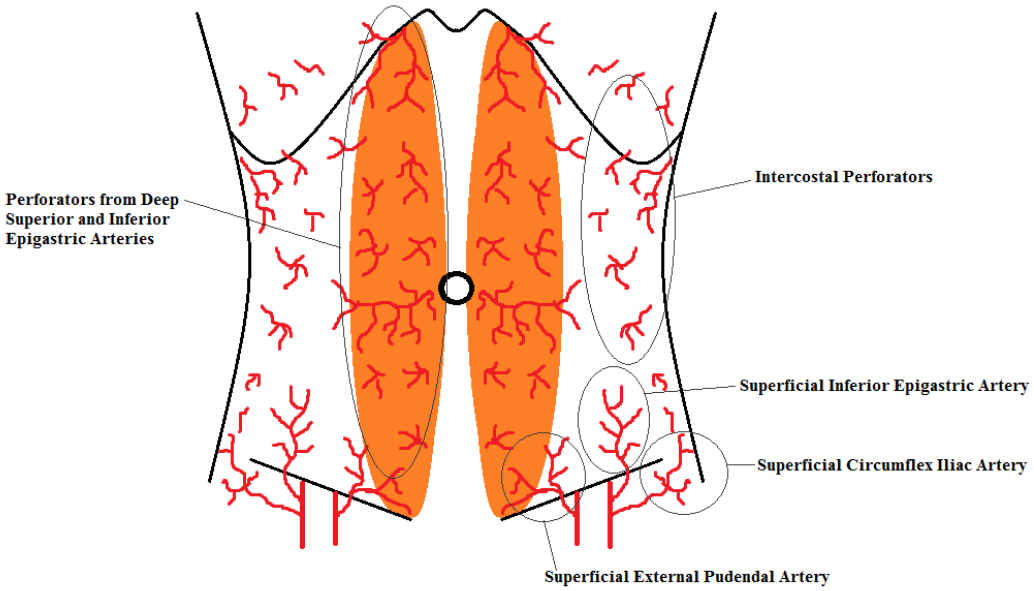

Figure 1.7: Arterial supply to the anterior abdominal skin and fat

Huge anatomical variation exists between individuals as to the presence or absence of these perforating vessels, their location, branching patterns, and their anastomoses.[34]

Constantly, however, the main vascular supply to the anterior abdomen comes from the bilateral epigastric arcades consisting of the superficial and deep superior and inferior

epigastric vessels. Of note the superficial superior epigastric artery and vein are often absent in humans and as such are usually disregarded anatomically.

The deep superior epigastric artery (commonly referred to as the superior epigastric artery) arises from the internal thoracic artery at the level of sixth intercostal space and runs anterior to the transversus thoracis muscle, passing inferiorly through the diaphragmatic origins on the xiphoid process and costal margin remaining anterior to the transversus abdominus muscle. It is initially deep to the rectus abdominus muscle but then perforates the fascia branching diffusely and sending perforating vessels through the muscle to supply it and the overlying soft tissue/skin. It is accompanied by the superficial epigastric vein / venae comitantes which is a continuation of the internal thoracic vein / venae comitantes.

The deep inferior epigastric artery arises from the external iliac artery just superior to the inguinal ligament. It travels through the extra-peritoneal tissue obliquely and in a medial direction along the medial edge of the deep inguinal ring continually passing superiorly to eventually pierce the transversalis fascia and enter the rectus sheath. As with the superior epigastric artery at this level it branches widely and sends perforating branches through the muscle to supply both it and the overlying soft tissue/skin. The artery is accompanied by the deep inferior epigastric vein / venae comitantes which drains into the external iliac vein.

The superficial inferior epigastric artery usually arises from the common femoral artery 2-5cm inferior to the inguinal ligament as part of a shared trunk with the superficial circumflex iliac artery; however other originating patterns can occur. It initially is deep to the cribriform fascia, but as it approaches the inguinal ligament it penetrates through and runs superiorly in the subcutaneous tissues. It is accompanied by venae comitantes, which drain into the femoral vein, and also the superficial inferior epigastric vein, which runs medial to the artery and drains into the saphenous bulb.

The main vascular supply to the anterior abdomen comes from the superior and deep inferior epigastric system. Both these systems have diffuse anastomotic connections with each other cranial to the level of the umbilicus at their terminal branches. It has been shown that the deep inferior epigastric arterial system is the more dominant; providing a more significant arterial blood supply to the anterior abdominal skin[35]. The angiosome supplied by the Deep Inferior Epigastric Artery (DIEA) can be seen in Figure 1.8.

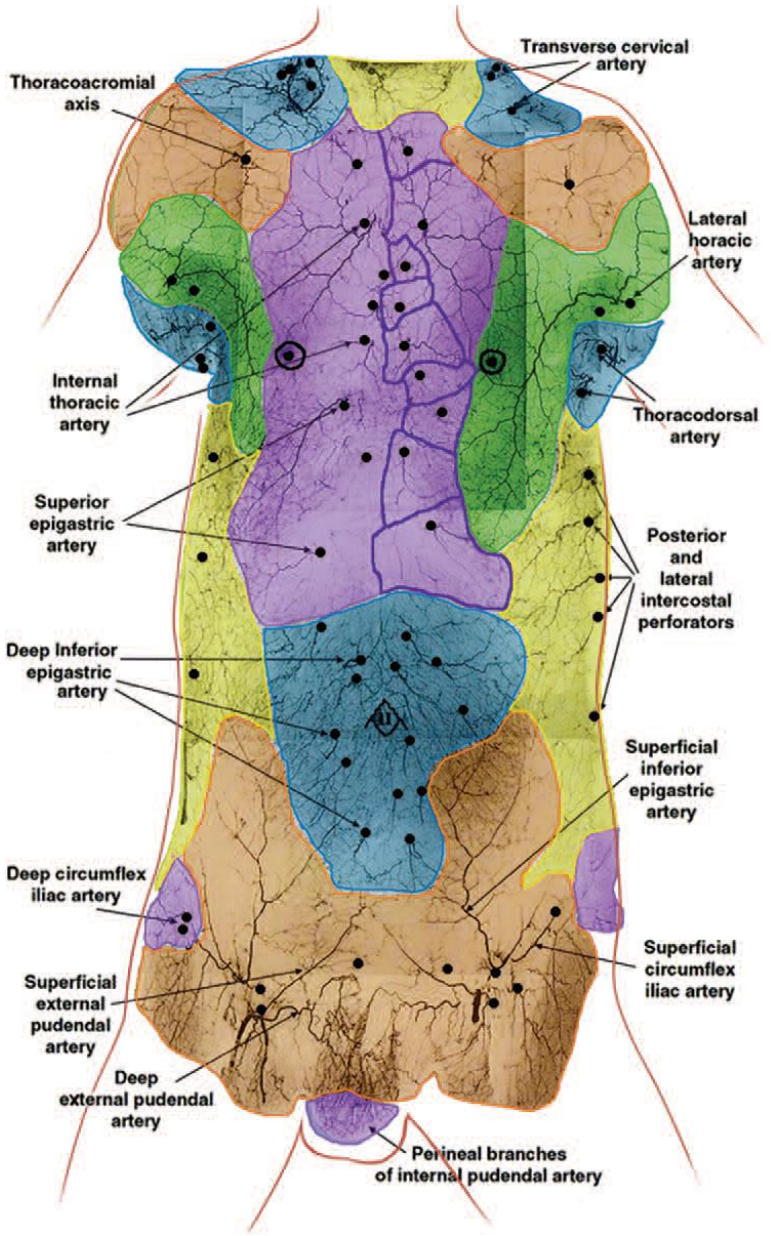

Figure 1.8: Human angiosome map showing area supplied by DIEA
Image reproduced with permission from Wolters Kluwer Health, Inc. (Taylor et al. 2011)[21]

The number of main DIEA trunks can vary (between one and four) but they consistently gives rise to a medial row and a lateral row of perforators through the deep fascia to supply the anterior abdominal tissue[36]. (See Figure 1.9)

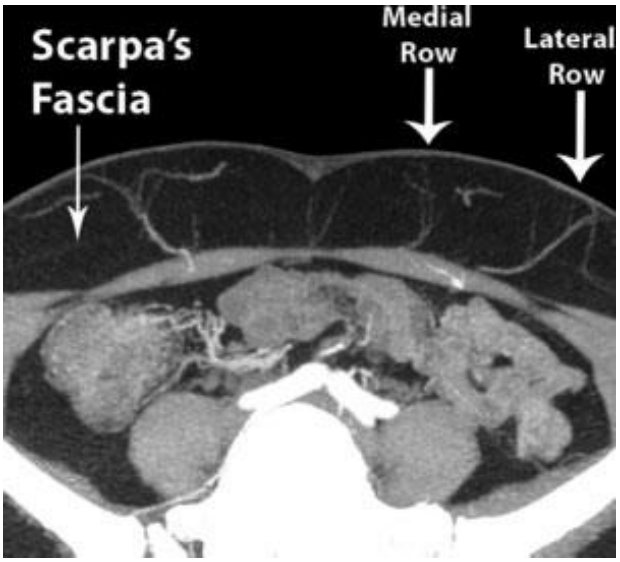

Figure 1.9: CT angiogram showing medial and lateral rows of the DIEA
Image reproduced with permission from John Wiley and Sons. (Rozen et al. 2011)[36]

The largest perforating vessels from the DIEA medial and lateral rows are located at its terminal branches 3-5cm around the umbilicus[37] . The perforating vessels will take a direct course through the deep layer of adipose tissue prior to reaching Scarpa's fascia where they then branch on the superficial surface of Scarpa's fascia or in the subdermal plexus[38].

As well as anastomosing with the superior epigastric vessels these para-umbilical perforating branches also show widespread anastomoses with other perforating branches of the deep inferior epigastric, superficial inferior epigastric, superficial circumflex iliac and intercostal arteries[37].

The connections between these cutaneous perforating vessels are through either "true" or "choke" (cf. 1.2.1). True anastomoses are those where the calibre of joining vessel remains the same, in comparison to choke anastomoses whereby the calibre of the joining vessel is smaller, but has the potential to dilate and improve vascular flow[12,13,16].

In contrast to the dominant arterial supply to the lower abdominal tissue arising from the DIEA, the dominant venous drainage usually occurs via the superficial venous system, in particular the SIEV[39,40]. Deep branches from the SIEV arise throughout its course (particularly in the peri-umbilical area) and these branches drain into the venae comitantes of the DIEA perforators[41].

1.6 Abdominal Tissue Transfer in Breast Reconstruction

Autologous tissue transfer using abdominal tissue as a donor site has been used for decades. The benefits of this site are the volume of tissue available for harvest, appropriate colour match (when skin paddle required); good tissue pliability; robust vascular supply and relatively low donor site morbidity.

These abdominal tissue flaps are based upon blood supply from the superior or inferior epigastric vessels. Traditionally these flaps were pedicled based upon the superior epigastric vessels and incorporated the rectus abdominus muscle along with the underlying pedicle. However, as time progressed these flaps have evolved to be routinely based upon the inferior epigastric vessels (the dominant anterior abdominal skin/fat blood supply as discussed above) and rather than being pedicled, are raised as free flap alternatives, which are transferred to the recipient site via microsurgical techniques. Furthermore the need to incorporate the rectus abdominus muscle along with the pedicle during flap raising has declined over time, with many choosing to raise the flap as a perforator based alternative.

1.6.1 TRAM Flap

The first use of a one stage abdominal based chest wall reconstruction was in 1974, when Tai & Hasegawa described the use of a "transverse abdominal flap" consisting of skin and fat from the upper abdomen, based upon the perforating vessels of the superior epigastric artery and vein, to reconstruct the chest wall in 5 patients following radical operations for recurrent breast cancer[42]. However, these flaps were utilised solely for chest wall coverage rather than the recreation of an aesthetic breast mound.

The first published description of breast reconstruction in terms of cosmesis (i.e. creating a breast mound) by using an abdominal tissue based flap actually broke the trend mentioned above in terms of historical evolution. Holmstrom, in May 1979, described an experimental free tissue transfer reconstruction from the lower abdomen based upon the deep inferior epigastric vessels, which he carried out on two patients[43]. He termed this the "free abdominoplasty flap" due to the fact that the overlying skin and fat was essentially what was discarded at the time of an abdominoplasty. At this time, Holmstrom noted the good vascular supply to the flap but commented on the precarious venous drainage, as unfortunately the flap failed in one of these patients secondary to venous thrombosis.

A few months later, in October 1979, Robbins published his technique of using a pedicled rectus abdominus myocutaneous flap to reconstruct a post-mastectomy wound[44]. He used a vertically orientated islanded flap, incorporating tissue from the upper and lower abdomen, on the same side as the mastectomy defect based upon the superior epigastric vessels. Drever[45] and Dinner[46] subsequently went on to publish their experience; techniques and refinements on using a vertically based rectus abdominus myocutaneous type flap for breast reconstruction.

In 1982, Hartrampf et al published their findings on using an ellipse of skin and fat obtained from the lower abdomen for breast reconstruction[47]. This transversely based flap; which they named the horizontal lower rectus abdominus flap (more commonly referred to as the Transverse Rectus Abdominus Myocutaneous (TRAM) flap in current literature) could be harvested based upon the superior epigastric pedicle of either the ipsilateral or contralateral rectus abdominus muscle to the mastectomy defect. They published their success of such a flap in 8 patients and also discussed the successful use of this type of flap in a bilateral breast reconstruction. They commented that this flap had advantages in terms of easily hidden donor site scarring in the supra-pubic area and also that the superior pedicle to the flap was well protected as it did not need to be identified. In this key paper, they also discuss the vertical rectus abdominus musculocutaneous (VRAM) flap as previously described by the authors above[44-46] and provide another transverse alternative (horizontal upper rectus abdominus flap) based upon the upper abdominal tissue, which they reserved for patients with infra-umbilical scars. A diagram showing the various flaps described is shown in Figure 1.10.

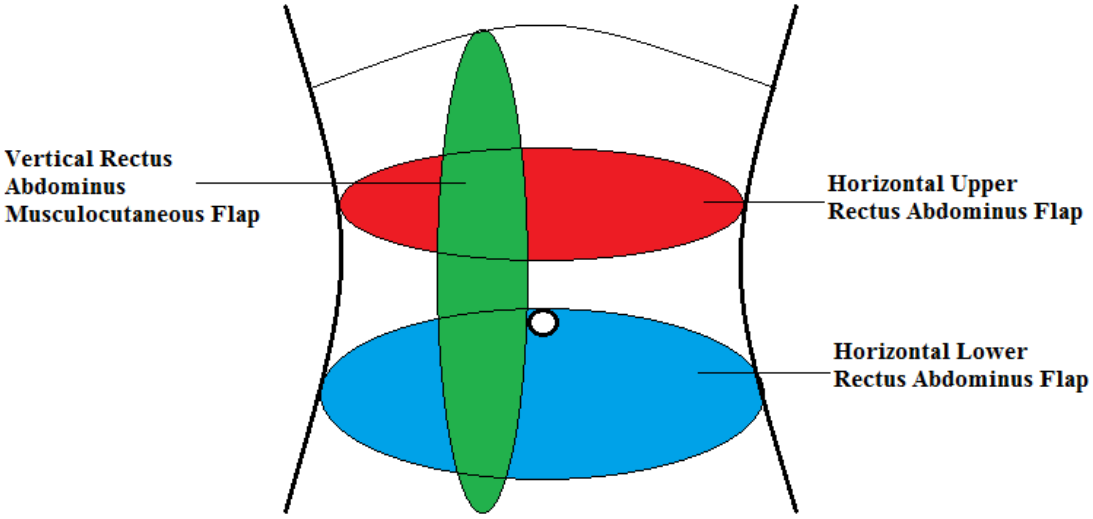

Figure 1.10: Abdominal flaps based on the Rectus Abdominus muscle.
As described by Hartrampf et al. 1982[47]

1.6.2 DIEP Flap

The next major breakthrough in terms of the lower abdominal tissue donor site came in 1989, when Koshima and Soeda, recognising the disadvantages associated with the harvest of the rectus abdominus muscle, described a muscle preserving alternative coining the term "perforator flaps"[48]. They described the use of such a flap in two cases; one as a pedicled flap to reconstruct a groin defect following a malignant lymphoma resection and the other as a free flap used to reconstruct an intra-oral defect following a squamous cell carcinoma excision. It was a natural evolution of the established VRAM/TRAM flap with the benefit that it did not rely upon harvesting a portion of the rectus abdominus muscle. Instead the perforating blood vessels supplying the abdominal tissue could be carefully dissected through the muscle tissue to their vessels of origin (the Deep Inferior Epigastric Artery and Vein) leaving the muscle tissue behind. The flap became known as the DIEP flap secondary to the vessels which supplied it. They presumed at the time of publication that this flap overcame the problems of post-operative herniation and additional bulkiness associated with the rectus abdominus myocutaneous flap, whilst still having the benefit of a similar area of vascular perfusion as the TRAM flap. However, they did point out that this flap could be technically demanding in terms of the perforator dissection and also the variability in terms of perforator size and location.

The first published description of the use of the DIEP flap in breast reconstruction was in 1994 by Allen and Treece[49]. Recognising the benefits that such a flap could provide in terms of the large volume of tissue available, they published their experience of using a free DIEP flap for breast reconstruction in 15 patients. Over the subsequent years the flap gained popularity worldwide with others, including Blondeel and Hamdi et al publishing their experiences of utilising the flap to provide a successful breast reconstruction[50,51].

Since then the DIEP flap has become a workhorse flap for breast reconstruction with many authors publishing their results in large series.

1.6.2.1 TRAM Flap vs DIEP Flap

The choice of a TRAM or DIEP flap is controversial amongst many surgeons. Modifications to the TRAM flap since its conception have been to adopt a muscle/rectus fascia sparing approach during flap raising, this involves harvesting only a small portion of the rectus muscle and fascia around the perforating blood vessels rather than harvesting the complete section of muscle. Advocates of this technique feel that it is at least comparable, if not better, to the DIEP flap, since only a small portion of the muscle is removed with or without a small portion of fascia then the recovery of the patient is likely to be better with less chance of herniation whilst foregoing the time-consuming technical difficulty of intramuscular pedicle dissection.

The literature varies greatly on the comparison between free TRAM and free DIEP flap patients' donor site morbidity. Despite the preservation of muscle tissue, as occurs in DIEP flap raising, there is still the potential complication of donor site abdominal bulging to occur, without any evidence of herniation. The rate of abdominal bulging post DIEP flap transfer quoted varies in the literature between 0.7-5%[52-54]. Nahabedian et al in 2005 attempted to ascertain whether risk factors such as previous abdominal surgery, diabetes mellitus, age, bilateral reconstructions, smoking, previous childbirth, plication or mesh use were associated with increased rates of abdominal bulging but the results were not statistically significant[55]. They did, however, find that previous pregnancy may have a slightly protective mechanism to reduce the rate of abdominal bulging[55]. Blondeel et al, found that those patients having breast reconstructions with free TRAM flaps had a weaker abdominal wall and higher rate of abdominal bulging, in comparison to those having free DIEP flap breast reconstructions[52]. This is in direct contrast to other authors who have found no statistically significant difference between TRAM or DIEP flaps with regards to

hernia or abdominal bulging[55-58]. Kroll also identified that it appears that DIEP flap patients have a lower requirement for post-operative opiate analgesia and thus DIEP flap donor sites may be less painful than those of TRAM donor sites[59]. A meta-analysis in 2009 concluded, that patients with TRAM flap donor sites have a higher rate of abdominal wall morbidity, in comparison to those with DIEP flap donor sites[60].

Another significant difference between the free TRAM and free DIEP flap is in terms of their venous drainage. The classical DIEP flap venous drainage relies solely upon the perforating (usually one or two) veins it has been raised upon. In contrast the free TRAM flap, which is raised as a composite block including muscle rather than dissecting the perforators free to their point of origin, will have a more abundant venous drainage system as there are likely to be more venous perforators within the block of harvested tissue. As such, venous congestion complications of free TRAM flaps are much rarer than those experienced in free DIEP flaps[61].

With regards to flap related complications, it appears that the free TRAM is more robust than that of the free DIEP flap and this may be due to the differences in their blood supply, as discussed above. It has been well documented in some studies that there is a significantly higher incidence of major flap complications in those patients undergoing DIEP flap breast reconstruction[57,58]. The same meta-analysis from 2009 also concluded that there is a higher rate of flap related complications (such as fat necrosis or partial flap loss) in those patients undergoing free DIEP flap surgery in comparison to free TRAM flaps[60].

1.6.3 SIEA Flap

Another lower abdominal flap which can be used for breast reconstruction is that based upon the superficial inferior epigastric artery (SIEA). This flap was first described in breast reconstruction by Grotting in 1991[62] and, much like the DIEP, many others have since advocated its use believing that it is the best option in terms of free lower abdominal tissue flap for breast reconstruction[63-65]. The benefit of the SIEA flap is that it requires no intrusion into the rectus sheath and injury to the rectus abdominus muscle. However, the anatomy with regards to the pedicle of this flap is extremely variable between patients and studies have shown that in many patients the artery is unsuitable for microvascular transfer or even completely absent (quoted absence between 13-51% and unsuitability between 13-70% dependent upon the literature)[65-67]. Many surgeons will routinely assess the

favourability of the SIEA flap during lower abdominal tissue harvest and if suitable proceed with this flap rather than a TRAM/DIEP flap due to the ease of harvesting, excellent donor site and rapid recovery of patients post-operatively[65,67]. A proposed algorithm based on their experience of 99 SIEA flap reconstruction by Speigel and Khan suggests only proceeding with SIEA flap reconstruction if the artery diameter is >1.5mm[68]. However it has been reported that SIEA flaps have a much greater risk of flap related problems in terms of necrosis, arterial complications, need for re-exploration and failure rate in comparison to DIEP flaps[69].

1.6.4 Lower abdominal "zones"

Scheflan and Dinner were instrumental in providing a classification and division system as to the blood supply to each part of the lower abdomen when raised as a flap based upon the epigastric vessels. They published their results of using a uni-pedicled TRAM flap and described a method of classifying different areas of the flap based upon their impression of perfusion to each area. They divided the lower abdominal ellipse upon which the flap was to be based into four equal areas which they named "zones". Each zone of the flap was numbered I-IV in terms of degrees of diminishing perfusion (i.e. Zone I best perfusion and Zone IV worst perfusion). Zone I overlies the perforating vessels, Zone II is across the midline adjacent to perforating vessels. Zone III is on the ipsilateral periphery to the perforating vessels and Zone IV is on the furthest periphery on the contralateral side from the perforators[70,71].

Despite this original description with regards to classification of perfusion zones being carried out by Scheflan and Dinner, the zone's became known colloquially as "Hartrampf's Zones" due to his previously published work on TRAM flaps[47]. (See Figure 1.11)

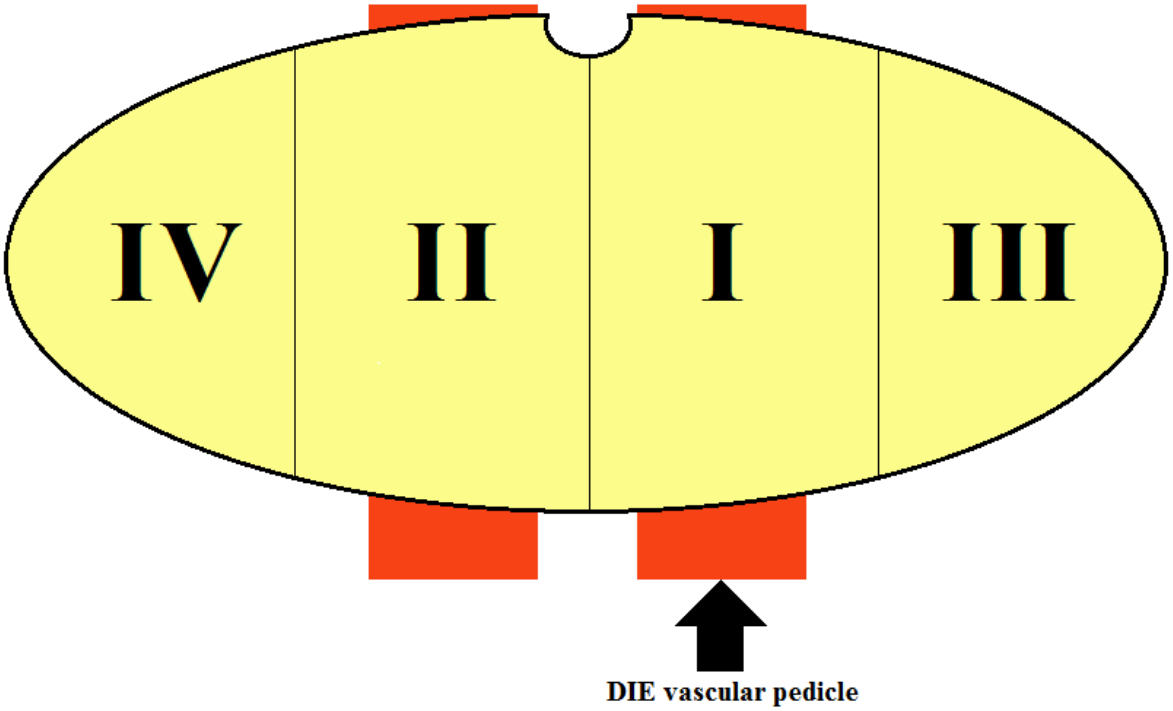

Figure 1.11: Hartrampfs' Zones of Perfusion

In a subsequent publication by Dinner and Scheflan highlighting refinements in their technique, they had changed their classification system by switching "Hartrampf's zone II/III[72]. (See Figure 1.12)

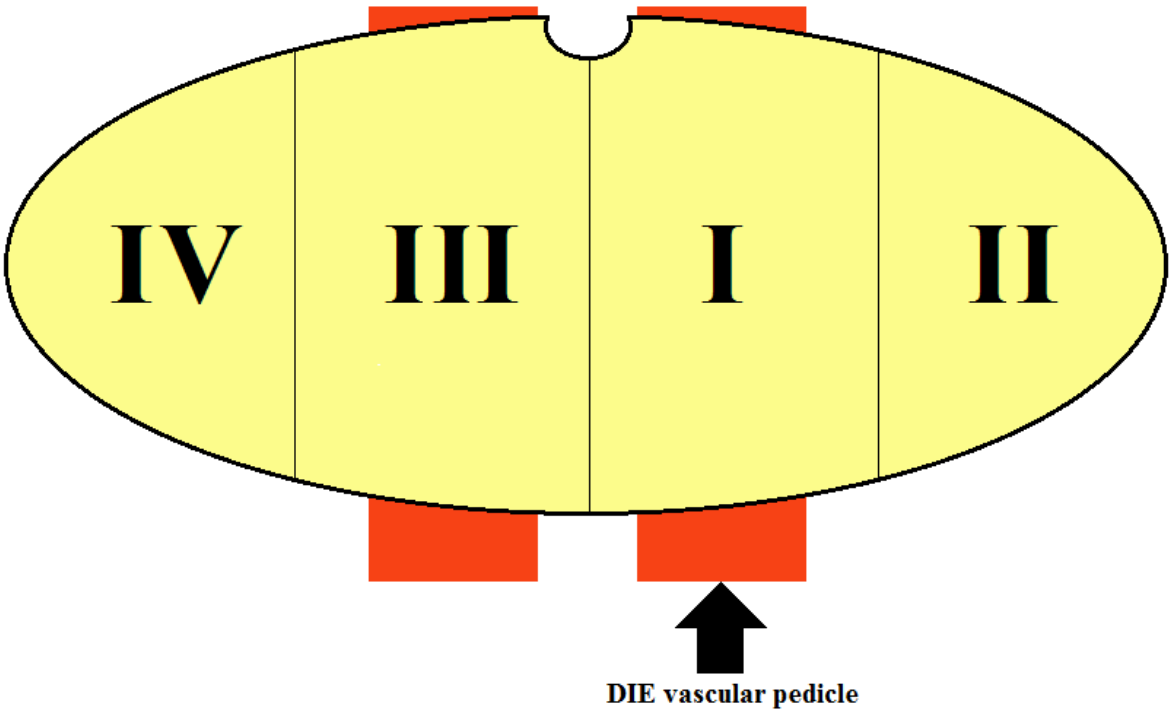

Figure 1.12: Dinners' Zones of Perfusion

These zonal classification systems were originally designed specifically with regards to a uni-pedicled TRAM flap based upon the superior epigastric vessels but they have become

universally adopted for the description purposes of flaps based upon the Deep Inferior Epigastric Vessels in modern practice and literature.

Both classifications were based upon observations and clinical impressions rather than scientific evidence. More recent studies assessing perfusion to the specific zones using ultrasonography[73]; Indocyanine green angiography[74] and tissue oxygenation[75] would agree that Dinner's revised zonal classification is more accurate, as perfusion does not appear to occur as easily across the midline. Controversially, despite the revised publication by Dinner advocating the change in Zone II/III, the original Hartrampf's zones are still commonly used.

Despite the disputes over the flap classification system in terms of using Hartrampf's or Dinner's zones; there is no dispute with regards to the location and perfusion characteristics of Zone I and Zone IV. For the purposes of this thesis Hartrampf's classical zones will be utilised in discussion.

The perforasome concept offers an alternative classification system. Rozen et al proposed a perforasome type classification for lower abdominal perfusion when based upon a single perforator from their findings in cadaveric dye injection studies and CT angiography[76,77]. This system divides the lower abdominal zones based upon whether a medial or lateral row perforator has been used in flap raising. As with Hartrampf's and Dinner's Zones, Zone IV remains the furthermost from the perforating vessel and the least well perfused. (See Figure 1.13)

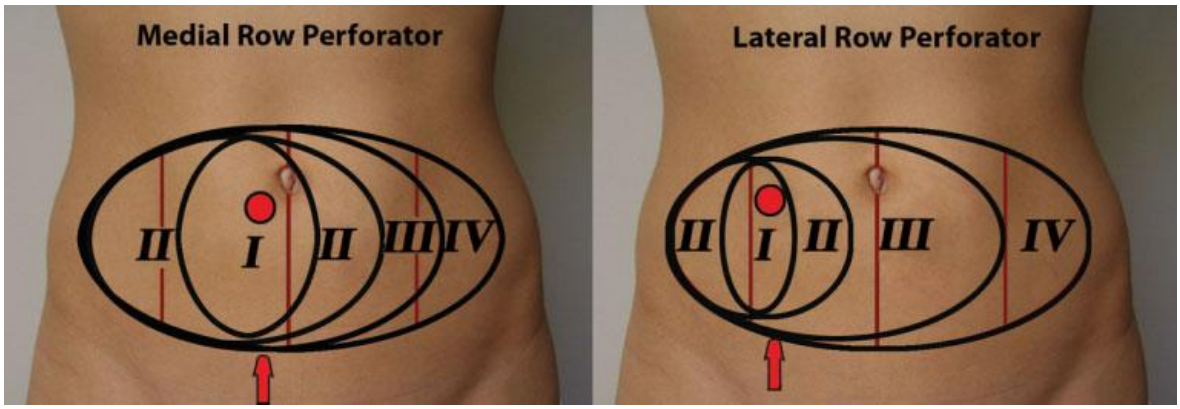

Figure 1.13: Perforasome Zones of perfusion based on medial (left) or lateral (right) row perforators
Image reproduced with permission from John Wiley and Sons. (Rozen et al. 2010)[76]

1.6.5 Vascular related complications associated with DIEP flaps

Despite the widespread use of the DIEP flap in breast reconstruction significant issues associated with poor vascularity of the flap can occur.

1.6.5.1 Venous Congestion

When the venous return of a flap through the venous anastomosis is not adequate, then parts of the flap or the entire flap can become congested; clinically presenting as a rapid skin paddle capillary refill with subsequent swelling and discolouration of the flap skin paddle. This is often the earliest sign of a failing flap and can signify that there may be a problem with the venous anastomosis (i.e. thrombosis or kinking). This usually necessitates an immediate return to theatre to assess the patency of the venous anastomosis to the flap.

In the standard DIEP flap, the total venous outflow of the flap is directed through the perforating venae comitantes accompanying the arterial perforator(s) chosen to base the flap upon into the DIEV. Experimentally, it has been shown that venae comitantes are not the best choices of venous drainage in free flaps and that draining veins should be situated far enough away from the feeding artery to improve drainage[78].

Problems associated with venous congestion resulting in compromised perfusion to DIEP flaps are well documented and it is the most likely vascular related complication.[41,54,61,79-82]

Excluding technical or unfavourable geographical problems (kinking) with perforator dissection or the primary DIEV anastomosis, venous congestion in the DIEP flap can still occur leading to potential partial or complete flap failure. Patent DIEV anastomosis with concomitant venous congestion has been reported to arise between 2.1 to 27 percent of the time in the literature[53,61,83,84].

The causes of venous congestion in the DIEP flap are poorly understood however several theories as to why venous congestion occurs have been described, including inadequate calibre of the perforating veins[85]; inadequate venous midline crossover[41,61,81] and an inadequate communication between the superficial and deep venous system[61,83].

With regard to the calibre of the perforating veins Kroll suggested that in order to allow adequate venous outflow in a DIEP flap then a perforating vein with a diameter of at least

1mm should be selected[85]. However, this was only based on his own experience rather than any anatomical or scientific research.

Others have theorised that inadequate midline crossover of the venous architecture in DIEP flaps may be a significant risk factor in the development of venous congestion particularly to Zone IV of the flap. In an anatomical injection study by Blondeel et al in 2000 they found that communicating venous branches crossing the midline were absent in 36% of specimens they examined and the only venous communications were via the subdermal plexus[61]. Arguably however, their study group was small consisting of only 15 cadaveric and 3 abdominoplasty specimens and limited by the fact that they would be unable to assess the contribution of the opening of oscillating veins in non-living specimens. A similar anatomical study by Schaverien et al in 2008 also found absence of midline venous crossover vessels in 1 out of 12 (8%) specimens they examined[81]. A larger in-vivo study consisting of 100 patients by Rozen et al in 2009 found an absence of midline crossover in 14% of the cohort[41].

The relationship of the deep and superficial vascular system is particularly interesting and its importance has gained significant evidence in the literature over the past 15years.

Blondeel initially proposed that the venous communication between the DIEV and the SIEV may be inadequate in patients with an SIEV >1.5mm and that in these patients the superficial venous system is required to drain the flap[61].

Tran et al agreed with this proposition in their study to assess microvascular complications of DIEP flaps and advocated that the SIEV should always be initially identified and preserved as a "lifeboat vessel". 11% of their DIEP flaps suffered intra-operative venous congestion despite patent DIEV anastomosis and 45% of these required additional bypass anastomosis to the SIEV to successfully salvage[83]. They also suggested that a very small diameter SIEV may be positive predictive indicator as it may correlate to a better communication and a deep system dominant enough to drain the flap[83].

The most convincing evidence of the correlation between the deep and superficial system was in a study published by Schaverien et al in 2010 assessing the relationship between venous congestion and intra-flap venous anatomy in 54 DIEP flaps using contrast enhanced MRA they found that DIEP flaps raised on venous perforators which lack a

direct communication with the SIEV system are significantly more likely (p<0.0001) to be complicated by venous congestion[86].

1.6.5.2 Fat Necrosis

Fat necrosis can be classified as partial flap necrosis without any skin paddle changes. This complication arises when areas of fat throughout the flap are not adequately perfused however the overlying skin paddle still has an adequate blood supply and remains healthy.

This can occur weeks to months after the flap reconstruction and often presents as firm lumps within the body of the reconstruction, non-healing wound edges plus or minus discharge. Furthermore, this fat necrosis can be of particular distress to breast reconstruction patients due to their history of breast cancer as they are often extremely concerned with finding firm lumps within their reconstruction.

Some degree of fat necrosis can be considered a relatively common complication of DIEP flap surgery, especially when larger flaps are required. The rate of fat necrosis associated with DIEP flaps vary within the literature quoted anywhere from 2.8-17.1%[85,87]. A recent meta-analysis by Khansa et al in 2013, including 70 articles deemed suitable, found the rate of fat necrosis in free DIEP flaps to be 14.4%[88].

However, the rate of absolute fat necrosis may be vastly higher than that actually presenting clinically or being reported. A study by Peeters et al, showed that evidence of fat necrosis was found ultrasonographically in 35% of all their DIEP flap patient cohort, whilst only being clinically detectable by physical examination in 14%[89].

Despite the problems of fat necrosis, many of the patients can be managed conservatively with reassurance and dressings if required. In the same study by Peeters et al, only 7% of the 35% of their patients affected by fat necrosis required a return trip to theatre[89]. However, in patients with a large degree of fat necrosis a return to theatre for open debridement or lipo-suction to the area may be required. Large volumes of fat necrosis can lead to significant volume deficit of the flap, contour irregularities and the need for subsequent revision procedures.

1.6.5.3 Flap Failure

Free flap failure is a catastrophic event for both patient and surgeon. It most commonly occurs in the immediate/early post-operative period as a result of vascular compromise to the pedicle of the flap. The causes of vascular compromise are usually attributed to thrombosis at the site of micro-anastomosis of the artery or vein. Venous problems are more commonly encountered and often are secondary to unfavourable pedicle geometry (kinking of the pedicle), technical aspects of the anastomosis or due to external pressure on the pedicle i.e. haematoma. Every effort is taken to ensure free flap survival with careful tissue handling/dissection of the pedicle; meticulous microsurgical anastomosis techniques and stringent post-operative care/monitoring of the patient and flap. Any evidence of flap compromise should necessitate an immediate return to the theatre for evaluation of the flap and pedicle anastomoses. Despite these interventions and precautions there is still a quoted acceptable rate of free flap loss of up to 5%[90]. With regards to the DIEP flap which has become a popular well-established choice in modern microsurgical breast reconstruction some experienced authors are quoting free DIEP flap failure rates of less than 1%. Gill et al had a 0.5% flap failure rate their series of 758 free DIEP flaps for breast reconstruction[53]. This appears to be similar to that quoted for free TRAM flaps as shown by Vega et al who quoted a free flap failure rate of 0.3% in their series of 500 free TRAM flaps[91].

Late free flap failure (that occurring after 7 days) is incredibly rare and is often associated with infection or recurrence of tumour at the recipient site[92].

1.6.6 Vascular augmentation of DIEP flaps

Regardless of the cause of venous congestion, a number of studies have attempted to salvage congested DIEP flaps through the use of vascular augmentation, by anastomosis of an additional vein[50,61,80,82-84,93-100].

Flap vascular augmentation procedures are often referred to as “supercharging” or “turbocharging” of the flap depending upon the vascular configuration of the additional anastomosis(es). This nomenclature however can be confusing and the terms are often incorrectly used interchangeably in clinical practice or even in the literature. A useful analogy to remember the terms and configurations they describe was provided by Semple, who suggests thinking of flaps like an engine in a motor vehicle. Supercharging refers to boosting the engines performance from an external source and turbocharging refers to

using the engines own exhaust to provide additional power[101]. These are the generally accepted definitions in current practice[102,103]. An example to illustrate "supercharged" and "turbocharged" flaps is shown in Figure 1.14.

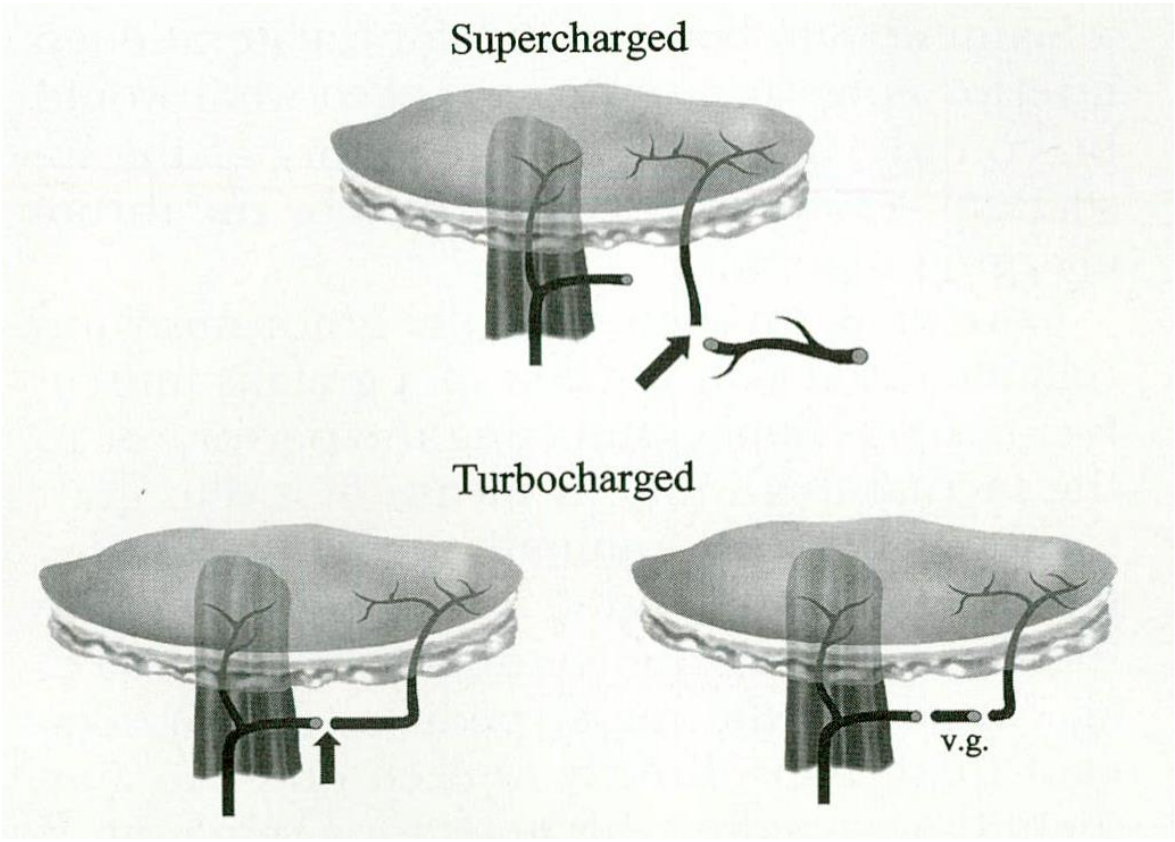

Figure 1.14: Illustration representing a supercharged and turbocharged flap
Note the lower right image refers to the use of an inter-positional vein graft. Image reproduced with permission from Wolters Kluwer Health, Inc. (Hallock 2000)[102]

However, further disparity exists as to whether the terms "supercharging" or "turbocharging" should be used for only additional arterial anastomosis(es) or venous anastomosis(es) and which terms should be used when.

Given the difference in expert consensus opinion regarding the terms, and indeed what they imply, in an effort to reduce confusion within this thesis the terms "supercharging" and "turbocharging" will not be utilised. Instead the terms arterial augmentation; venous augmentation and combined arterial/venous augmentation will be used to clearly explain when an additional arterial input, additional venous output or combined arterial/venous input/output is used respectively.

Whilst the use of vascular augmentation has been documented widely in DIEP flap surgery over the past 15 years, the evidence is limited to isolated case reports or case series based upon salvage procedures performed once venous congestion has already occurred.

In one of the early large case series of DIEP flap breast reconstructions Blondeel described his experience of the first consecutive 100 flaps performed[50]. He was the first to describe venous augmentation of the DIEP flap via an external source. He mentions in the discussion of this paper that two obese patients had inadequate venous drainage and that the problem was overcome through vascular augmentation, using a free arterial transposition graft between the SIEV and the venae comitantes of the IMA. Shortly after Villafane et al went on to describe a similar successful salvage using a saphenous vein interposition graft from the SIEV to circumflex scapular vein labelling the SIEV as a "lifeboat "[100].

Wechselberger et al in 2001 published the benefits of venous augmentation from their experience of 37 free DIEP flap breast reconstructions. In 3 of these flaps they noted significant venous congestion despite patent anastomosis of the main DIEV to the IMV. These flaps were salvaged by venous augmentation by anastomosing the SIEV to an additional recipient vein out-with the flap in a procedure they termed "venous superdrainage"[84]. A number of other surgeons have since described their salvage of congested free abdominal flaps utilising venous augmentation of the flap by anastomosing DIE venae comitantes or the flap SIEV to various other recipient veins including the perforating intercostal[82], chest wall[83], thoracodorsal[97], cephalic[80,97],basilic[93], lateral thoracic[84] and even external jugular veins[97].

Utilising components within the flap to augment venous drainage has also been described as a method to salvage the congested DIEP flap. Rohde and Keller initially described their technique of DIEP flap salvage by anastomosing the ipsilateral SIEV to the proximal end of one of the flap venae comitantes[99]. Liu et al in 2007, then described a similar technique of salvage but in a reverse-flow pattern by anastomosing the ipsilateral SIEV of the flap to the distal end of one of the flap venae comitantes providing additional reverse flow venous drainage[95]. However, concerns were raised about the reverse flow technique as potential valves present within the vein may prevent retrograde flow[98].

Recognising the need for venous salvage procedures in those flaps showing intra-operative venous congestion, despite patent venous anastomosis and no clear consensus in the literature Galanis et al proposed an elegant intra-operative algorithm in 2014[104]. (See Figure 1.15)

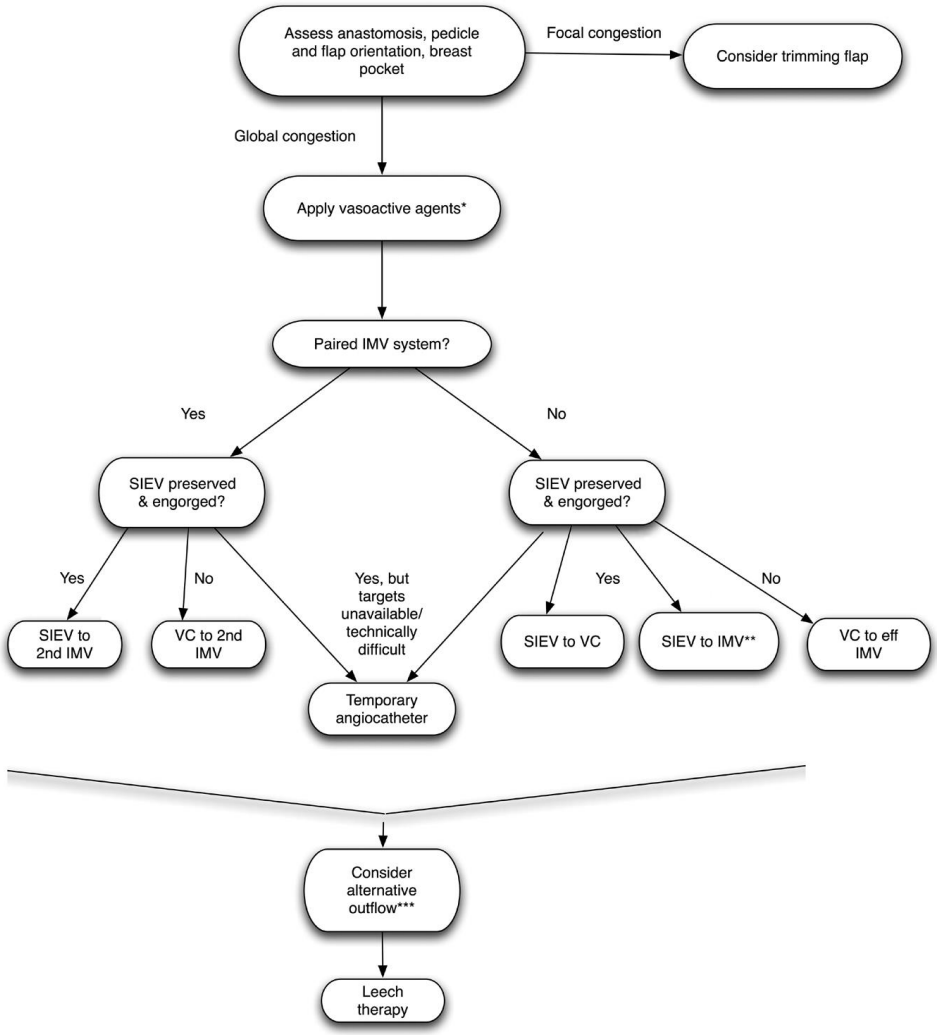

Figure 1.15: Suggested algorithm for management of intra-operative venous congestion Image reproduced with permission from Wolters Kluwer Health, Inc. (Galanis et al. 2014)[104]

Some surgeons have started to routinely carry out venous augmentation procedures on their flaps, in an effort to reduce the risks of venous congestion.

Enajat et al in 2010, published the results of a large retrospective study of 564 consecutive DIEP flap cases performed by four surgeons comparing those DIEP flaps which had one venous anastomosis (273) versus those flaps (291) which at the time of the original operation had an additional primary venous anastomosis to either a second venae comitantes or the SIEV[105]. In this study they found that use of two venous anastomoses significantly reduced the number of cases of post-operative venous congestion (0 flaps) in comparison to the group which only had one venous anastomosis (7 flaps) without increasing overall complication rate. They recommend that where possible all flaps should have an additional venous anastomosis performed. Whilst this is a large study comprising of 564 flaps, it is inherently flawed by the fact that it was retrospective in nature and the cohorts were not randomised. The choice to perform a primary second venous anastomosis

was on an individual surgeons' preference and one of criteria for performing a second primary anastomosis was intra-operative suspicion of venous congestion.

Boutrous in 2013 published his similar opinion that where possible all DIEP flaps should have double system (deep and superficial) venous drainage[79]. In his case series of 352 free DIEP flaps used for breast reconstruction he performed an additional venous augmentation procedure in 311 of the flaps by additionally anastomosing the SIEV from the DIEP flap to an internal mammary perforating vein or internal mammary venae comitantes. Out of the double venous drainage cohort there was one flap requiring return to theatre for venous congestion (0.3%); in the remaining 41 single venous drainage flaps two required return to theatre (4.9%). He believed this was a statistically significant reduction however comparison between both groups is difficult due to the completely different cohort sizes and also the fact that those patients in the non-double venous drainage group could be considered as having non favourable venous anatomy to begin with if the original venous augmentation procedure was not possible.

Whilst all instances above represent venous augmentation to treat venous congestion, arterial augmentation can also be performed to improve arterial flow, however, this is often not an issue in a standard DIEP flap.

1.7 Assessment of DIEP flap flow/perfusion

Laser Doppler Flowmetry and ICG angiography are two well established non-invasive methods of assessing superficial flap blood flow/ tissue perfusion. Ultrasonic transit time flowmetry provides an invasive measurement of intravascular blood flow. These techniques were used in the methodology of this thesis and will be discussed in detail below.

1.7.1 Laser Doppler Flowmetry

In 1964 Yeh and Cummins carried out an experiment using a continuous wave helium neon laser beam directed at small polystyrene spheres immersed in water. They demonstrated that the scattering of the laser light which occurred could be measured and utilised to assess flow within fluids using the principle of the Doppler shift effect[106]. After the initial discovery another group of researchers working at Brown Engineering Company (Huntsville, Alabama) realised the technique could be used not only to assess flow rates within fluids but also within gases and designed a machine capable of measuring this

which they called a Laser Doppler Flowmeter[107]. The advantages of this technique became widely apparent as it offered a reliable, accurate non-invasive method of measuring flow even at low velocity.

1.7.1.1 History of Laser Doppler Flowmetry in Plastic and Reconstructive Surgery

In 1975 Stern was the first to use Laser Doppler Flowmetry to assess skin perfusion in humans. He realised that the technique offered a rapid method by which to assess changes in skin perfusion and foresaw potential clinical applications in assessing patients with vascular disease and also the "assessment of the viability of burns and grafts[108]. Sterns' initial predictions were correct and the technique became established in the medical world for a variety of specialities ranging from rheumatology to plastic surgery. In plastic surgery the use of Laser Doppler Flowmetry has become well established in the burn subspecialty to aid in the estimation of burn depth[109-111] and in the microsurgery subspecialty to aid in the monitoring of free flaps[112-116].

1.7.1.2 Principles

Laser Doppler is a non-invasive technique which allows the measurement of superficial microvascular blood flow within tissues.

In the assessment of skin perfusion it operates generally upon the following principles. Laser light (usually in the near-infrared/infrared spectrum) is generated and directed at the target tissue from a stationary point. Upon contact the laser light will have several interactions with the target tissue including absorption, transmission, scatter and reflection. The scattered and reflected laser light energy from the tissue can be measured using specialised photoreceptor sensors within a receiver. The scattered and reflected laser light energy will have different characteristics dependent upon the type of tissue it interacts with. Interactions with static tissue will produce much less scattering of the laser light and thus will have a lesser effect upon the frequency of the reflected beam. However interactions with moving tissue (such as red blood cells moving within intravascular spaces) will produce a greater degree of scattering and thus broaden the frequency of the reflected laser beam known as the doppler shift effect. The difference between the frequency of the reflected laser light from static tissue and that occurring from moving tissue is detected by the receiver and the velocity of the moving tissue (i.e. red blood cells)

calculated thus allowing a measure of blood flow and in turn; perfusion of the tissue to be calculated.

The perfusion of the tissue as obtained by laser doppler measurement is commonly expressed as “flux” (which is the number of red blood cells multiplied by their velocity) and this in turn is converted to arbitrary perfusion units by software within the laser doppler machine often in graphical displays which allow statistical comparison between different areas of interest.

1.7.1.3 Methods of Laser Doppler Measurement

The laser light energy can be delivered to the target in two ways.

The laser light energy can be delivered (from a distance) to the target tissue via a low intensity focused laser light beam which is scanned across the target area using moveable mirrors and the resultant “doppler shift” assessed (from a distance) via photoreceptor in the receiver. This technique is known as Laser Doppler Imaging (LDI) or “scanning laser doppler” and allows relatively large areas of tissue to be measured in a short period of time.

Another method of delivering the laser light energy is via optic fibres. Generated laser light is transmitted from the source via an afferent optical fibre to an optical probe which must be in direct contact with the target tissue. As well as the afferent optical fibre terminating in the probe there is usually one or more efferent optical fibre(s) originating from the probe which capture the scattered and reflected laser light from the tissue and transmit it to photoreceptors in a receiver allowing the doppler shift and thus blood flow to be calculated. This technique is known as Laser Doppler blood flow monitoring. As a result of the probe being in direct contact with the tissue and the relatively short distance between the afferent and efferent fibre(s) only the blood flow within relatively small areas of tissue can be measured; albeit very accurately. This technique is useful for measuring blood flow in areas which are difficult to access using a laser beam (i.e. within body cavities).

1.7.2 Indocyanine Green Angiography

1.7.2.1 History of Indocyanine Green Angiography

Indocyanine Green (ICG) is a tricarbocyanine dye initially developed as a cyan forming layer in the production of Technicolor film by Dr Brooker and Heseltine of the Eastman Kodak Research Laboratories.

In 1955 an executive of Eastman Kodak Research Laboratories was a patient undergoing treatment by cardiologist Dr Irwin Fox at the Mayo Clinic. Coincidentally at the same time Dr Fox was searching for a biocompatible dye that could be detectable in blood and recruited the aid of his patient in this search. The executive sent a variety of dyes to Dr Fox for evaluation; amongst which ICG was one. The ICG dye met the characteristics which Dr Fox required; however it was very unstable in its aqueous form. Subsequent refinements to the dye were undertaken by a pharmaceutical company (Hynson, Westcott & Dunning Inc. Baltimore, Maryland) producing a stable lyophilised form and Dr Foxs' team began further trials[117].

Initially ICG dye was used in assessing cardiac output[118] and then hepatic blood flow/function.[119] It was not until 1969 that the first use of ICG in absorption angiography was undertaken by Kogure et al when they obtained detailed images of the cerebral vasculature of a canine brain [120] and subsequently fundus circulation in a monkey[121]. These early angiography studies relied upon invasive intra-arterial injections of ICG as it was believed that intravenous injections of the dye failed to obtain acceptable results and as such was not deemed appropriate for human use.

Hochheimer described a technique using ICG dye intravenously in cats for near-infrared absorption angiography purposes in 1971[122] solving the requirement for intra-arterial administration and in conjunction with Flowers' went on to describe the first successful human near infra-red absorption angiography imaging of large choroidal vessels using ICG the following year[123].

Further research and improvements upon their technique; altering the concentrations of dye and camera systems, led to the development of near-infrared fluorescence angiography using ICG dye allowing visualisation of the smaller blood vessels within the eye and blood flow within them[124]. The potential application of the ICG angiography process in

obtaining detailed images of the vasculature of the human eye were quickly realised and the technique has been used extensively in the speciality of ophthalmology since[125].

Further to the successful medical uses of ICG angiography listed above, interest began to grow for the potential application of the technique in surgery when Raabe et al described the first use of ICG angiography intra-operatively to assess real time blood flow through the vasculature in neurosurgical procedures[126]. Since then further applications of the technique have been found in not only Neurosurgery but Cardiac; Vascular; Oncological; Hepatic; Breast and Plastic and Reconstructive surgery[127].

1.7.2.2 Indocyanine Green Angiography in Plastic and Reconstructive Surgery

In a specialty such as Plastic and Reconstructive Surgery where fundamental knowledge of the vascular perfusion of tissues is essential it is of no surprise that ICG angiography should have an established role.

Rubben et al first alluded to the potential use of ICG angiography in Plastic and Reconstructive surgery when they published their successful experimental results of using ICG angiography to assess skin flap perfusion in animal models and a select patient cohort in 1994[128]. However, it was not until 8 years later when Holm et al. utilised the technique to intra-operatively assess the perfusion/viability of skin flaps[129] and the post-operative monitoring of free flaps[130] that ICG angiography became popular in the speciality. Since then a wide variety of applications in the realms of plastic surgery have been found not only in the design and assessment/monitoring of flaps but also in terms of lymphography[131]; sentinel lymph node biopsy[132] and burn depth estimation[133].

1.7.2.3 Principles

ICG dye absorbs energy in wavelengths of the electromagnetic spectrum between 600-900nm and fluoresces strongly when exposed to wavelengths between 750-950nm. Upon injection into the living human circulation ICG dye rapidly binds to plasma proteins (predominantly albumin) and remains confined to the intravascular space circulating around the body.

By utilising a laser light source emitting energy in the wavelength between 750-900nm the dye will fluoresce. This fluorescence is not visible to the human eye however can be

detected by an appropriate optical video detector and the real-time images recorded. The laser light source and detector are usually coupled within the same machine allowing simultaneous fluorescence and detection. The half-life of the dye in human circulation is very short at between 150-180seconds[134] and thus the capture sequence must be commenced soon after injection. As a result of the rapid excretion of the dye; repeated measurements can be taken after alteration of variables without risk of artefact or having to wait for long periods of time in contrast to fluorescein which can take up to 18 hours to clear[135].

A laser of the appropriate wavelength and a fluorescence detector can be used to allow real-time visualisation of blood flow. Metabolisation of the dye occurs solely via the liver parenchymal cells and the by-products are excreted via bile salts; no cellular absorption occurs and thus toxicity is low[136].

1.7.3 Intravascular flow measurement using ultrasonic transit-time flow meter

1.7.3.1 History of ultrasonic transit-time flow meters

The first ultrasonic transit-time flowmeter was invented by Franklin et al and described in a paper published in 1962 in which they discussed the potential of their invention and that it could allow flow within a blood vessel to be continuously measured[137]. However it was not until 1978 that Drost described the theoretical basis of the transit-time volume flow principle through which these devices could accurately measure the velocity of fluids [138]. The advantages of ultrasonic transit-time flow measurement is that the probes utilised do not require to be in direct contact with the vessels being assessed and are not influenced by vessel internal/external diameter, vessel shape or the blood flow profile. The recordings obtained via the technique are stable and calibration is also unnecessary as there is little zero-drift from baseline[139].

1.7.3.2 Principles

Intravascular flow can be detected through the process of transit-time ultrasound flow theory. This theory dictates that when ultrasonic energy moves from a point of origin to a destination through a flowing liquid (in this case blood) the time taken to reach the destination is altered. If the ultrasonic energy is moving with the direction of flow transit time is decreased and conversely if the ultrasonic energy is moving against the direction of

flow transit time is increased. Providing that the distance between the point of origin and destination is fixed and the normal transit-time between these two points known, then by measuring changes in transit time occurring secondary to the liquid through which the ultrasonic energy is passing, it allows the calculation of liquid velocity.

1.8 Volume/skin requirements in DIEP flap transfer and associated problems with Zone IV

Acceptable outcome in DIEP flap breast reconstruction is not solely based upon successful flap transfer but also upon being able to transfer an adequate volume of tissue to shape such tissue to meet the reconstructive needs of the patient.

On the occasions when large volume reconstructions are required (i.e. large contralateral breast), large skin paddles required (delayed breast reconstructions) or reconstructions requiring additional projection (less ptotic breast) then the majority of the lower abdominal pannus may be required as a donor site. As an example Zone IV can be useful to provide more volume and skin particularly when performing delayed reconstructions of large breasts.

Despite the widespread utilisation of the free DIEP flap in breast reconstruction there are well recognised flap related vascular complications[41,54,61,79,80,82-84,86]. This is particularly true of Zone IV as it is the area of the flap which receives the least perfusion/drainage from the perforating vessels and is therefore the most likely to suffer from perfusion related issues such as ischaemia, venous congestion and fat necrosis. Despite some authors reporting variable success with the inclusion of Zone IV in their DIEP flaps[61] others have commented that Zone IV almost always suffers from problems[83]. In addition there is the belief that inclusion of Zone IV may compromise the overall arterial perfusion to the other Zones of the DIEP flap[83]. Secondary to this many surgeons routinely excise and discard Zone IV from the DIEP flap prior to flap transfer to the defect site. As a result the volume of the DIEP flap available for transfer is reduced by approximately 15-20% and can be difficult to shape to fit the geometry of a natural breast.

There are various strategies described to increase the volume/projection of free DIEP flap breast reconstructions including dividing the DIEP flap in the midline and “stacking” each hemi-flap on top of each other[140-142]; stacking an SIEA flap on top of a hemi-DIEP

flap[142,143] or in the extreme stacking a Superior Gluteal Artery Perforator (SGAP) or Profunda Artery Perforator (PAP) flap on top of a DIEP flap[144].

However, these procedures all have a significant learning curve in terms of arranging flap and pedicle geometry, require additional dissection; require additional operating time or in the case of the stacked DIEP/SGAP/PAP flap combinations an additional donor site out-with the abdomen.

Others advocate delayed augmentation of DIEP flaps using lipomodelling as the simplest method to address any volume deficiencies or contour irregularities[145,146]. However, this often leads to the requirement of multiple additional procedures for the patient.

If Zone IV of the DIEP flap could reliably be included without increasing the risk of flap related complications then this may allow surgeons the necessary volume or skin paddle required for reconstruction rather than having to resort to additional or second stage procedures.

1.8.1 Pro-active vascular augmentation of DIEP flaps

Vascular augmentation of the DIEP flap through the additional anastomosis of an extra artery / vein or both at the time of the original operation is another option to potentially improve blood supply to the flap and increase prospective flap volume.

Whilst most vascular augmentation procedures are performed as salvage to treat the congested DIEP flap (cf. 1.6.6), some authors are now proposing pro-active vascular augmentation of DIEP flaps to improve blood flow to the flap or to increase the size of the flap which can be taken. Such procedures have previously been described to augment the blood supply of TRAM flaps[101,147-152] but are only recently being described exclusively in DIEP flap breast reconstructions.

The first cases of vascular augmented DIEP flaps for breast reconstruction were actually described in 2002 by Mu et al who performed 5 cases of vascular augmented DIEP flaps using both ipsilateral and contralateral DIE vessels attached respectively to the proximal and distal ends of the IM vessels[153].

However it was not until 2007 when Hamdi et al later described their use of vascular augmented DIEP flaps that the technique received widespread acknowledgment[154]. In their

series of 16 free DIEP flap breast reconstructions inclusion of all Zones of the flap was required secondary to the need for a large volume reconstruction or issues with abdominal vascular supply (i.e. previous scarring on one side of the flap). Various augmentation configurations were utilised in this case series. 13 of the flaps underwent vascular augmentation procedures using the IM vessels to supply the main pedicle and various intra-flap anastomoses. The remaining 3 flaps had vascular augmentation procedures using the thoracodorsal vessels attached to a secondary pedicle to provide blood supply to the flap in addition to the main pedicle IM vessel anastomoses. Hamdi et al also attempted to provide a classification system for the various types of flap configurations. (See Figure 1.16)

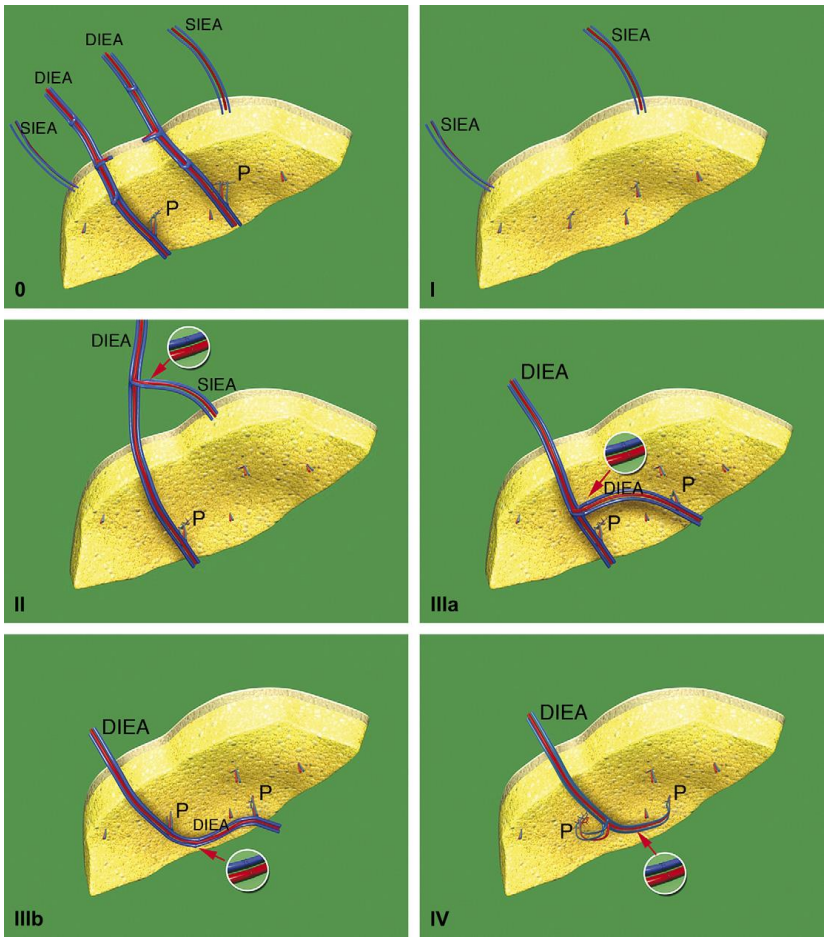

Figure 1.16: Proposed classification system for abdominal perforator flap and vascular augmentation configurations
Image reproduced with permission from Elsevier Ltd. (Hamdi et al. 2007)[154]

Since the description by Hamdi et al a variety of other authors have reported their experiences in providing large volume reconstructions with vascular augmented DIEP flaps and suggested various modifications to the above classification system[155-161].

1.9 Potential concerns of vascular augmentation of abdominal flaps

There are numerous documented cases concerning the use of vascular augmented flaps; mostly as a salvage procedure to improve venous drainage in congested flaps [50,61,80,82-84,93-100], or as an attempt to utilise larger more robust flaps[101,147-158,160,161]. However, the results of these cases are simply reported in terms of free flap survival or failure with little in the way of scientific evidence supporting their use.

Whilst the experimental animal studies have provided some evidence that vascular augmentation of abdominal flaps in rats leads to increased areas of flap survival; these are all post-operative outcome assessments with no account for the in-vivo effects on blood flow or perfusion occurring at the time of augmentation procedure[162-167]. Also it would appear from some of these experimental studies that arterial augmentation is more important than venous augmentation[166,167].

Though it may seem logical that increasing the number of blood vessels used to supply a flap will lead to an increase in flap perfusion and improvement in flap outcome; recent evidence has shown that counter-intuitively this may not be the case. An elegant experimental animal and in-vivo human study was published by Douglas et al in 2014 assessing the effect of perforator number and location on total pedicle blood flow and on Zone IV skin and fat perfusion of DIEP flaps[168]. In the first part of this study carried out on twenty abdominal flaps in rats they found that there was a significant decrease in main pedicle blood flow when the flaps were based on more than a solitary perforator. In the second part of this study they assessed in-vivo using LDI and ICG angiography the effect of perforator number on Zone IV perfusion and interestingly found that basing flaps on more than one perforator resulted in significantly lower perfusion to Zone IV of the flap. In their discussion they suggest that their findings could be accountable to changes in pressure gradients within the flap and that by adding additional perforators the overall venous filling pressures to this area of the flap are reduced resulting in poorer perfusion. The results of this study would corroborate the findings published by Gill et al in 2004 in which they found a significant positive correlation between increasing the number of perforators used to supply flaps and flap related complications, including partial flap loss, in a retrospective review of 758 DIEP flaps[53].

1.10 Outcomes of DIEP flap breast reconstruction

The outcome of DIEP flap surgery is due to far more than flap survival or the presence or absence of any of the potential complications discussed previously. Although complete or partial flap failure in itself is an important outcome better measures are now required for audit and in many areas justification of funding. Given the primarily aesthetic aim of breast reconstruction; i.e. replacing the excised breast tissue for something similar in terms of size, shape and volume; then the major outcomes are patient satisfaction and post-operative cosmesis.

1.10.1 Patient Reported Outcome Measures

Traditional outcome assessment in the field of Plastic Surgery has focussed on surgeon/peer opinion and analysis of photographic evidence or complication rates. However, whilst this data is important to consider, it fails to take into account individual patient perceptions of the surgical results and the profound effect that surgical interventions can have on a patients' quality of life. In an effort to address this issue specially designed questionnaires, known as Patient Reported Outcome Measures (PROMs), have been developed that quantify health-related quality of life and/or other significant outcome variables (e.g., satisfaction) from the patient's perspective[169]. The results obtained via these questionnaires have become increasingly important in clinical practice and also plastic surgery research.

Whilst capturing of any patient response to treatment is crucial, in order to ensure their satisfaction with the results, it is important to do so in a reliable and reproducible manner. Concerns have been raised that in plastic surgery research many of the studies using PROMs to assess surgical outcome have utilised questionnaires which have not been adequately validated or designed according to established guidelines[170]. The development of validated PROM questionnaires with particular reference to the population that they are assessing rather than generic questionnaires is paramount to ensure that the data observed is reliable.

Studies assessing the outcomes of breast reconstruction and breast aesthetic surgery lend themselves well to the use of PROMs as it is well established that overall patient satisfaction is the most important factor in determining the success of any form of surgery dealing with aesthetics[171]. However, a systematic review by Pusic et al in 2007 highlighted

that there were major issues with the PROM questionnaires used in the field of breast surgery as only seven of two hundred and twenty three studies utilised appropriate psychometrically validated PROMs for a breast population[172]. The use of such generic questionnaires in a breast population can lead to a lack of sensitivity, failing to identify specific problems encountered by breast surgery patients, over-estimating treatment effects and leading to reporting bias[173].

The BREAST-Q, developed in 2009 by Pusic et al, is a PROM questionnaire designed to study the impact and effectiveness of breast surgery from the patient perspective[174]. It covers six domains involved in breast surgery; satisfaction with breasts, overall outcome, process of care, and psychosocial, physical and sexual well-being. It can be utilised in a modular fashion for breast augmentation, reduction or reconstruction. Since its introduction in the plastic surgery literature the BREAST-Q has become well validated in breast research and outcome-reporting[175].

With regards to the superiority of abdominal based reconstruction versus the other treatment options, utilising PROMs as an assessment tool, there is much conflicting evidence in the literature.

A retrospective study of by Alderman et al in 2000 assessing determinants of patient satisfaction after breast reconstruction found that in the 212 patients those opting for autologous based abdominal flap reconstructions were more than twice as likely to be satisfied with their reconstruction than those patients with implant based reconstructions[25]. Similar retrospectives studies by Christensen et al[26] and Saulis et al[31] assessing the outcomes of 263 and 268 women respectively found that women who had opted for autologous reconstruction over implant based reconstruction were significantly more satisfied with their reconstruction.

In a large study by Yueh et al in 2010 comparing outcomes of 538 patients undergoing various types of autologous flap (TRAM, DIEP or LD) or implant based breast reconstructions. They found that patients undergoing DIEP flaps had the highest satisfaction rates and that 80% of all DIEP patients were happy with their reconstruction[33]. This corresponds to the results of a study by Damen et al who reviewed satisfaction rates after DIEP flap breast reconstruction and found that in the 72 woman they reviewed 94% were satisfied with the appearance of their breast reconstruction when dressed and 89% felt that the reconstructed breast felt like their own[176].

Other studies have shown that there may be no real difference in satisfaction or that implant based reconstructions may have higher rates of patient satisfaction. In a study of 206 patients by Andrade et al they could find no difference in terms of patient satisfaction between implant or autologous tissue reconstruction[177]. A later study by Fogarty et al in 2004, assessing patient satisfaction after TRAM flap reconstruction or implant based reconstruction found similar results, in that whilst the mean patient satisfaction in the TRAM group was higher than the implant group, it did not reach statistical significance[178]. However, this study was small consisting of data comprised from only 57 patient questionnaires. In a larger study of 168 patients, Spear et al found that patients with implant reconstructions had the highest satisfaction scores in comparison to those with autologous based reconstructions[179].

1.10.2 Aesthetic Outcome

Advocates of DIEP flap breast reconstructions state that they offer a natural shape, feel and good aesthetic outcomes[49-51,53]. This relates to the evidence available in the literature concerning aesthetic outcomes of abdominal flap breast reconstructions.

Alderman et al in 2000 found patients undergoing autologous based abdominal flap reconstructions were 4.72 times more likely to be satisfied from an aesthetic point of view than those undergoing implant based reconstructions[25]. A similar finding was made by Tonseth et al, in that DIEP flap based reconstruction scored significantly higher from an aesthetic point of view, in terms of shape, symmetry, volume, position and consistency, in visual analogue scales than those having expandable implant based reconstructions[180].

A study in 2003 by Cocquyt et al, showed that patients undergoing a DIEP flap breast reconstruction had superior results in terms of cosmesis, as assessed by expert panel, to those patients having breast conserving surgery procedures[181].

In their comparison of 538 patients, Yuet et al found that patients undergoing autologous breast reconstruction with abdominal based flaps were happier from an aesthetic point of view than those undergoing implant based, or latissimus dorsi flap reconstructions but could find no difference in terms of aesthetic satisfaction whether a TRAM or DIEP flap was utilised[33].

Despite finding higher patient satisfaction rates amongst implant based reconstruction patients, Spear et al found significantly higher expert panel rated aesthetic outcomes in the TRAM cohort of patients in their study[179].

Furthermore, since the tissue being used is the patient's own, rather than foreign materials, then these aesthetic results tend to be longer lasting, as they are not subject to complications, such as capsular contracture which can change the shape of the appearance at a later stage. Autologous tissue will also reflect changes in a patients' body habitus over time; i.e. losing or gaining weight will also effect the volume of their reconstruction.

A five year retrospective review of breast reconstruction patients by Weichman et al in 2015, found that those patients who underwent free abdominal based reconstructions were more satisfied with the appearance of their breasts than those patients who underwent implant based reconstruction[32].

1.10.3 Problems associated with outcome assessment

Whilst some studies suggest that overall patient satisfaction is linked to cosmetic result[177,182], it is obvious from the conflicting patient satisfaction and aesthetic outcome evidence listed above that this may not be the case. It is well known that post-operative patient satisfaction questionnaires may be flawed, as patient perceptions of satisfaction can be influenced by other beliefs, such as general acceptance or gratitude for their care and that satisfaction ratings should be interpreted with caution as they may hide other areas of concern[183-185]. This may help to explain why some patients are delighted with their cosmetic outcome, despite their surgeon critically believing it to be a poor result or vice versa. This topic was investigated in a study by Beesley et al in 2012, who retrospectively investigated the reasoning behind patient answers to a previous breast reconstruction satisfaction audit questionnaire[186]. In this study they found that the main influence on patient overall satisfaction was due to far more than cosmesis but also by the patient's relationship with their medical practitioners; the role of their reconstruction in their cancer journey and their clinical outcomes. It may also be that a patient's responses may be influenced by the point at which they are questioned upon their reconstructive journey[187].

1.10.4 Conventional subjective methods of aesthetic assessment

Given the confounding factors in determining overall patient satisfaction and links to aesthetic outcome through questionnaires, it is clear that we must have other reliable assessment tools to record and assess pre and post-operative aesthetic outcomes. Without adequate outcome assessment measures, it becomes problematical to compare and contrast the various types of breast reconstruction available to each patient. However, due to the subjective nature of cosmesis, as discussed previously, the current assessment methods we have are lacking.

Conventional methods of post-operative aesthetic outcome assessment of breast reconstruction surgery have often relied upon comparing post-operative still photographs (See Figure 1.17) coupled with ratings of various aspects of the reconstruction obtained by panel assessment.

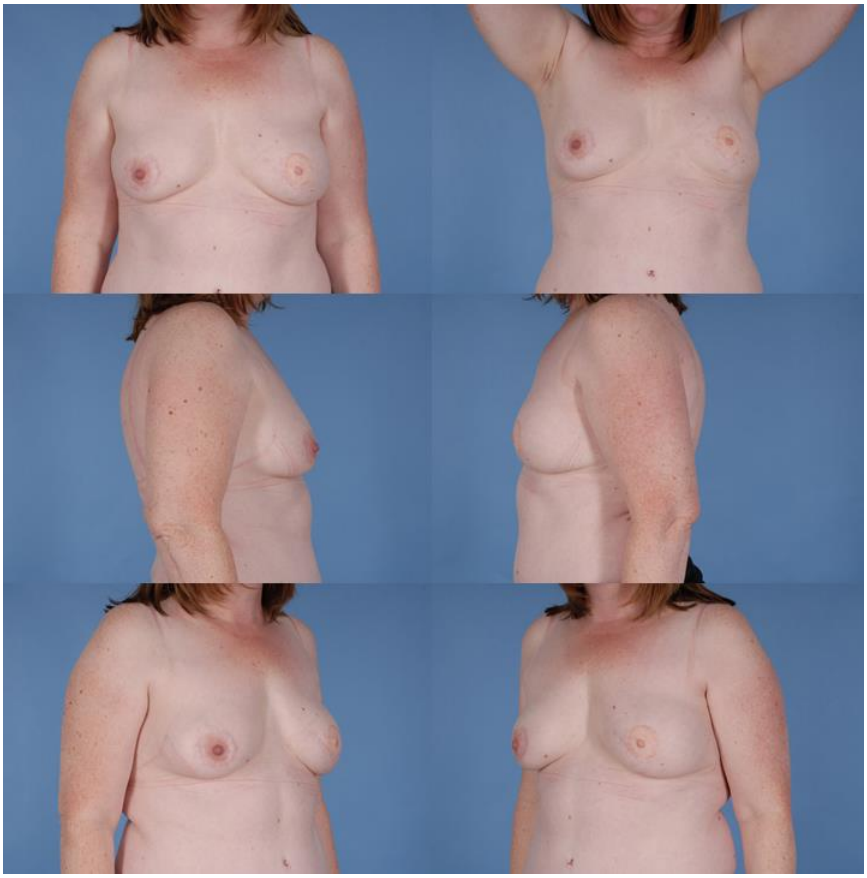

Figure 1.17: Conventional still photograph views obtained post breast reconstruction

Whilst many studies have utilised this technique for assessment of cosmesis post breast conserving surgery or reconstruction,[188-196] there is huge variation in terms of methodology as shown in a systematic review by Potter et al in 2011[197]. There have also been concerns

that the methodology employed in these studies lack precision and are difficult to reproduce[189].

Furthermore many of the researchers involved in the aforementioned studies will statistically analyse the results obtained from panel ratings to show the degree of inter-rater reliability between the members of the assessment panel[189,190,198-200]. A greater degree of inter-rater agreement between panel members is believed to relate to more scientifically robust results.

Whilst still photographs are able to convey a certain degree of information to the panel, they are inherently limited by the fact that they are two-dimensional in nature, offer no demonstration of breast mobility and are subject to great panel dispute. Breast mobility is of particular interest in terms of autologous tissue transfer, as it is believed that autologous tissue reconstructions move much more like a natural breast, in comparison to implant based reconstruction and this may explain the trend towards patients reporting higher levels of aesthetic satisfaction; however this cannot be visualised on static photographs.

1.10.5 Novel objective methods of aesthetic assessment

In an effort to improve upon the flaws described above, other more objective assessment methods have been described, such as the use of computer software designed to take objective measurements from two dimensional digital photographs with subsequent computer analysis of various indices linked to symmetry[198,199,201].

This technique has been further improved upon through the use of three-dimensional imaging systems; allowing the capture of a greater amount of information[202,203]. The objective data obtained from three-dimensional imaging system shows promise in being more accurate than traditional subjective assessments[200]. However, these complex systems are all very expensive, require vast user expertise in terms of set-up and analysis and are not available in the majority of plastic surgery units.

1.11 Summary

In summary, breast reconstruction using free abdominal tissue, such as the DIEP flap, offers many advantages to the patient and appears to be linked to high satisfaction in terms of cosmesis, despite inadequate techniques of post-operative aesthetic outcome assessment. Unfortunately, it is evident that problems can occur with the DIEP flap particularly with

regards to its vascular supply and venous congestion leading to potential compromise of the flap. Zone IV of the flap is the most liable to suffer these problems and as such is commonly discarded in an effort to prevent flap related complications but these can still occur regardless. In an effort to allow the harvest of larger flaps or to salvage compromised flaps, strategies in the form of vascular augmentation procedures have been described, but there is little research into the effect that these procedures have on flap perfusion. Further research into the effects on perfusion of these procedures may allow the harvest of larger DIEP flaps with a better vascular supply.

Chapter 2: Outline of Completed Studies

Use of abdominal flaps, including the free DIEP flap is well established in reconstruction of the breast following cancer. However, there are issues identified in terms of post-operative outcome assessment of such reconstructions and the problems associated with Zone IV DIEP flap perfusion.

As such the following studies have been undertaken:

2.1 The effect of vascular augmentation on DIEP flap Zone IV perfusion utilising the contralateral SIEA/SIEV

The aim of this study was to assess in-vivo the effects of vascular augmentation on DIEP flap Zone IV perfusion by augmenting the flap using the contralateral SIEA, SIEV or both in combination.

2.2 The effects of vascular augmentation on abdominal flap Zone I / IV perfusion and main pedicle arterial blood flow in an experimental animal model

The aim of this experimental study was to investigate the effects of vascular augmentation upon flap Zone I / Zone IV perfusion and also the effect this intervention has upon the main pedicle arterial blood flow in a rat abdominal flap.

2.3 The use of real-time digital video in the assessment of post-operative outcomes of breast reconstruction

The aim of this study was to assess whether standardised real-time digital video footage was a valid assessment tool in assessing post-operative outcomes and to compare its use against standardised static photography.

Chapter 3: Materials and Methods

3.1 Materials and Methods for Chapter 4

3.1.1 Ethical Approval

Ethical approval for this study was sought and granted by the West of Scotland Research Ethics Committee (REC 4 - 11/WS/0117).

3.1.2 Statistical Design

This was a pilot study. Determining sample size for a pilot study is known to be difficult and the literature concerning this issue is limited. Van Belle[204] and Julious[205] suggested the "rule of 12" for pilots studies as they found that increasing a sample size to 12 participants had a large effect upon the confidence intervals of observed data; but increasing the sample size further did not. A sample size of 12 is also considered practical for small scale studies whilst still being able to provide valuable preliminary information[206]. This was discussed with a medical statistician and it was felt that a sample size of 12 would feasibly provide enough data to drive a statistically valid study or indeed provide data to generate power calculations for further larger scale studies in this area.

3.1.3 Patient Selection

Patients due to undergo breast reconstruction with free unilateral DIEP flaps by Mr IR Mackay were identified from the surgical waiting list at Canniesburn Plastic Surgery Unit, Glasgow Royal Infirmary.

3.1.3.1 Inclusion Criteria

1. Female breast reconstruction patients

2. Age between 18-70 years

3. Due to undergo either immediate or delayed unilateral free DIEP flap breast reconstruction

3.1.3.2 Exclusion Criteria

1. Bilateral reconstructions

2. History of hyperthyroidism or thyroid adenoma (ICG dye contraindicated in such patients)

3. History of allergic reaction to iodine or ICG dye

4. ASA grade ≥ 3

5. Patients with abdominal scarring

3.1.3.3 Consent

Potential participants were approached with an information sheet about the study. Written informed consent was obtained from all patients.

3.1.4 Clinical assessment

Patient information including demographics, past medical history, drug history and social history were collected, as per standard pre-operative breast reconstruction surgery, during each patients' pre-operative assessment and this information stored in the patients notes.

3.1.5 Randomisation Process

In order to assess the contribution of each individual component of the superficial vascular system on the perfusion of Zone IV four different intervention sequences were required. These sequences were as follows

- Both SIEA and SIEV clamped (traditional flap)

- Both SIEA and SIEV unclamped (arterial and venous augmentation)

- SIEA unclamped and SIEV clamped (arterial augmentation)

- SIEA clamped and SIEV unclamped (venous augmentation)

The order in which each intervention sequence was carried out was randomised via an independent internet based randomisation service; utilising true randomness generation via atmospheric noise[207], for every patient prior to the commencement of their operative procedure.

3.1.6 Environmental Conditions

All procedures/measurements were carried out in the same theatre under the same controlled environmental conditions.

Previous studies with regards to measurement of cutaneous blood flow using Laser Doppler suggest that capillary blood flow within skin is stable when environmental temperatures are between 17-28 degrees Celsius[208,209] thus ambient theatre temperature was set at a level between 25 and 27 degrees Celsius and was recorded prior to and during the measurement process to ensure that it was kept constantly within this range.

It has been shown that reflected light can interfere with LDI scan results in terms of falsely high flux readings[209]. In order to standardise environmental lighting and reduce possible interference from reflected light sources during the scanning process the main operating theatre lights were turned off and the internal lighting dimmed to lowest possible level. Windows were temporarily occluded to block any other external sources of light from entering the theatre.

During the scanning capture period of the study all entrances to the theatre were closed and signs placed to prevent intrusion and interruption to the study.

3.1.7 Laser Doppler Imaging

3.1.7.1 Laser Doppler Imaging Machine Specifications

A MoorLDI2-IR laser Doppler blood flow imaging system (Moor Instruments, Axminster, Devon, UK) owned by the Canniesburn Research Trust was used during the human study. The device is portable, based upon a mobile stand and consists of an imaging head, monitor and computer. The imaging head is linked to an articulated arm allowing easy positioning over the study area; containing an infra-red laser diode operating at 785nm optical capture device and also a digital video/photo capture device which can display images in real time. The central computer contains the software necessary to capture, process and then store the LDI images for each patient study. The monitor allows real-time viewing of the digital video feed intra-operatively and to review the captured LDI images post-operatively. (See Figure 3.1)

Figure 3.1: MoorLDI2-IR laser Doppler blood flow imaging system
Image reproduced with permission from Moor Instruments Ltd. 2015

This system was serviced and calibrated prior to the commencement of the study by Moor Instruments, Axminster, Devon, UK.

3.1.7.2 Preparation for Laser Doppler Imaging

The LDI machine was brought into the theatre suite and turned on prior to the commencement of each patients operation, as per manufacturers' guidance that a 30 minute period should be allowed to elapse from initialisation of the system to first scan sequence. Once the 30minute period had passed the LDI machines calibration was ensured using the supplied sealed calibration block and the machine recalibrated if required to ensure validity of scan results. The machine was then placed to the side within the operating theatre until required for the first scanning sequence.

3.1.7.3 Technique of Laser Doppler Measurement

The LDI machine was positioned prior to the start of the first scanning sequence. In order to standardise the positioning of the LDI imaging head and ensure that the same area of the

flap was scanned in each subsequent scanning sequence the mobile base of the LDI machine was locked in position and its articulating arm set at the furthermost point with the area of the flap to be scanned within the imaging window. The imaging head height was set at 30cm from the flap and this position locked. The mobile base was not moved between individual scanning sequences and only the articulating arm was fully retracted and fully extended between scanning sequences.

Moor BDA version 5.3 software was used to acquire images due to its ease of use, greater reliability and consistency. Scanning parameters were set as medium scan type (resolution of 64) on a large vertical rectangle at 4milliseconds/pixel. Each LDI scanning sequence took exactly two minutes. Scanning sequences for each patient in the study were stored on the hard drive for subsequent analysis.

3.1.8 ICG Angiography

3.1.8.1 ICG Angiography Machine Specifications

A SPY® intra-operative fluorescence vascular angiography system developed and manufactured by Novadaq® (Novadaq Technology Inc., Mississauga, Ontario, Canada) was used in the human study. This device was supplied to our plastic surgery unit on loan by CardioLogic® (CardioLogic Ltd, Yorkshire, United Kingdom) the UK distributor of the Novadaq SPY imaging technology. (See Figure 3.2)

Figure 3.2: SPY® intraoperative imaging system

The device is portable and easily movable around the theatre suite. It consists of an imaging head, monitor, computer and optical storage drive. The imaging head is linked to an articulated arm allowing easy positioning over the study area, containing both a laser operating at 805nm (to cause fluorescence of the ICG dye) and an optical capture device which can record in real time the resultant fluorescence. The central computer contains the software necessary to capture; process and then store the image sequences on the optical storage drive for each patient study. The monitor allows real-time viewing of the fluorescence intra-operatively and to review the captured sequences.

This system was serviced and calibrated prior to the commencement of the study.

3.1.8.2 Preparation for ICG Angiography

The SPY® imaging system was brought into the theatre suite and turned on prior to the commencement of each patients operation. The machine was then placed to the side within the operating theatre until required for the first ICG angiography scanning sequence.

The ICG dye used in this study was ICG-Pulsion® manufactured by Pulsion Medical Systems (PULSION Medical UK, Middlesex, United Kingdom). This comes in a sterile vial containing a lyophilised form with approximately 25mg of active ICG and sodium iodide as a preservative. This was reconstituted with 5ml of sterile water prior to yield a constitution of 5mg/ml.

3.1.8.3 Technique of Indocyanine Green Angiography Measurement

The SPY® imaging system was positioned prior to the start of the first scanning sequence. In order to standardise the positioning of the SPY® imaging head and ensure that the same area of the flap was scanned in each subsequent scanning sequence the mobile base of the SPY® machine was locked in position and its articulating arm set at the furthermost point with the area of the flap to be scanned within the imaging window. The imaging head height was set at 40cm from the flap and this position locked. The mobile base was not moved between individual scanning sequences and only the articulating arm rotated between scanning sequences.

2ml of reconstituted ICG dye (5mg/ml) (cf. 3.1.8.2) was injected into a peripheral access line immediately followed by a fluid bolus of 20mls of sterile water. 20 seconds was allowed to elapse to allow the dye to fully enter the circulation (as per the manufacturers' guidelines) and then the scan of flap skin paddle using the SPY® intraoperative imaging system was commenced. Immediately following the scan of the flap skin paddle a subsequent scan was performed upon the fat component of the flap to assess fat perfusion. Each scanning sequence lasted 38 seconds and sequences were stored on hard drive for subsequent analysis.

Between each change in variable to be assessed and subsequent scanning sequence; a time interval of 10 minutes was allowed to pass in order for the dye to be fully eliminated from the circulation as was the protocol in a previous study carried out within our unit[168] and shown in other published work[210].

2ml of ICG dye (5mg/ml) was required for each combined skin/fat scanning sequence and a total of 40 mg was used upon each study participant.

3.1.9 Operative Procedure and Measurement Process

To improve standardisation of the results, a single Consultant Plastic Surgeon (Mr I R Mackay) was responsible for the raising of all DIEP flaps within the research cohort.

Each patient was marked pre-operatively, with the borders of the abdominal flap being defined as per the surgeons' routine practice. The upper incision curves down bilaterally from the superior umbilicus to the left and right anterior superior iliac spines. This incision can be extended more laterally beyond the anterior superior iliac spines, if required to prevent "dog ear" deformity, dependent upon each patient's body habitus. The lower incision is designed like a skewed "W" with the lowest points being approximately 8 cm lateral to midline and a slightly upward curve over the mons pubis area. (See Figure 3.3)

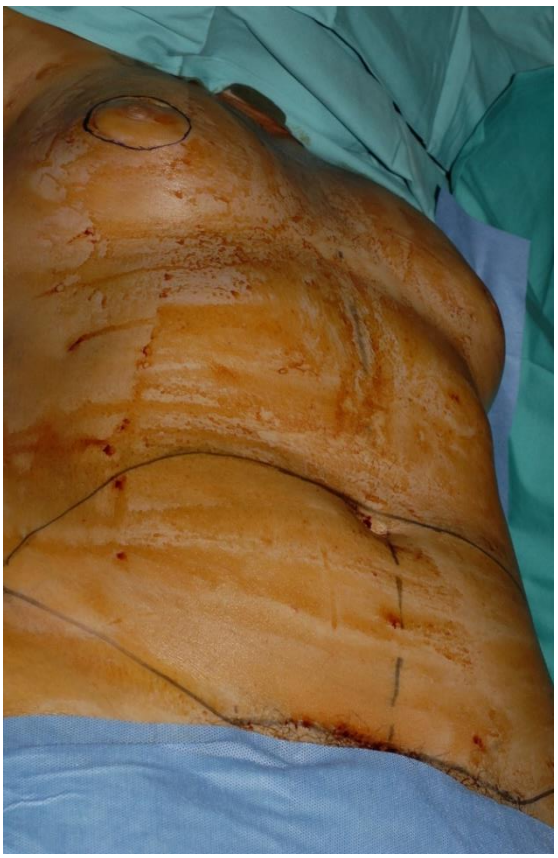

Figure 3.3: Patient prepped and draped for surgery to commence with pre-operative markings visible

All procedures were carried out under General Anaesthesia by one of three senior consultant anaesthetists. The nature of the study had been discussed in detail with each anaesthetist prior to commencement of each procedure in an effort to ensure that each patient was kept as haemodynamically stable as possible during the measurement process. Patient variables (heart rate and systolic/diastolic blood pressure) were recorded every 5

minutes throughout the operation. Patient core temperature was also recorded every 15 minutes through the use of a nasal temperature probe. Maintenance of patient temperature was aided by the use of Bair Hugger™ blanket covering areas of the patient out-with the operative field.

Raising of each DIEP flap was carried out as standard from lateral to medially bilaterally; however considerable additional care was taken to identify and preserve the superficial vascular system (SIEA/SIEV), initially on both sides. (See Figure 3.4)

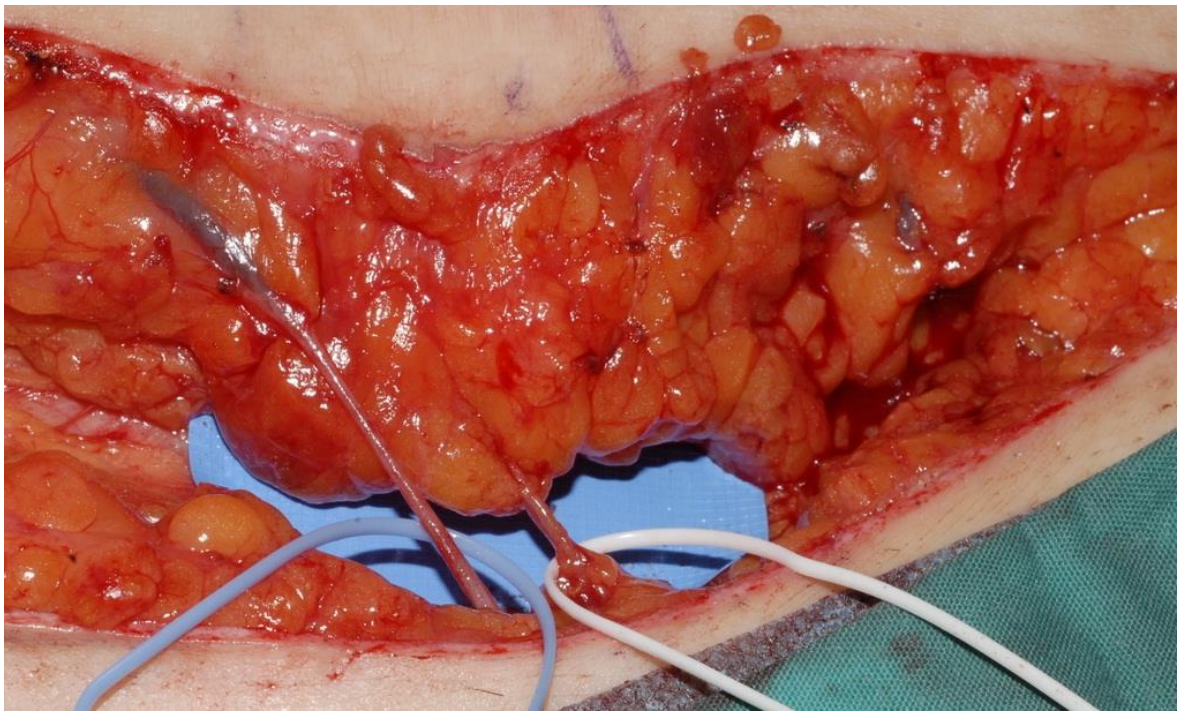

Figure 3.4: Right SIEA (white vessel sloop) and SIEV (blue vessel sloop) dissected free

Concomitantly the patient underwent mastectomy (unless delayed) by one of two Consultant Breast Surgeons. The medial 4th costochondral cartilage was removed and the underlying internal mammary artery (IMA) and internal mammary vein (IMV) prepared to act as recipient vessels for microvascular transfer.

After deciding upon which side to base the flap (dependent upon the adequacy of the perforating blood vessels in terms of size, dissection required and pulsatility); the perforator(s) were dissected to their origin from the main pedicle (DIEA/DIEV). (See Figure 3.5)

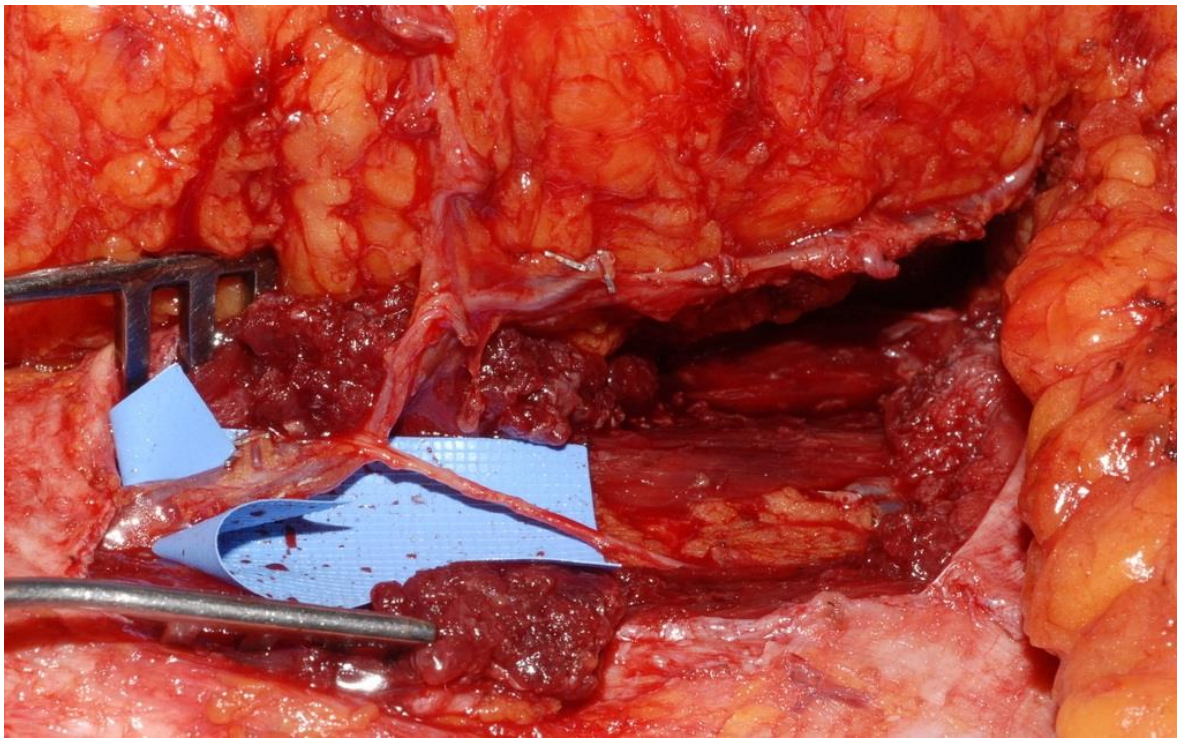

Figure 3.5: Patient 12 in study showing DIEP flap based on a left large medial row and small lateral row perforator with an intramuscular course
The preserved motor nerve can be seen crossing over the top of the pedicle

The ipsilateral superficial vascular system to the main pedicle was divided, leaving the contralateral superficial vascular system intact.

The DIEP flap was completely islanded on only the perforators supplied from the main pedicle (DIEA/DIEV) and contralateral superficial vascular system (SIEV+/-SIEA).

The flap was divided into the classical Hartrampf's zones of perfusion (I-IV) and these areas marked. The flap was then temporarily secured in its anatomical position with several silk sutures on the side not being assessed (i.e. contralateral side to zone IV), as shown in Figure 3.6.

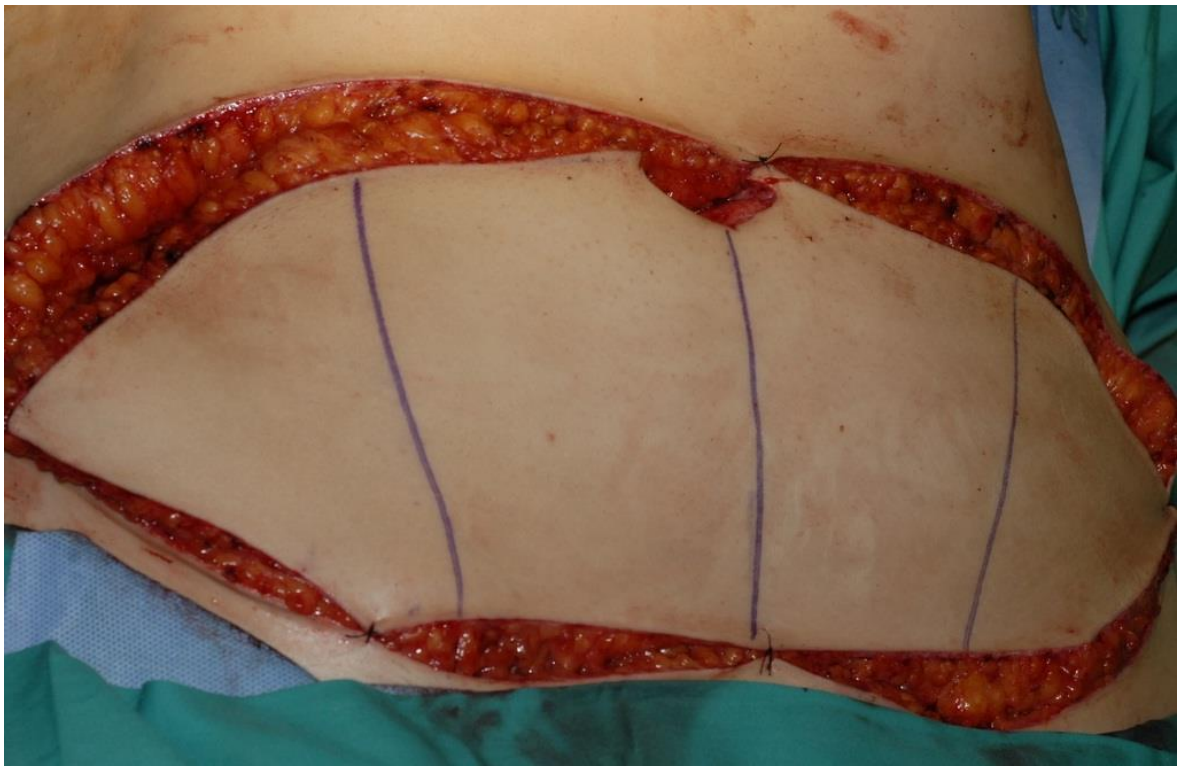

Figure 3.6: Left DIEP flap divided into Zone I-IV and secured in place prior to scanning.

Single arterial and venous Acland clamps (3A and 3V respectively) were used to occlude the SIEA, SIEV or both as per the randomised intervention sequence (cf. 3.1.5). Following each clamping/unclamping intervention a period of 5mintues was allowed to elapse prior to allow perfusion within the flap to stabilise and reactive hyperaemia to settle[211].

Perfusion measurements using LDI (cf. 3.1.7.3) and ICG angiography (cf. 3.1.8.3) were obtained.

Each measurement sequence was carried out as per the flowchart diagram shown in Figure 3.7.

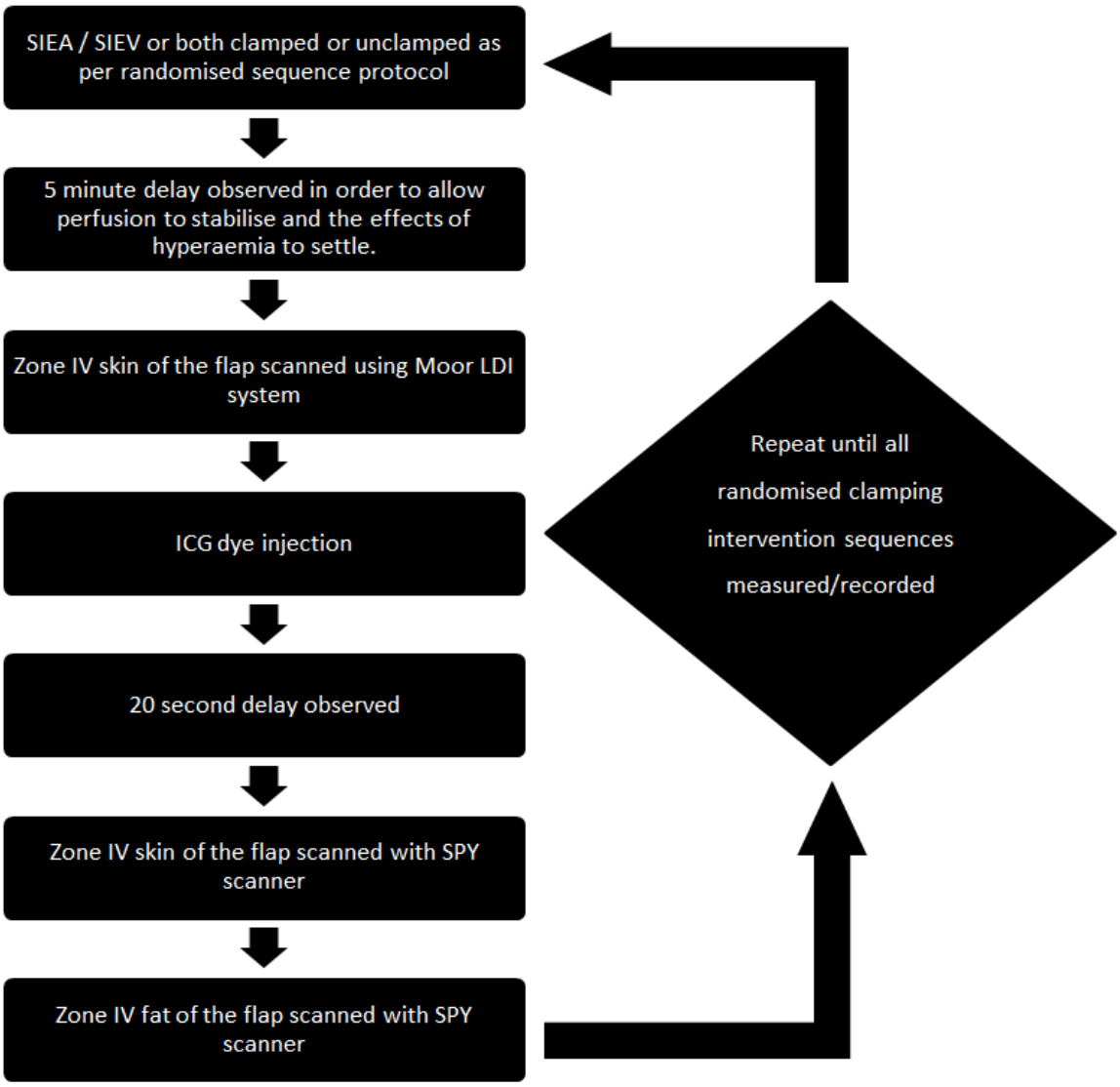

Figure 3.7: Flowchart summarising clamping intervention and scanning process

Upon completion of the measurement process the patients operation proceeded as standard, with Zone IV of the flap being discarded (surgeons' routine practice), the flap detached completely and transferred to the recipient site. The DIEA/DIEV was anastomosed to the IMA/IMV end to end using 9.0 sutures; flap inset, rectus sheath repaired and the abdomen closed via an abdominoplasty.

3.1.10 LDI Scan Analysis

Moor BDA version 5.3 software was used to analyse all captured patient LDI images. This post-processing software allows objective measurements to be made on each of the captured LDI images. A region of interest can be set for only a specific area within the entire LDI image to be assessed independently; in this case Zone IV of the flap.

In order to ensure that the same region of interest was set for each patients flap, between the varying scan sequences, the region of interest was always measured as the area encompassing 1cm from the flap peripheral margins to 1cm within the marked Zone IV border. By measuring 1cm from the flap periphery this also helped to reduce the artefact created by the slight curvature of the cut edges which can influence light reflection and lead to inaccurate flux readings[209].

Median flux values of Zone IV perfusion values for each patient were obtained. Median LDI flux values were used as previous published work by Tollan et al assessing similar aspects of DIEP flap perfusion has suggested that median LDI flux values give more accurate results[212].

3.1.11 ICG Scan Analysis

Captured SPY imaging sequences were analysed via SPY-Q analysis toolkit software. This post-processing software allows objective measurements to be made on each of the captured study sequences. A region of interest can be set for the area to be reviewed within the image sequence and baseline fluorescence intensity for this area can be calculated for the duration of the capture sequence. The baseline fluorescence intensity can then be used for quantitative comparisons between imaging sequences.

In order to ensure that the same region of interest was set for each patients flap, between the varying scan sequences, the same process was utilised as for LDI Scan analysis (cf. 3.1.10) with the region measured at 1cm from the flap periphery.

Mean Zone IV skin and fat perfusion values for each patients scanned sequences were obtained.

3.1.12 Statistical Analysis

All results were analysed using Minitab version 16 (Minitab Inc., State College, PA).

Continuous data was assessed for normality using the Anderson-Darling test of Normality. A repeated measures analysis of variance (ANOVA) test was used to assess for statistically significant differences between each of the clamping/vascular augmentation interventions. Where $p<0.05$ in the repeated measures ANOVA test the Bonferroni correction method

was used to assess for pairwise differences between each vascular augmentation intervention and the control group (traditional flap).

Since it was well established that the SIEA may be absent in up to 13-35% of patients[65-67] it was proposed that should the SIEA be absent in any of our study cohort that the SIEV clamping and unclamping still be utilised for analysis. In such cases when the SIEV was clamped the result obtained was placed in the "Both Clamped" and when the SIEV was unclamped the result was placed in the "SIEA Clamped / SIEV Unclamped" category to allow for correlation. Separate sub-analysis excluding those patients where the SIEA was not present was undertaken to ensure accuracy of the results.

3.2 Materials and Methods for Chapter 5

3.2.1 Ethical Approval

This study was approved by the University of Glasgow Ethical Review Panel. A personal investigator licence (60/12807) and project licence (60/4415) under the terms and conditions of the Animals (Scientific Procedures) Act 1986, was granted by the UK Government Home Office.

3.2.2 Statistical Design

As this was a pilot study measuring the effects on perfusion and flow of vascular augmentation of rat abdominal flaps there was not sufficient data in the literature upon which to base a power calculation.

The Home Office and The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), a UK based scientific organisation dedicated to reducing the number of animals used in research, suggest the following resource equation when deciding upon animal numbers to be used in small non-routine pilot studies such as our own.

E = N (number of animals per treatment x number of treatments) - T (number of treatments)[213].

They state that E (the sample size) should be a number between 10 and 20, to use the minimal number of animals possible whilst providing scientifically valid results.

10 animals were proposed to be used in this study comprising of two treatment groups (traditional vs vascular augmented) and thus the following resource equation was made:

E = ((10 x 2) – (2)) = 18

This fell within the recommended sample size of 10-20 as stated above. However the resource equation wasn't entirely applicable to our study as the above equation considers the number of treatment arms to require two separate animal cohorts. In our study we were proposing to perform each treatment arm upon the same abdominal flap of a single animal using the clamping intervention as described above rather than requiring a separate cohort of animals. As such rather than requiring 20 animals (10 in each cohort) to perform our

research upon we would only require 10 overall. After discussion with the Veterinary Surgeon representing the Biological Research Division, Veterinary Research Facility, University of Glasgow he suggested the addition of a further 2 animals to our proposed cohort of 10 to account for any unexpected complications or mortalities secondary to anaesthesia or procedure during the experiment.

Thus 12 animals were proposed to be utilised in this experiment and the ethical review panel and Home Office were satisfied that this adequately reduced the number of animals required whilst still providing scientifically valid results.

3.2.3 Animals

Male out-bred Sprague Dawley rats were chosen to be used for this research study. This decision was made following advisement from the Veterinary surgeon from the Biological Research Division, Veterinary Research Facility, University of Glasgow.

3.2.4 Anaesthesia and Peri-operative care

All animals in this study had the same anaesthetic protocol devised by the named Veterinary surgeon for the project. Anaesthetic induction was carried out by the named Veterinary Surgeon and maintenance of their anaesthesia and peri-operative care provided by specialist animal technicians under his direct supervision.

Animals were weighed pre-operatively; results recorded and their anaesthesia requirements calculated. Induction was carried out via administration of gaseous Isofluorane in an animal containment box combined with further intra-peritoneal injection of Inactin® (thiobutylbarbitole). Following induction all animals received an additional intraperitoneal injection of atropine to reduce secretions.

Maintenance inhalational anaesthesia (N_2O/Isoflourane/O_2) was provided to each animal via voluntary respiration through the use of a nasal cone secured to each animals' snout.

Each animal in the study had their heart rate and oxygen saturations continuously monitored via the use of a Nonin 8500AV pulse oximeter probe (Nonin Medical Inc., Plymouth, MN, USA) attached to a hind limb.

Each animals' temperature was maintained through the use of an ambient heat source situated in proximity to the animal. Core temperature was monitored throughout the procedure through the use of a rectal probe and the heat source moved as necessary to maintain an adequate temperature between 37-38°C. The rectal probe temperature monitor was also connected via analogue output to a Powerlab® 400 System to allow continuous capture and recording of temperature data throughout the measurement period.

3.2.5 Randomisation Process

The side upon which to base the flap and the order of scanning (traditional or vascular augmented) was randomised via an independent internet based randomisation service[207].

3.2.6 Environmental Conditions

All procedures were carried out in the same room within the same research facility with ambient temperature controlled between 21-24.5°C.

3.2.7 Laser Doppler Flowmetry

3.2.7.1 Laser Doppler Flowmetry Machine Specifications

A Moor VMS-LDF2 laser Doppler flowmetry system (Moor Instruments, Axminster, Devon, UK) obtained via loan from Moor Instruments to the Canniesburn Research Trust was used during the animal study. The device is small and portable, consisting of a central base unit containing a 785nm diode laser and two input/output channels for the attachment of optical probes. The base unit has its own digital display on the face panel but also has an additional analogue output connection. Optical probes supplied with the unit consist of a probe head (to be attached to the target area of tissue), optical cable and a probe tail which can be inserted into the base unit. (See Figure 3.8)

Figure 3.8: MoorVMS-LDF2 laser Doppler monitor
Image reproduced with permission from Moor Instruments Ltd. 2015

This system was serviced and calibrated by Moor Instruments, Axminster, Devon, UK prior to the commencement of the study.

3.2.7.2 Technique of Laser Doppler Measurement

Two optical probe heads were secured in direct perpendicular contact to the central portion of Zone I and Zone IV of the flap respectively using plastic probe head holders and adhesive stickers. Due to respiration causing the animals' abdomen to move and poor adhesion from the stickers supplied an additional piece of Micropore™ tape was placed over the top of the probe heads to ensure good contact of the probe head to the target tissue. Care was taken to avoid any external pressure on the probe head whilst placing the tape to prevent any falsely decreased perfusion readings. Real-time LDF capture sequences were recorded for each study intervention using Moor-VMS-PC for Windows software and stored for subsequent analysis.

3.2.8 Microvascular Flow Measurement

3.2.8.1 Microvascular Flow Measurement Machine Specifications

Microcirculation transit-time flow probes (Transonic V series type 0.5V; Transonic Systems Inc., Ithaca, NY, USA.), were used during this study to assess microvascular flow

through the main pedicle DSEA. These probes are designed to be able to assess flow within vessels of between 0.25-0.5mm in outer diameter, are specifically calibrated for blood temperature of approximately 37°C and can detect flow rates from 0-50ml/min. The probes operate at an ultrasound frequency of 7.2Hz, can detect flow rate changes of 0.05ml/min and have a relative accuracy of ±3%. Probes had been calibrated for use in a study performed the previous year.

Probes consist of a handle (within which runs the cabling to connect to an analogue input) connected to a probe body and reflector. The space between the probe body and reflector create a natural lumen also known as the ultrasonic window. (See Figure 3.9)

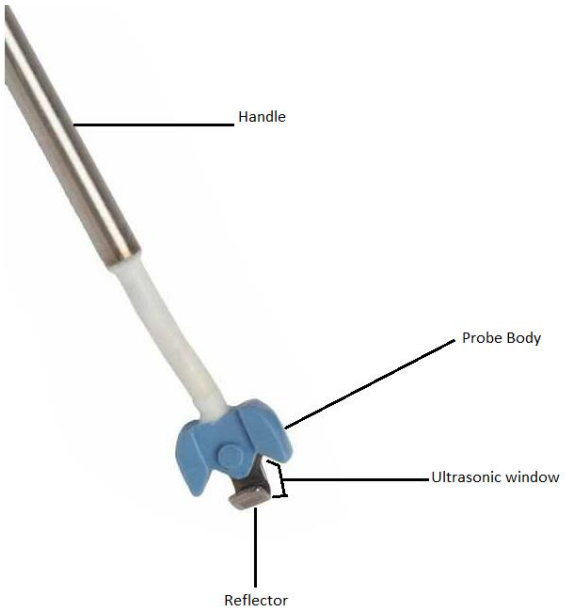

Figure 3.9: Microcirculation flow probe

Two transducers present on either side of the probe body simultaneously convert electrical energy to ultrasonic energy and ultrasonic energy back to electrical energy. The ultrasonic signals are passed back and forth through the ultrasonic window, this is shown in Figure 3.10.

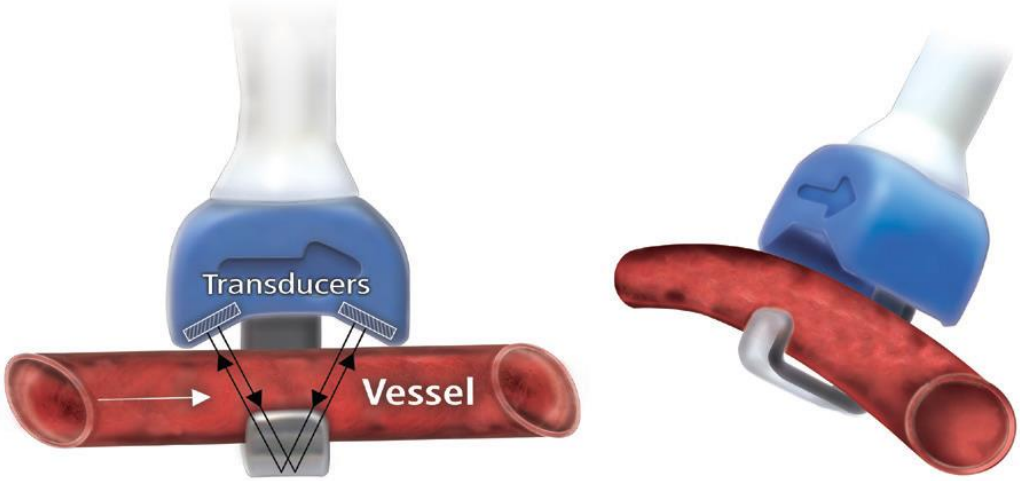

Figure 3.10: Mechanism through which the flow probe operates
Image reproduced with permission from Transonic System Inc. (2015)

The time taken for these signals to reach the opposite transducer can be detected in terms of changes in the electrical signals sent and received using a flowmeter.

Flow probes were attached to a transit-time ultrasonic flowmeter (T204, Transonic Systems Inc.) which converts the analogue signal received from the flow probe into an estimation of mean flow in mL/min and this is displayed on the digital display on the face panel of the machine. Given the small size of the vessels studied and microvascular probes used, the flowmeter was set on low-flow setting which allows a more accurate assessment of intravascular flow within small vessels by recording separate flow measurements every 0.0025 seconds. This data was subsequently digitised through an analogue output connection to a Powerlab® 400 System (AD Instruments Pty Ltd.). The Powerlab® 400 System was in turn connected via USB cable to a laptop PC running Chart V5.56 for Windows (AD Instruments Pty Ltd.) software allowing continuous capture of flow data.

3.2.8.2 Preparation for intra-vascular flow measurement

Flow probes were soaked in sterile saline (maintained at a temperature of 37°C) for at least ten minutes prior to the measurement process commencing. This was in line with manufacturer recommendations to eliminate a random-drift in zero offset.

3.2.8.3 Technique of intra-vascular flow measurement

Flow probes were sited within the incised rectus sheath and held firmly in a stable position using an external stand and microvascular clamp. The DSEA was placed within the lumen

of the probe and drops of sterile saline (maintained at a temperature of 37°C) were used to submerge the area and act as an acoustic couplant. A five minute interval was observed after the placing of the microvascular flow probe prior to obtaining any measurements to allow for stabilisation of the vessel and flow following positioning within the lumen of the probe.

Continuous capture and recording of flow data was made using the Powerlab® 400 System / PC interface (cf. 3.2.8.1).

3.2.9 Experimental Procedure

3.2.9.1 Flap Raising

To ensure standardisation of the results obtained all animal flaps were raised in the same manner by the same surgeon (thesis author). Following anaesthetic induction (cf. 3.2.4) each animal's abdomen was depilated using a mechanical razor.

Animals were positioned supine upon a dissecting board and their limbs extended. The periphery of the abdominal flap was marked and the flap subdivided into four Zones to mimic Hartrampf's Zone of perfusion (cf. 1.6.4) (See Figure 3.11)

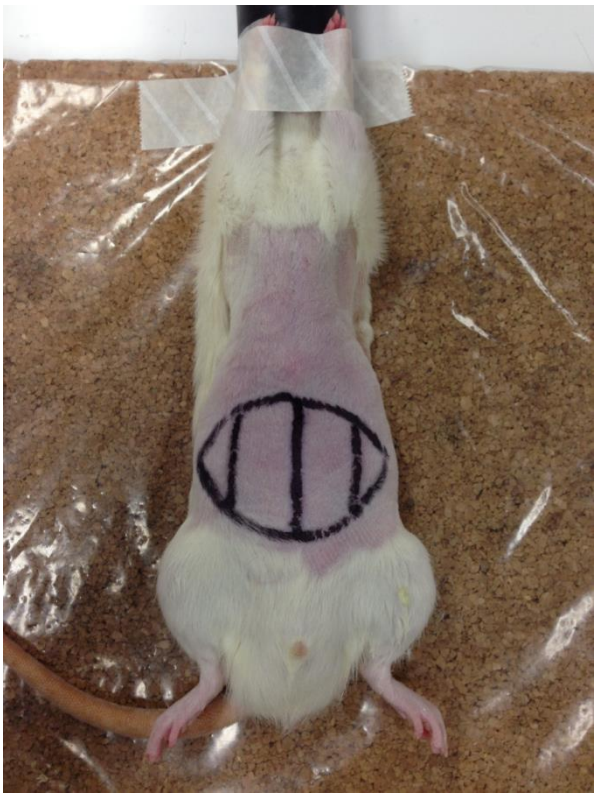

Figure 3.11: Anaesthetised Sprague Dawley rat in supine position with abdominal flap marked

Raising of each abdominal flap was carried out as per similar technique to the method described by Oskar et al[214] under the aid of an operating microscope and is described below.

Initial incision through the skin and fat to the level of the muscle fascia/rectus sheath was made using a disposable 15 blade scalpel on one side of the flap. Haemostasis was achieved throughout the procedure via the use of bipolar diathermy. Upon reaching the level of the muscle fascia/rectus sheath microsurgical scissors were used to raise the abdominal flap in a lateral to medial fashion until the perforating blood vessels supplying that side of the flap were identified. The perforating vessels were carefully dissected free circumferentially and the dissection continued until the midline was approached. (See Figure 3.12)

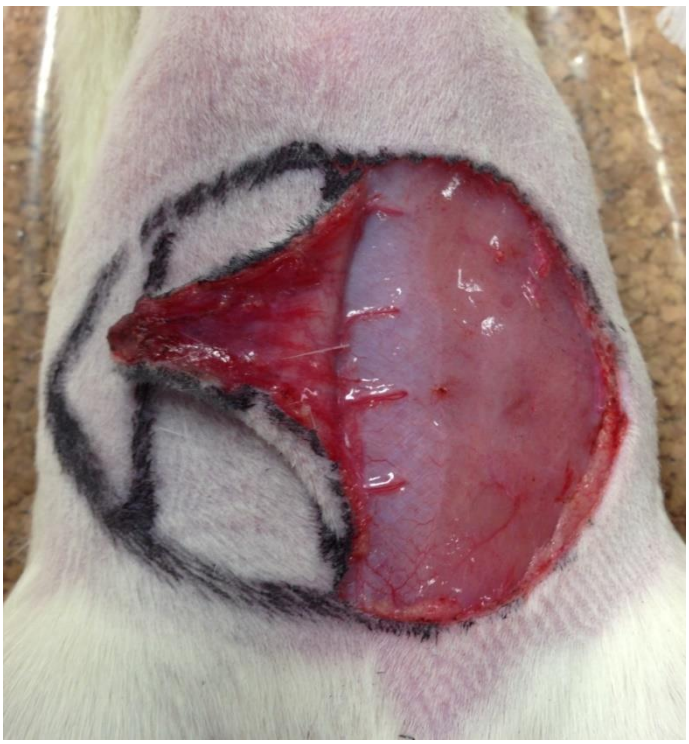

Figure 3.12: Left half of the abdominal flap raised from lateral to medial with the perforating vessels supplying the skin and fat dissected free and clearly visible

At this stage the other side of the flap was raised following the same method to that described above until the flap was completely islanded on only the perforating blood vessels on both sides of the flap. Once islanded an incision was made in the rectus sheath on both sides of the abdomen parallel to the perforating blood vessels. (See Figure 3.13)

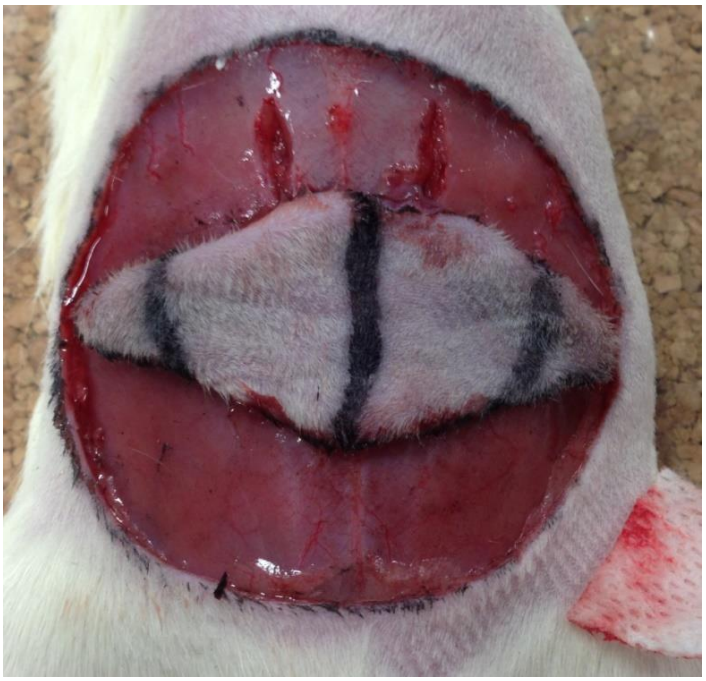

Figure 3.13: Abdominal flap completely islanded on perforating blood vessels with the bilateral incisions through the cranial rectus sheath visibile

Once the main deep superior epigastric vascular pedicle was identified cranially bilaterally it was dissected in a cranial to caudal direction taking extreme care to preserve the perforating blood vessels. Dependent upon the results of flap randomisation (cf.3.2.5), on the side which the flap was to be based the cranial most perforating blood vessels supplying that side was identified and all caudal perforating vessels cauterised. (See Figure 3.14)

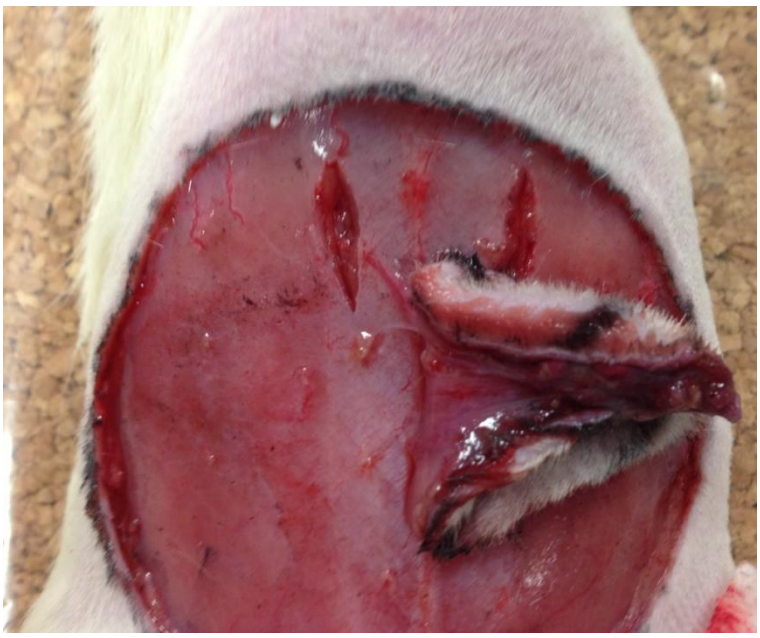

Figure 3.14: Flap based on right side with the solitary cranial perforator clearly visible All caudal perforators on this side have been cauterised

The solitary perforating blood vessels were then carefully dissected through the rectus sheath (taking a small cuff of rectus sheath and rectus muscle to preserve the integrity of the extremely small perforators) to their point of origin from the main deep superior epigastric pedicle. Caudal to the origin of these perforating vessels the deep superior epigastric artery (DSEA) and vein (DSEV) were ligated using 10.0 Ethilon sutures.

On the contralateral side of the flap a similar process was undertaken although the most caudal perforator was selected and dissected completely free to its origin and all cranial perforating blood vessels cauterised above the rectus sheath and ligated at their respective origins from the DSEA and DSEV using 11.0 Ethilon sutures. (See Figure 3.15)

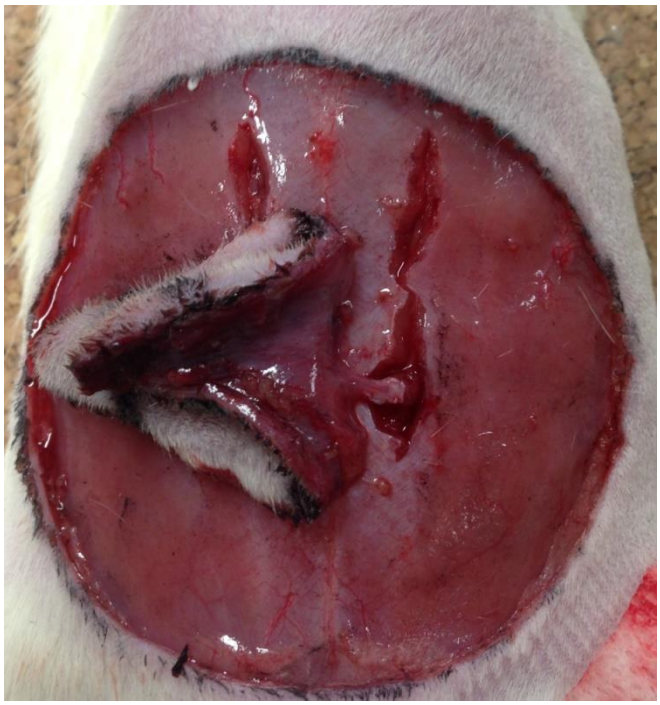

Figure 3.15: Dissection of the caudal most perforator on the contralateral side of the flap

The DSEA and DSEV were then ligated caudal to the dissected caudal-most perforating vessels using 10.0 Ethilon sutures. At the cranial-most point where the DSEA and DSEV are emerging from below the rib-cage on this side of the flap the DSEA and DSEV were dissected free.

In summary at this stage of completed flap dissection the flap is obtaining its vascular supply from a pedicle containing the cranial most perforating artery and vein from the DSEA and DSEV (main pedicle) on one side of the flap and the contralateral DSEA and DSEV pedicle (augmentation pedicle) containing only the caudal most perforating artery and vein.

A transit-time ultrasound flow probe (Model 0.5V, Transonic Systems Inc.) was then inserted through the incision in the rectus sheath and the main pedicle DSEA supplying the flap was placed within the flow probe (cf 3.2.8.3). The flow probe was secured externally in position using a clamp attached to a stand.

The flap was then sutured back into its original position taking care to avoid any tension using 5.0 Ethilon sutures. (See Figure 3.16)

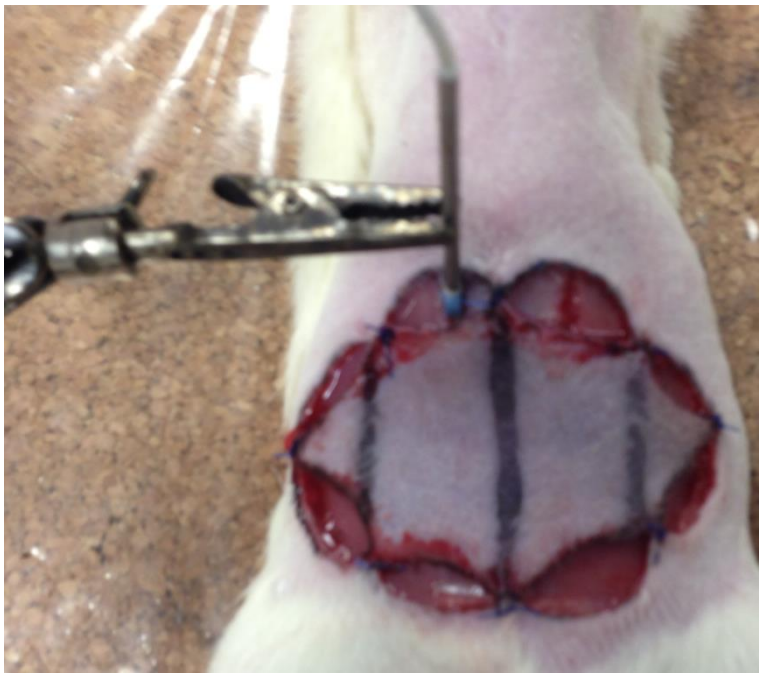

Figure 3.16: Flap completely raised and secured in anatomical position
The transit-time ultrasound flow probe placed around the right DSEA can be observed held in position with a clamp

MoorVMS-LDF2 laser Doppler monitor optical probes were then attached to Zone I and Zone IV of the flap respectively (cf. 3.2.7.2).

The set-up of the multiple machines used in this experiment (MoorVMS-LDF2 laser Doppler monitor; T204 flowmeter, Powerlab® 400 system) is shown in Figure 3.17.

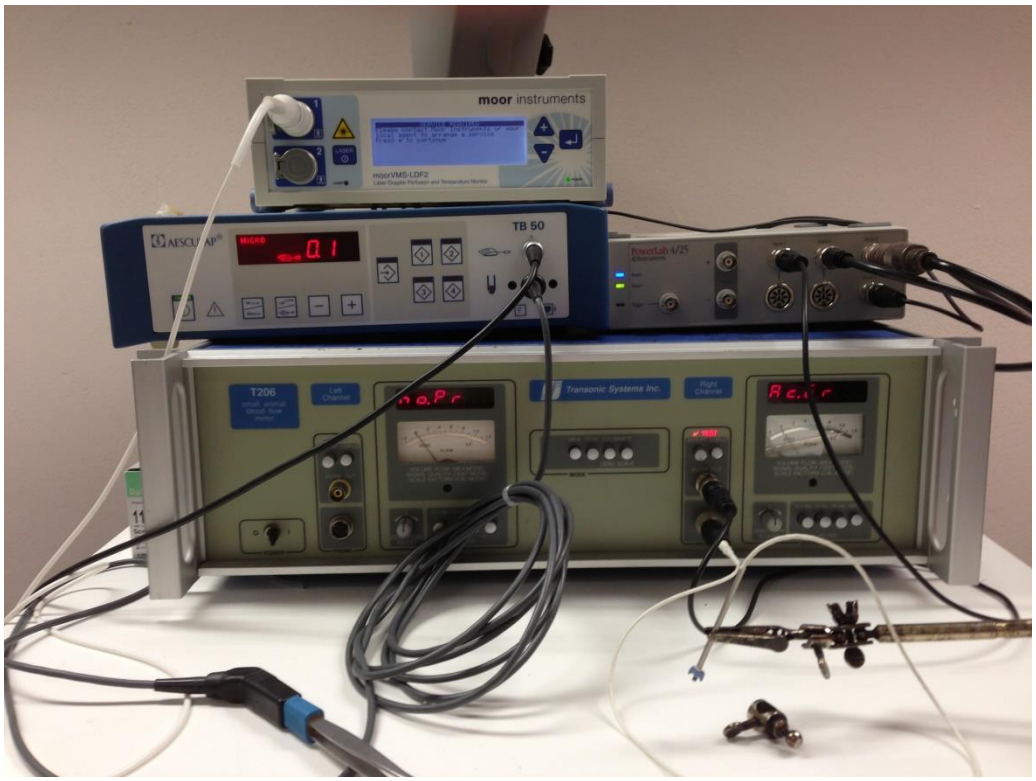

Figure 3.17: Equipment set-up in animal experiment model

If the first scanning sequence had been randomised to assess the traditional flap initially then the contralateral DSEA/DSEV pedicle was occluded using a removable Acland B1-V microvascular clamp.

3.2.9.2 Measurement Process

Upon completion of flap raising (irrespective of clamped or non-clamped pedicle) there was a five minute interval observed prior to the commencement of the measurement process to allow the flap to stabilise and the effects of any reactive hyperaemia to settle[168,211].

Prior to each measurement sequence animal heart rate and oxygen saturation were recorded.

LDF perfusion readings obtained through the optical probes attached to Zone I and Zone IV of the flap were recorded for a period of one minute (cf. 3.2.7.2) and the results stored for subsequent analysis.

Concurrent flow rate measurements through the main ipsilateral SEA were obtained for the same one minute period (cf. 3.2.8.3) and recorded in Chart V5.56 for Windows for subsequent analysis.

Once these measurements were complete the clamp was either placed on or removed from the contralateral DSEA and DSEV and a further 5 minute interval was observed to again allow for reactive hyperaemia to settle.

At the end of the measurement process all animals were humanely terminated via a method approved under schedule 1 to the Animals (Scientific Procedures) Act 1986.

3.2.10 Laser Doppler Flowmetry Analysis

Moor-VMS-PC for Windows was used to analyse the data obtained via the MoorVMS-LDF2 laser Doppler monitor optical probes. Subsequent analysis of captured sequences utilising this software allow for the calculation of mean flux results over the entire capture period.

Mean flux values were obtained from Zone I and Zone IV in the traditional and vascular augmented flaps.

3.2.11 Intravascular Flow / Temperature Analysis

LabChart Reader Version 8 (AD Instruments Pty Ltd.) for Microsoft Windows was used to analyse all data captured by the PowerLab® 400 system. This software allows the user to analyse and interrogate the digital data generated by the Powerlab® 400 System and its analogue inputs.

Mean core temperature values for the entire procedure and mean flow rate through the main pedicle DSEA (for the one minute observation period of the study) were obtained.

3.2.12 Statistical Analysis

All results were analysed using Minitab version 16 (Minitab Inc., State College, PA).

Continuous data was assessed for normality using normal probability plots and an Anderson-Darling test of Normality. Data was found to be non-normally distributed and as such non-parametric tests were chosen for statistical analysis.

Wilcoxin Signed Rank tests were used to assess for any statistically significant differences in the results obtained from the traditional flaps and those obtained for the vascular

augmented flaps. Comparisons were made between Zone I and Zone IV perfusion; flow rate through main DSEA and pulsatility index pre and post vascular augmentation.

3.3 Materials and Methods for Chapter 6

3.3.1 Ethical Approval

Ethical approval for this study was sought and granted by the West of Scotland Research Ethics Committee (REC 1 – 12/WS/0082).

3.3.2 Statistical Design

A medical statistician was recruited to the research team to provide their input for this study in terms of sample size and analysis of the results.

A formal power calculation to judge the required sample size was not possible as this was a pilot study and as such there were no available studies on the topic upon which to obtain a reasonable estimate of the standard deviations required for a calculation.

Traditional studies utilising inter-rater agreement between responses have focussed upon assessing kappa scores (cf. 1.10.4). Simple kappa scores can only recognise absolute agreement or disagreement between raters and do not make any account of the varying levels of disagreement. A modification to the simple kappa score is that of weighted kappa scores; these scores measure varying degrees of disagreement by assigning different "weights" to each of the responses measured. The further apart the responses from each other the higher the weight assigned; i.e. higher levels of disagreement are associated with higher weight allocations.

On the statisticians advice utilising the suggestions by Norman and Streiner[46] who indicated that the sample size for weighted kappa would require too many guesses and invoke a rule of thumb; the minimum number of objects being rated should be more than $2c^2$ (where c is the number of categories).

For our purposes utilising an assessment grading based on four categories we should ideally have $2x4^2=32$ subjects.

As such it we deemed recruitment of 35 patients to be appropriate for the requirements of the study.

3.3.3 Patient Selection

Participants were recruited from the Canniesburn Plastic Surgery Unit areolar tattooing clinic. This was to ensure that all oncological, adjuvant and surgical reconstructive treatments had been completed.

3.3.3.1 Inclusion Criteria

1. Females aged 18 or more years

2. Breast reconstruction patients

3. Greater than six months post breast reconstruction

4. Due to attend for areolar complex tattooing

3.3.3.2 Exclusion Criteria

1. Bilateral reconstructions

2. Unilateral reconstruction less than six months ago

3. Breast reconstructions not specifically carried out following breast cancer excision

3.3.3.3 Patient Consent

Patients meeting the above criteria were written to with a patient information leaflet detailing the study in question. Patients who wished to participate in the study were met prior to their tattooing procedure or tattoo follow-up review and shown the instructional demonstrational digital video clip to ensure that they were fully informed as to what would be expected. Written informed consent was obtained and each patient was given a unique identifiable subject number. Patients were able to withdraw from the study at any time.

3.3.4 Clinical assessment

Information including patient demographics and details upon type of breast reconstruction surgery were collected.

3.3.5 Patient Satisfaction Assessment

Each patient in the study was asked to complete a 22 item Breast Cancer Treatment Outcomes Scale (BCTOS) questionnaire developed by Stanton et al with regards to their opinion on their reconstruction[215] (See Appendix B). This questionnaire requires the patient to compare their reconstruction with the non-reconstructed breast and to score on a simple 1 (no difference) – 4 (large difference) scale for various aspects ranging from cosmesis to function.

3.3.6 Standard photography

All participants underwent standard breast photography as per current Canniesburn Plastic Surgery Unit Guidelines. These comprise of the following static photographic views of both breasts: antero-posterior with hands at sides, antero-posterior with hands raised above head, right lateral, left lateral, left oblique and right oblique. (See figure). Photographs were taken by one of two clinical photographers in the same dedicated photography studio using a Nikon D200® digital single-lens reflex camera. These digital photographs were transferred from the camera onto computer as JPEG image files.

3.3.7 Video capture

As no standard for digital video capture exists, a novel protocol for digital video capture was created by Mr A Malyon (Consultant Plastic & Reconstructive Breast Surgeon). This outlined the instructions for a sequence of movements (incorporating all views obtained by photography), deemed necessary to allow subjective assessment. (See Figure 3.18)

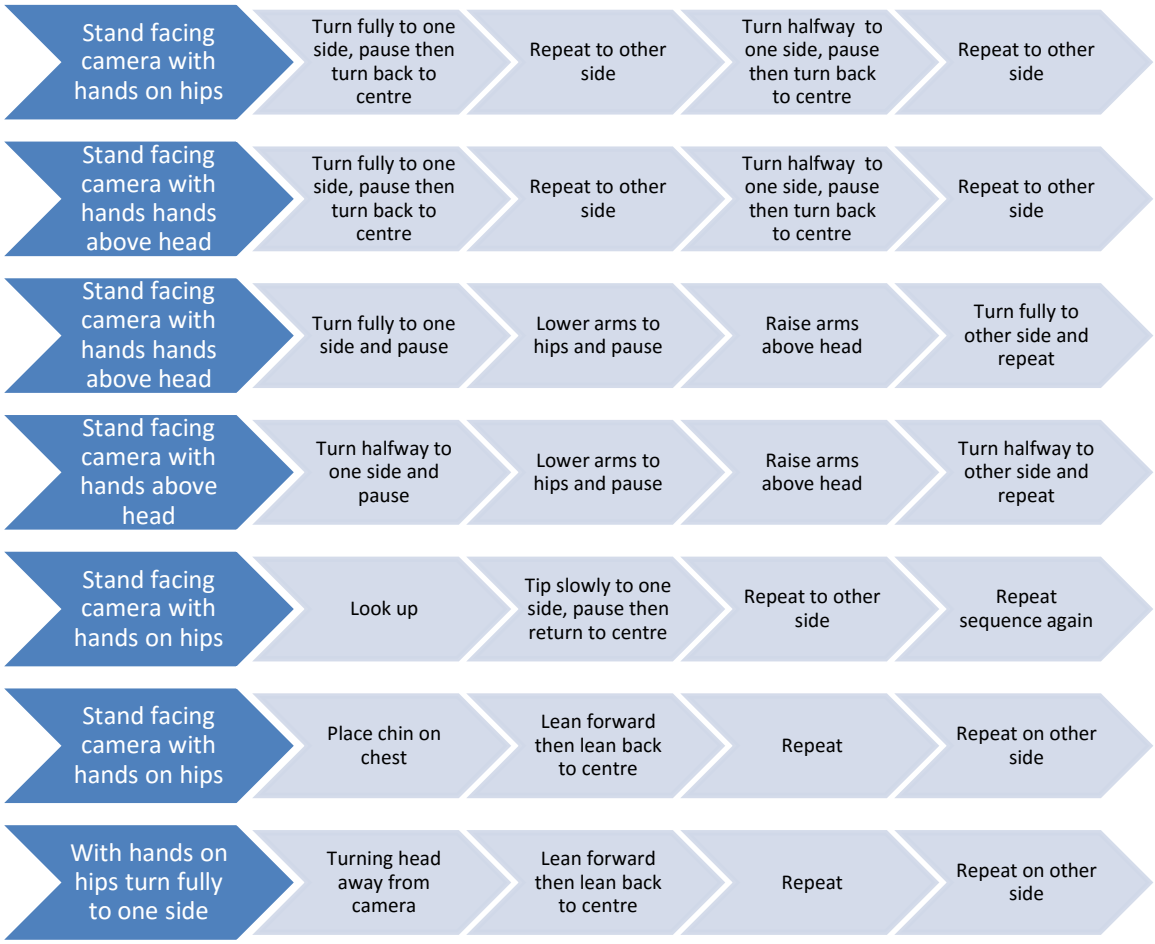

Figure 3.18: Scripted sequence of movements for digital video capture
Image reproduced with permission from Elsevier Ltd. (Gilmour et al. 2014)[216]

The movements were designed to protect patient anonymity at all stages and to prevent defining facial shots to be captured during the filming stages.

The services of a professional female model were utilised to perform the above scripted routine whilst undergoing digital video capture by a cinematographer within a dedicated studio of the Medical Illustration Department; Glasgow Royal Infirmary.

Narrated position and movement instructions were then added to the video footage to create an instructional demonstrational digital video clip providing both clear visual and audio instructions to the viewer.

Following the photography session the patients were moved to a filmography studio and underwent digital video capture whilst being requested to copy exactly the visual and audio instructions of the demonstration video which was being played to them concurrently via a televised feed in their direct view within the studio. All digital video capture was carried

out by a single cinematographer in the same dedicated studio using a Sony PMW-EX1® digital video camera. The video clips were then cropped using Final Cut Pro© software (Apple Inc.) and made into Windows® Media Audio/Video files.

3.3.8 Creation of image/video sets

The six digital photography images for each patient in the study were transferred onto a single Powerpoint slide (Microsoft Powerpoint 2010) to create an image set for each patient and their unique subject number was placed in the non-visible notes section to allow later identification. (See Figure 3.19)

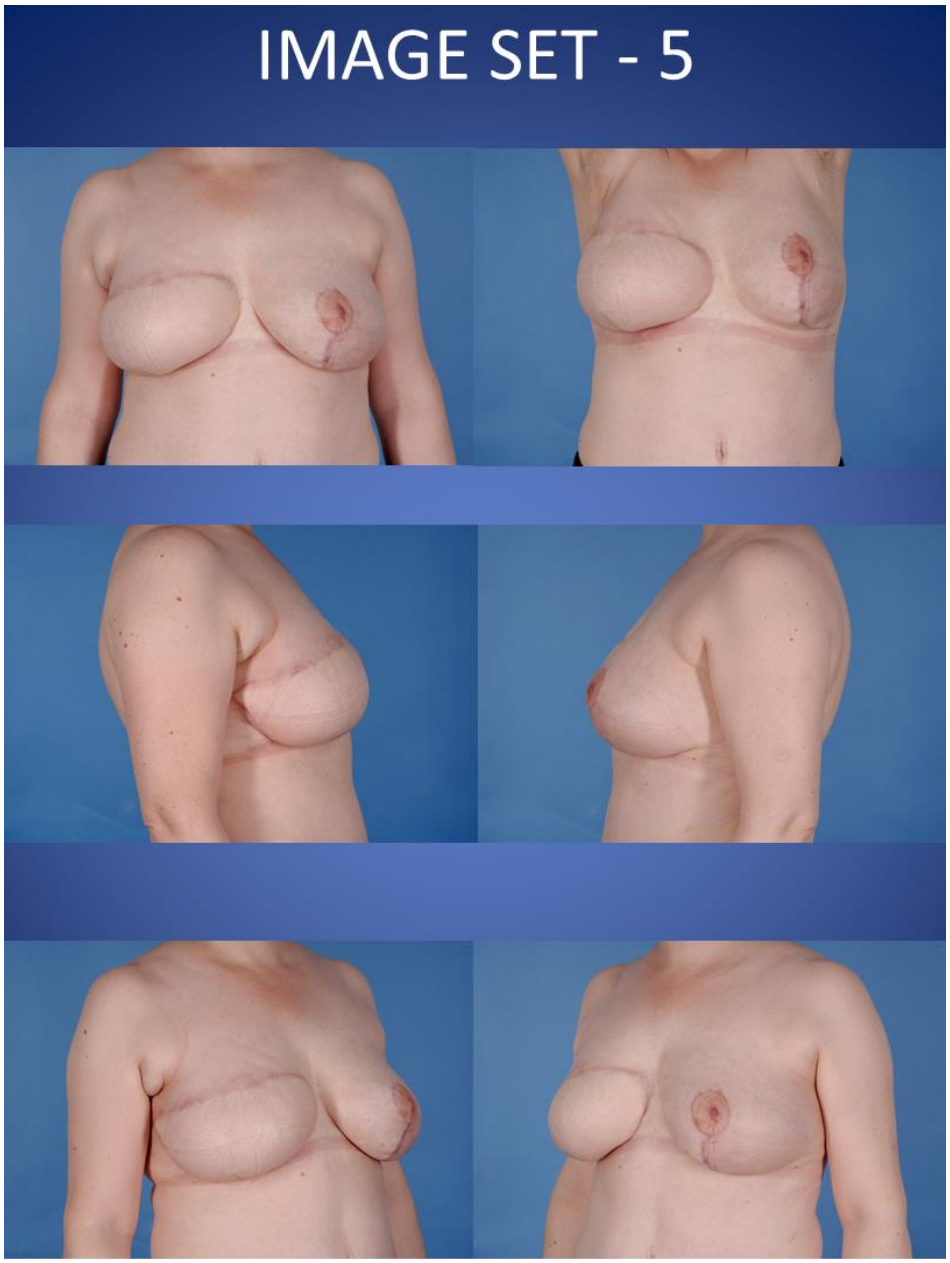

Figure 3.19: Example of an image set used in panel assessment presentation

Each separate patient slide was then compiled to create a complete Powerpoint presentation of all the patient photography image sets. The order of these patient slides was randomised using an online random list generator[207] and the heading of each slide named Image set 1,2,3 and so forth; yet being patient identifiable due to their hidden unique subject number. The same process was repeated to create a separate Powerpoint presentation comprised of the patient video sets.

3.3.9 Panel Assessment Process

A panel comprising of seven Plastic Surgeon (six Consultant Plastic Surgeons all with subspecialist interest in breast reconstruction and one senior onco-plastic breast fellow), five Consultant General Breast Surgeons and nine non-surgical allied health professionals was convened.

Each panel member was issued with a Panel Member Instruction sheet welcoming them to the panel and explaining the assessment process.

The panel members were issued with a simple assessment score sheet in which to record their views. The footer of each assessment score sheet was numbered with a unique panel member assessment number in order to ensure that each sheet was unique to each panel member.

The assessment score sheet asked panel members to record their opinions initially as to their overall opinion of the reconstruction on a simple 1-4 scale as originally devised by Harris[194]. (See Table 3.1)

Score	Rating
1	**Excellent** - Treated breast nearly identical to untreated breast
2	**Good** – Treated breast slightly different than untreated breast
3	**Fair** – Treated breast clearly different from untreated breast, but not seriously distorted
4	**Poor** – Treated breast seriously distorted

Table 3.1: Breast cosmesis assessment scoring scale (Harris et al. 1979)[194]

In addition the panel was also asked to rank their opinion on individual aspects of each reconstruction in terms of Size, Shape and Elevation (and in the case of the video clips Mobility) using a similar 1-4 scale as developed by Stanton et al[215]. (See Table 3.2)

Score	Rating
1	**No difference** between treated and untreated breast and area
2	**Slight difference** between treated and untreated breast and area
3	**Moderate difference** between treated and untreated breast and area
4	**Large difference** between treated and untreated breast and area

Table 3.2: Breast cancer outcomes treatment scale (BCTOS) (Stanton et al. 2001)[215]

The panel was then shown each photograph image set (cf. 3.3.8) as part of the Powerpoint presentation via a projector.

The panel was given approximately 45 seconds to record their opinions upon the static photograph image sets on the dedicated assessment sheets.

Following this the panel was shown the Powerpoint presentation containing the video clips (cf. 3.3.8). Panel members were given the duration of the entire video clip to record their findings; approx. 150 seconds.

Panel members were blinded as to the identity of each patient at all stages. Each panel member was also asked to provide their opinion on whether they found the still photographs or video clips more useful/informative in assessing the post-operative outcome of breast reconstruction.

At the end of the assessment process all panel assessment forms were collected and the results correlated and analysed.

The assessment process was completed in a single evening lasting approximately 3 hours and including a 45 minute refreshment break for the assessors.

3.3.10 Statistical Analysis

All results were analysed using Minitab version 16 (Minitab Inc., State College, PA).

Panel inter-rater agreement was calculated using Kendall's Coefficient of Concordance for both still photographs and video assessments. Kendall's Coefficient of Concordance was chosen due to the ordinal scale based nature of the assessment scores and the fact that this test allows for the measurement of agreement between multiple raters assessing the same sample. The test also takes into consideration the association between the rankings observed.

The values obtained range from 0 (no agreement) to 1 (complete agreement) and can be further interpreted using the guideline developed by Schmidt[217]. (See Table 3.3)

Score	Level of agreement
0.1	**Very weak** agreement
0.3	**Weak** Agreement
0.5	**Moderate** agreement
0.7	**Strong** agreement
0.9	**Unusually strong** agreement

Table 3.3: Guideline allowing interpretation of Kendall's Coefficient of Concordance Scores (Schmidt 1997)[217]

Overall median panel photograph and video scores for each patient were compared to the patient questionnaire self-scores relating to cosmesis (size, shape and elevation) using a Spearman's rank correlation test. This test was chosen due to the difference in assessment scales used by the patient (cf. 3.3.5) and panel (cf. 3.3.9), as it allows the measure of agreement between two sets of linear data. The levels of agreement between panel and patient can be interpreted in a similar manner to the scores above.

Chapter 4: The effect of vascular augmentation on DIEP flap Zone IV perfusion utilising the contralateral SIEA/SIEV

4.1 Introduction

Despite the growing volume of reported vascular augmentation procedures being performed in the literature (cf. 1.8.1) the scientific evidence supporting their use is poor. There are various experimental animal models[162-167] suggesting that these techniques lead to increased flap survivability but there is little in the way of in-vivo research.

The aim of this study was to assess in-vivo the effects of vascular augmentation on DIEP flap Zone IV perfusion by augmenting the flap utilising components of the contralateral SIE vascular system (SIEA & SIEV) and to assess whether arterial, venous or combined augmentation had the greatest effect.

4.2 Methods

DIEP flaps were raised in patients undergoing unilateral breast reconstruction and islanded on only the perforators supplied from the main pedicle (DIEA/DIEV) and contralateral superficial vascular system (SIEV+/-SIEA) (cf. 3.1.9). The flap was divided into Hartrampf's zones of perfusion (cf. 1.6.4). A randomised clamping sequence of the various components of the superficial vascular system was undertaken (cf. 3.1.5) and the resultant effects on Zone IV perfusion measured by LDI (cf. 3.1.7) and ICG angiography (cf. 3.1.8).

4.3 Results

4.3.1 Patients

Twelve consecutive patients due to undergo free unilateral DIEP flap based breast reconstruction meeting the inclusion/exclusion criteria were identified and recruited to this study which was performed from 01/02/12 to 14/02/13.

A summary of patient demographics is shown in Table 4.1.

Patient	Age (years)	ASA Grade	BMI (kg/m^2)	Smoking Status	Pre-operative Chemotherapy	Pre-operative Radiotherapy
1	52	1	30	Non Smoker	No	No
2	42	1	23	Ex-Smoker	2 years prior	No
3	39	1	29	Ex-Smoker	No	No
4	59	2	27	Ex-Smoker	Neo-Adjuvant	No
5	50	1	26	Non Smoker	No	No
6	34	2	24	Current Smoker	2 years prior	2 years prior
7	40	2	26	Non Smoker	No	No
8	33	1	28	Non Smoker	No	No
9	43	1	23	Non Smoker	Neo-Adjuvant	No
10	52	1	27	Non Smoker	No	No
11	54	1	27	Ex-Smoker	No	3 years prior
12	53	2	32	Non Smoker	No	No

Table 4.1: Summary of patient demographics

One total flap loss occurred in Patient 6 of our cohort. The other eleven flaps (92%) healed uneventfully with no recorded partial flap loss or fat necrosis. The mean number of post-operative days till discharge of patients in our cohort was 6.8 (range 6-9days).

One patient in our cohort suffered wide-spread metastases of her breast cancer in the post-operative follow-up period and unfortunately died 1 year post-operatively. All other patients were disease free at last clinical follow-up review.

The randomised order of clamping sequences for each patient is shown in Table 4.2.

Patient	Sequence 1	Sequence 2	Sequence 3	Sequence 4
1	Both Vessels Clamped	SIEA Clamped / SIEV Unclamped	Both Vessels Unclamped	SIEA Unclamped / SIEV Clamped
2	Both Vessels Clamped	Both Unclamped	SIEA Unclamped / SIEV Clamped	SIEA Clamped / SIEV Unclamped
3	Both Vessels Clamped	Both Unclamped	SIEA Unclamped / SIEV Clamped	SIEA Clamped / SIEV Unclamped
4	SIEA Clamped / SIEV Unclamped	Both Vessels Clamped	Both Vessels Unclamped	SIEA Unclamped / SIEV Clamped
5	Both Vessels Unclamped	SIEA Clamped / SIEV Unclamped	Both Vessels Clamped	SIEA Unclamped / SIEV Clamped
6	SIEA Unclamped / SIEV Clamped	Both Vessels Unclamped	Both Vessels Clamped	SIEA Clamped / SIEV Unclamped
7	Both Vessels Clamped	SIEA Clamped / SIEV Unclamped	Both Vessels Unclamped	SIEA Unclamped / SIEV Clamped
8	Both Vessels Unclamped	SIEA Clamped / SIEV Unclamped	SIEA Unclamped / SIEV Clamped	Both Vessels Clamped
9	SIEV Clamped	SIEV Unclamped		
10	SIEV Unclamped	SIEV Clamped		
11	Both Vessels Clamped	Both Vessels Unclamped	SIEA Unclamped / SIEV Clamped	SIEA Clamped / SIEV Unclamped
12	Both Vessels Unclamped	SIEA Unclamped / SIEV Clamped	Both Vessels Clamped	SIEA Clamped / SIEV Unclamped

Table 4.2: Randomisation order for clamping/unclamping interventions
No SIEA was identified in patients 9 and 10

Systolic/Diastolic blood pressure and heart rate were recorded on a 5minute basis for each patient during the 30 minute study intervention and flap perfusion measurement period. Patient core temperature was recorded every 15minutes. These results are shown in Table 4.3.

Patient	Mean Arterial Pressure (mmHg)	Heart rate (bpm)	Core temperature (°C)
1	64.3 (1.2)	62 (0)	36.9 (0)
2	57.7 (3.3)	72 (3.1)	37.6 (0.1)
3	78.2 (1.5)	63 (1.6)	37.6 (0)
4	61.5 (2)	67 (2.4)	36.5 (0)
5	76 (0.9)	73 (1)	36.8 (0)
6	65 (2.3)	64 (2.9)	37.1 (0.1)
7	72.6 (0.5)	80 (1.3)	37.3 (0.1)
8	88 (0)	90 (0.8)	37.6 (0.1)
9	65.6 (1.6)	60 (0.8)	36.7 (0)
10	69.7 (1.2)	54 (1.9)	37.1 (0.1)
11	63.7 (1.6)	49 (2)	36.3 (0)
12	68.5 (0.8)	55 (1.6)	37 (0)

Table 4.3: Patient peri-operative observations
(SD in brackets)

Patient mean arterial pressure and heart rate data during the study intervention and flap perfusion measurement period were plotted into the following box and whisker plots. (See Figure 4.1 and 4.2)

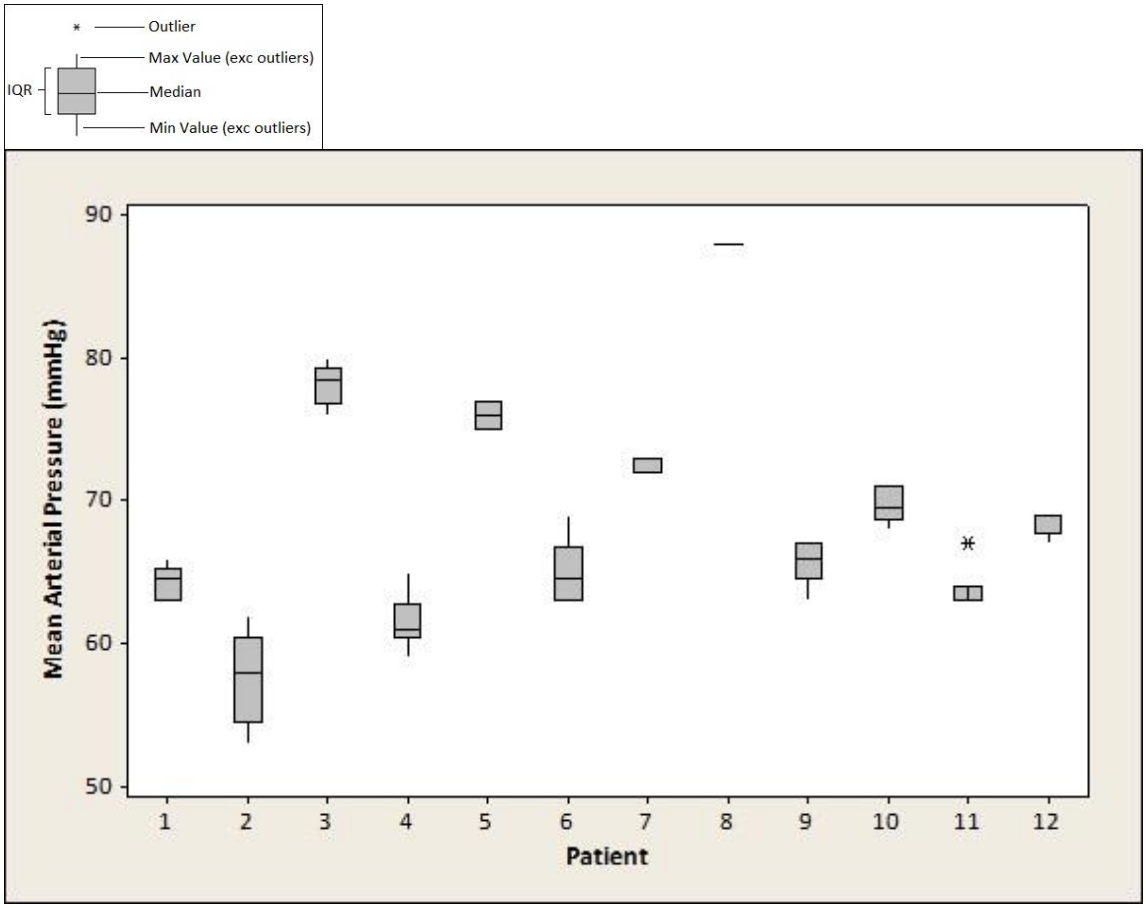

Figure 4.1: Boxplots of patient Mean Arterial Pressure (mmHg) during the investigation phase of the study.
Variation exists between patients but there is minimal variability in each individual patients MAP during the measurement process.

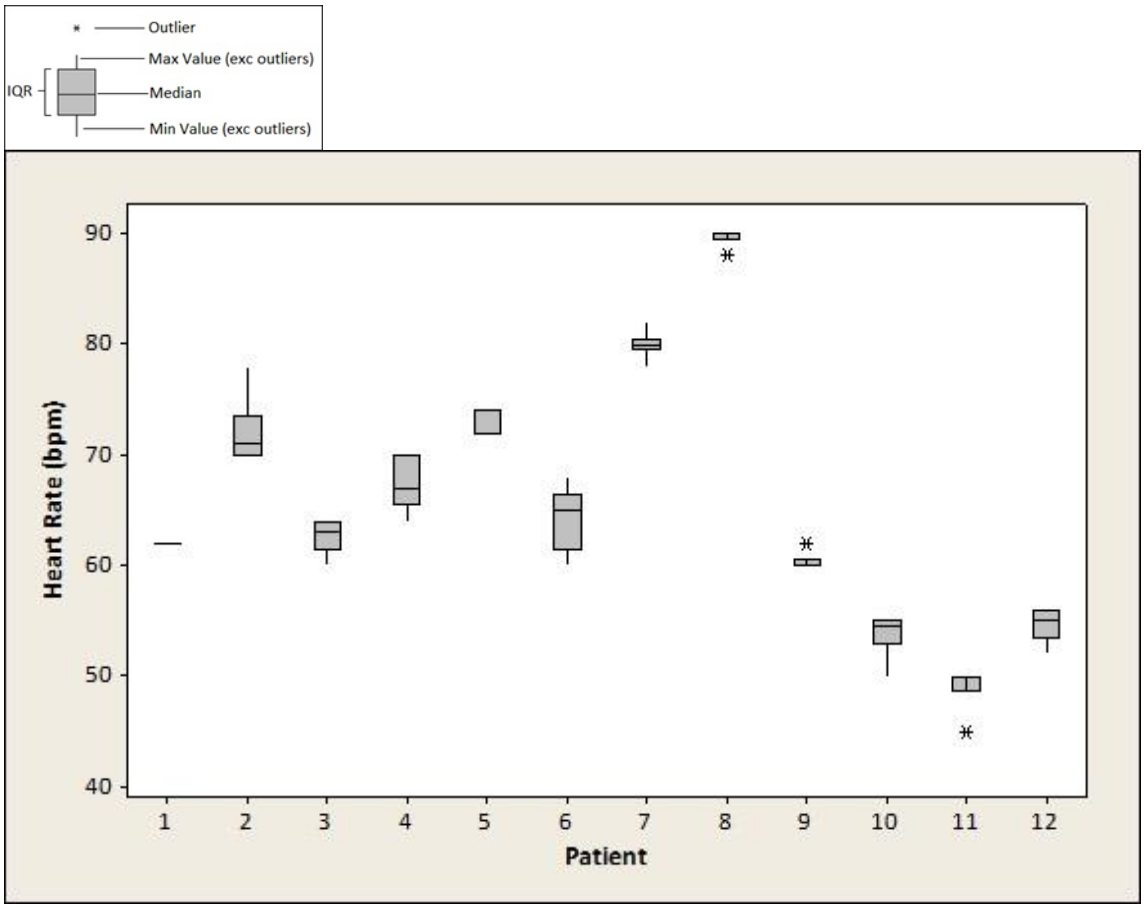

Figure 4.2: Box and whisker plot of patient peri-operative Heart Rate (bpm)
Variation exists between patients but there is minimal variability in each individual patients Heart Rate during the measurement process.

The average interquartile range for patients during the measurement process was 2.2mmHg and 2.1(bpm) for mean arterial pressure and heart rate respectively demonstrating cardio-vascular stability throughout the study measurement process.

4.3.2 Intra-operative Details

Intra-operative details for all patients are summarised in Table 4.4.

Patient	Type of Reconstruction	Mastectomy Side	Mastectomy Weight (grams)	Flap Side	Perforator Choice	Number of Perforators	Flap Weight (grams)	Flap Ischaemia Time (mins)
1	Immediate	Left	988	Right	Medial Row	1	731	73
2	Delayed	Right	NA	Right	Medial & Lateral Row	2	640	68
3	Immediate	Left	878	Right	Lateral Row	1	1007	80
4	Immediate	Right	Not Recorded	Left	Lateral Row	1	Not Recorded	57
5	Immediate	Right	825	Left	Medial Row	2	598	55
6	Delayed	Left	NA	Right	Lateral Row	2	565	81
7	Immediate	Right	466	Right	Medial Row	1	581	74
8	Immediate	Right	750	Left	Medial Row	2	864	96
9	Immediate	Right	730	Left	Medial Row	1	506	36
10	Immediate	Left	774	Left	Medial Row	1	774	82
11	Immediate	Right	590	Left	Lateral Row	1	715	74
12	Immediate	Right	743	Left	Medial & Lateral Row	2	942	59

Table 4.4: Summary of operative details for each patient in study

4.3.3 Zone IV Skin Perfusion assessed using LDI

A static image of each Zone IV of patient flap was obtained, along with a graphical overlay (flux image) demonstrating perfusion. Figure 4.3 shows such an LDI image; in this case Patient 11 from the study.

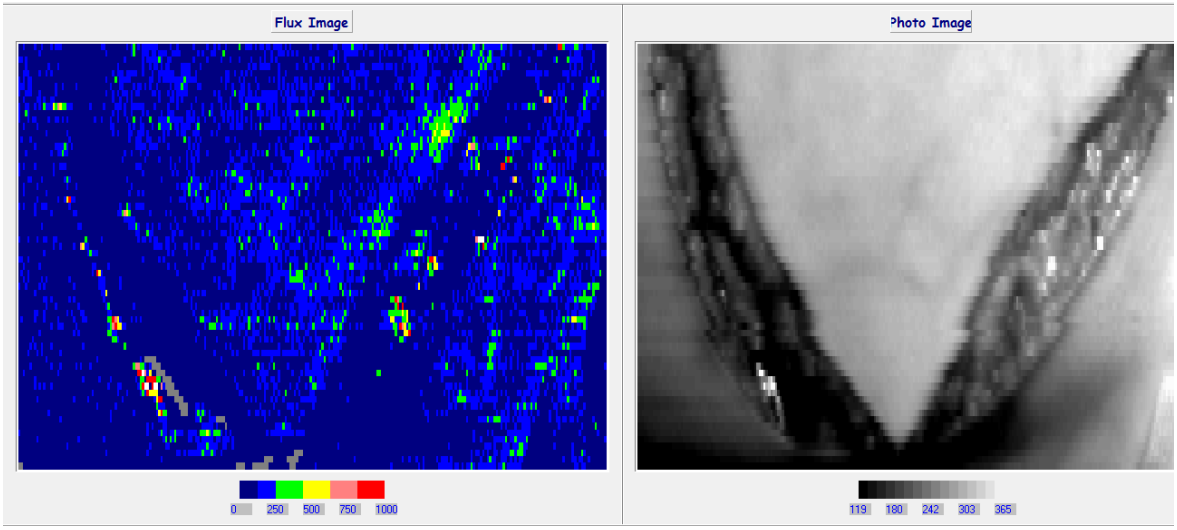

Figure 4.3: LDI flux and photo image of Zone IV obtained from patient 11 in this study As shown in the key low perfusion = blue, moderate perfusion = green/yellow, high perfusion = red

Unfortunately following servicing the LDI machine immediately malfunctioned with problems related to the live video feed resulting in catastrophic system failure. As a result the machine was not available for the primary patient LDI scanning sequence. The machine was returned to Moor and the problem subsequently resolved. Another software malfunction occurred during the study resulting in the inability to achieve LDI scans for patient 4 in the study.

The Median LDI Zone IV skin perfusion values for each patient assessed using LDI is shown in Table 4.5 and Figure 4.4.

Patient	Traditional flap (flux)	Venous augmentation (flux)	Arterial augmentation (flux)	Art / Vein augmentation (flux)
1	xx	xx	xx	xx
2	101	94	107	127
3	40	32	37	34
4	xx	xx	57	-
5	72	80	101	100
6	62	62	61	85
7	55	48	68	70
8	77	82	77	86
9	79	128	-	-
10	49	45	-	-
11	124	119	122	131
12	51	50	79	74
Median	67	71	77	86*

Table 4.5: Median Zone IV skin perfusion results assessed using LDI
*** P Value <0.01 (compared to traditional flap. Malfunction of the Moor LDI machine meant no LDI results were obtained for patient 1 and only one sequence for patient 4.**

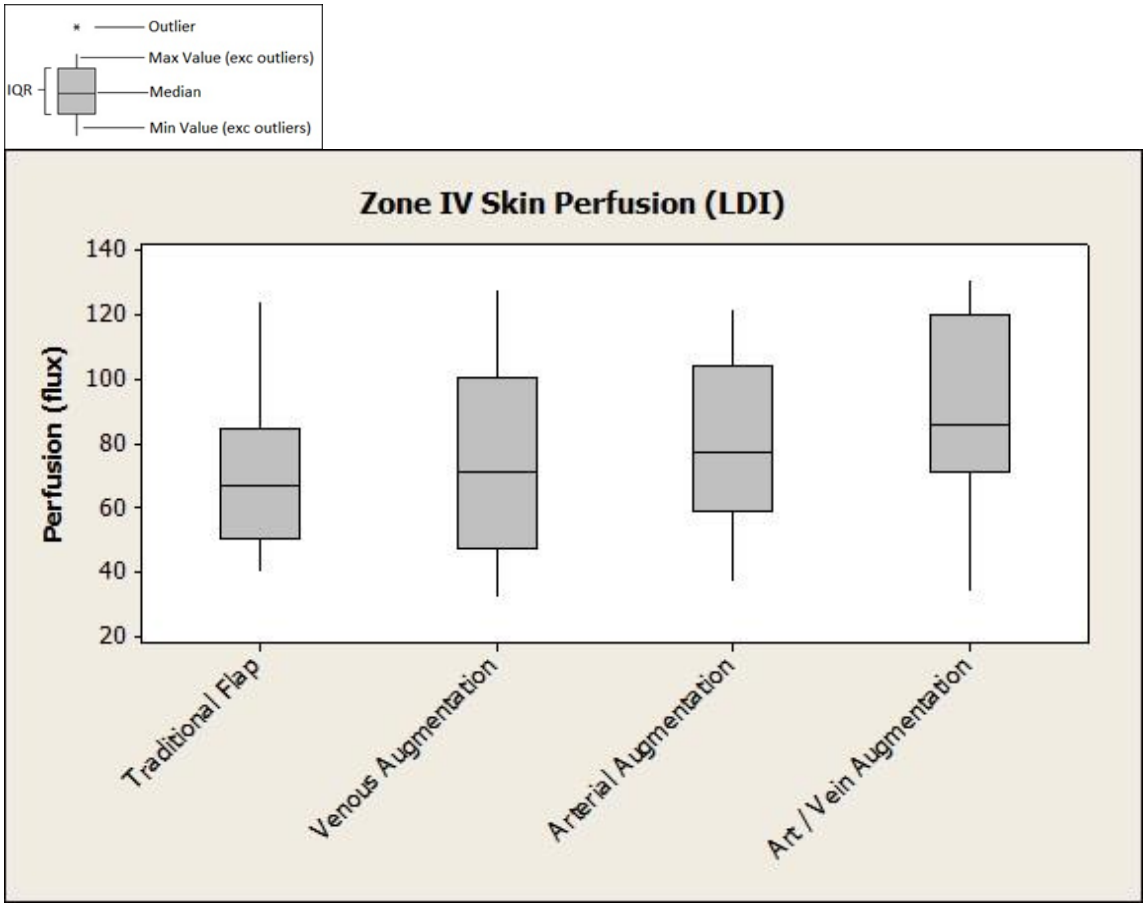

Figure 4.4: Box and whisker plot of median Zone IV skin perfusion for each intervention as assessed by LDI
In comparison to the median flux values observed for the traditional flap (67), there is an increase in skin perfusion upon SIEV (71); SIEA (77) and combined SIEA/SIEV (86) augmentation. The increased perfusion upon combined SIEA and SIEV augmentation was significant (*p*<0.01).

Perfusion data was widely distributed but the box and whisker plot shows a trend to increasing perfusion by unclamping the various components of the superficial vascular system. The largest observed increase in Zone IV perfusion is observed when the flap is augmented using both the SIEA and SIEV.

Data was found to be normally distributed and the results of a repeated measures ANOVA showed there to be a statistically significant difference between the clamping interventions. (p=0.008). (See Appendix A)

Assessment for differences between the traditional flap and the various vascular augmentation interventions by the Bonferroni correction method are summarised in Table 4.6

Type of vascular augmentation	Difference of mean perfusion traditional vs vascular augmentation (flux)	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Adjusted *p*-value
Venous augmentation	+3	-8.884	14.88	1.0000
Arterial augmentation	+11.19	-1.763	24.14	0.1073
Arterial and Venous augmentation	+18.06	5.112	31.01	0.0045*

Table 4.6: Bonferroni correction pairwise comparisons between traditional flap and vascular augmentation interventions

Zone IV skin perfusion was found to be significantly increased upon vascular augmentation of flaps with both the SIEA and SIEV (p=0.0045)

4.3.4 Zone IV skin perfusion assessed using ICG Angiography

A greyscale image of the area being scanned along with a correlating visual graphical display was also obtained. (See Figure 4.5)

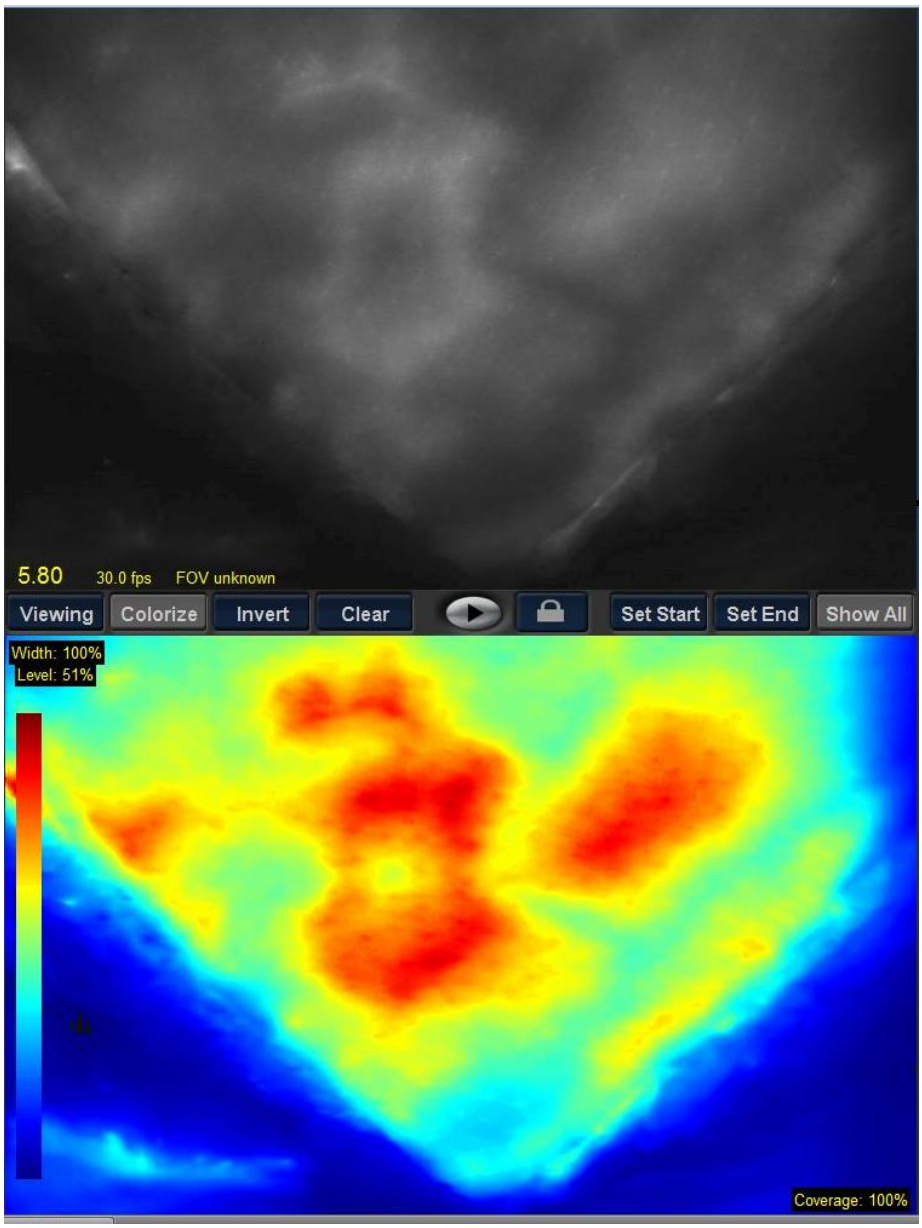

Figure 4.5: SPY scan of Zone IV of patient 11 in this study
low perfusion = blue, moderate perfusion = yellow/orange and high perfusion = red

The Zone IV skin perfusion values for each patient assessed using ICG angiography is shown in Table 4.7 and Figure 4.6.

Patient	Traditional flap (units)	Venous augmentation (units)	Arterial augmentation (units)	Art / Vein augmentation (units)
1	52	69	100	107
2	53	74	76	55
3	28	50	49	43
4	94	42	207	144
5	58	36	88	134
6	150	148	20	88
7	19	35	73	83
8	46	34	45	20
9	51	128	-	-
10	23	16	-	-
11	79	107	125	53
12	95	108	63	17
Median	53	60	75	83

Table 4.7: Mean Zone IV skin perfusion results assessed using ICG angiography

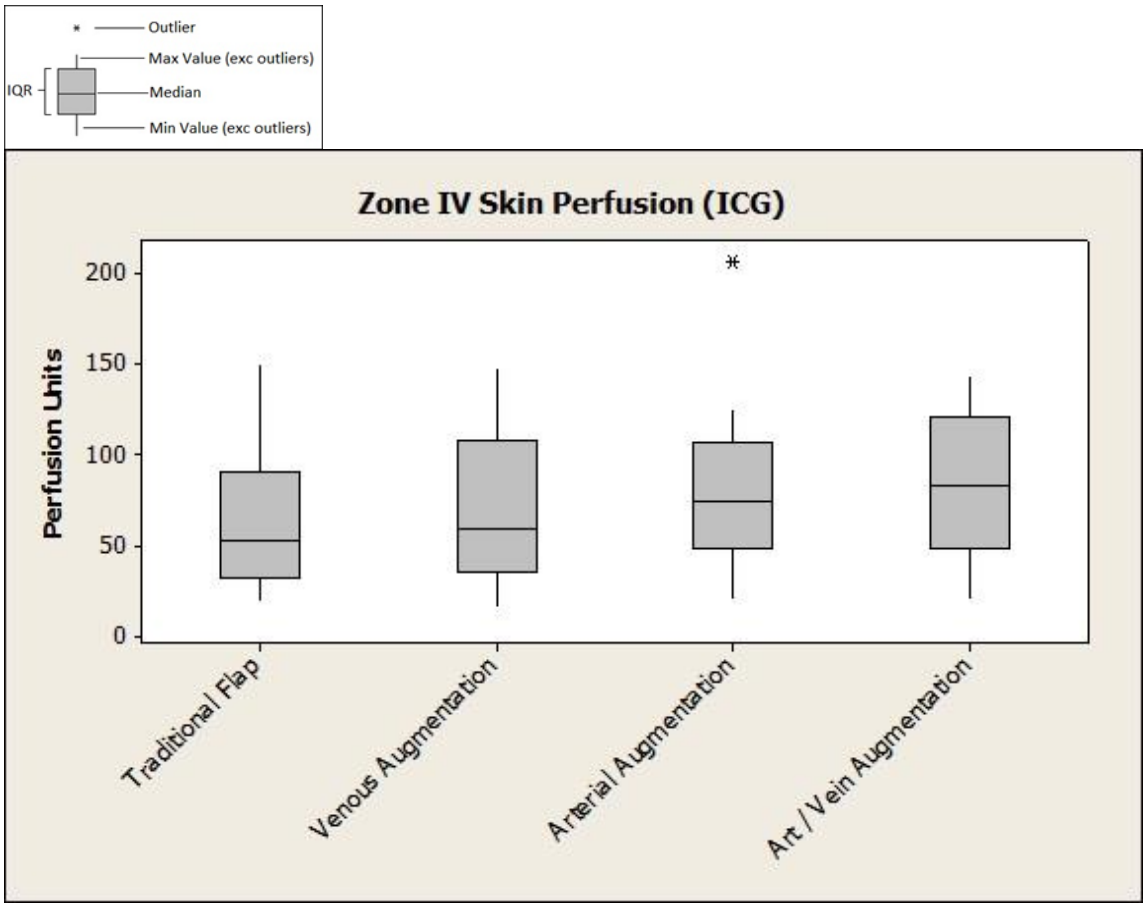

Figure 4.6: Box and whisker plot of median Zone IV skin perfusion for each intervention as assessed by ICG angiography
In comparison to the Traditional Flap (53) there is increased skin perfusion upon SIEV (60), SIEA (75) and combined SIEV/SIEA (83) augmentation. Although these results did not reach significance (p>0.05).

Skin perfusion as assessed by ICG angiography also shows a trend towards increasing perfusion as the SIE vascular components are unclamped.

Data was found to be normally distributed and the results of a repeated measures ANOVA showed that there were no statistically significant differences between the clamping interventions. (p=0.734). (See Appendix A)

4.3.5 Zone IV fat perfusion assessed using ICG Angiography

The Zone IV fat perfusion data obtained from ICG angiography is shown in Table 4.8 and Figure 4.7.

Patient	Traditional flap (units)	Venous augmentation (units)	Arterial augmentation (units)	Art / Vein augmentation (units)
1	35	36	71	64
2	31	111	58	90
3	45	26	26	44
4	61	19	119	68
5	47	51	58	80
6	53	89	29	50
7	19	21	53	67
8	48	34	55	32
9	22	77	-	-
10	23	17	-	-
11	27	50	50	130
12	67	56	64	59
Median	40	43	57	66

Table 4.8: Mean Zone IV fat perfusion results assessed using ICG angiography

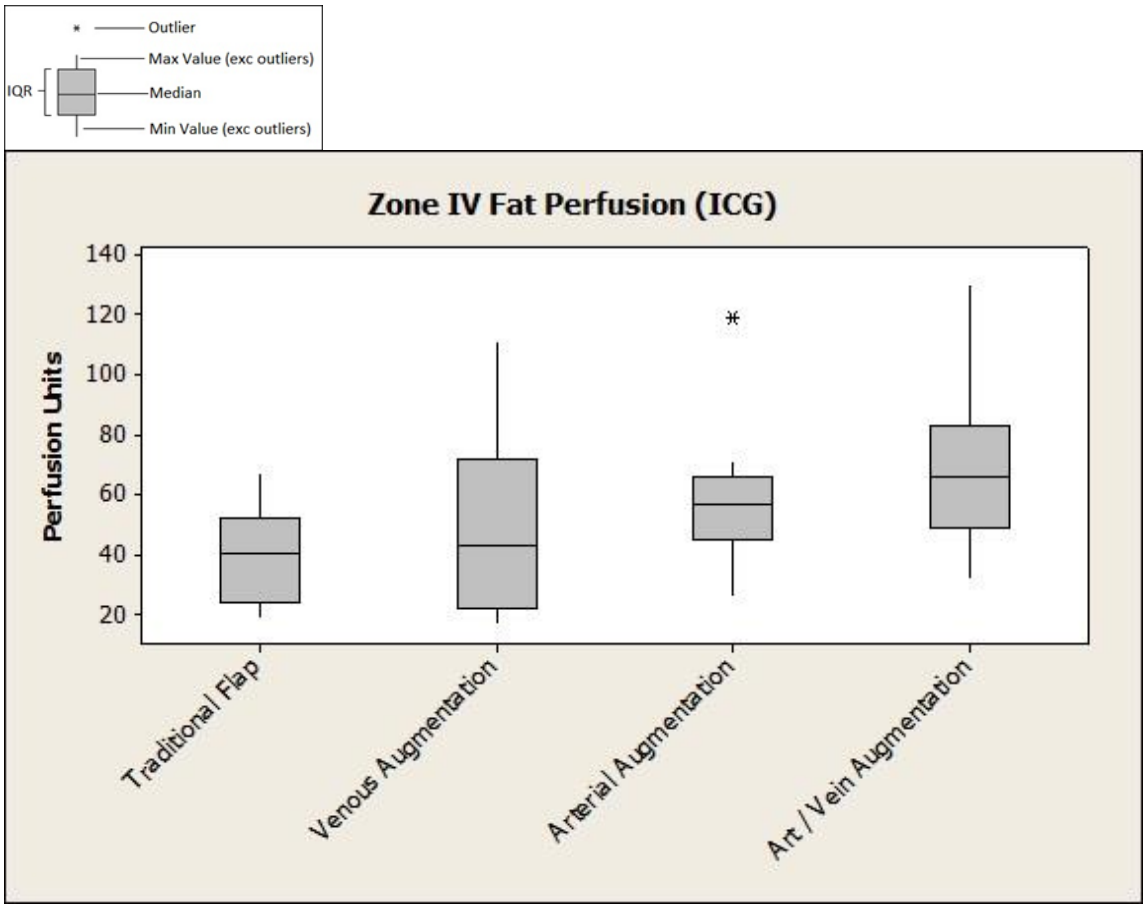

Figure 4.7: Box and whisker plot of median Zone IV fat perfusion for each intervention as assessed by ICG angiography
In comparison to the Traditional Flap (40) there is increased fat perfusion upon SIEV (43), SIEA (57) and combined SIEV/SIEA (66) augmentation. Although these results did not reach significance (*p*>0.05).

The above box and whisker plot is similar to both the LDI and ICG skin perfusion scan results and it appears that there is also a trend towards increasing Zone IV fat perfusion upon vascular augmentation of the flap. The largest increase in fat perfusion is when both the SIEA and SIEV are used in combination to augment the flap.

Data was found to be normally distributed and the results of a repeated measures ANOVA showed there were no statistically significant differences between the clamping interventions. (p=0.130). (See Appendix A)

4.3.6 Effect of Perforator Row

The effect of the perforator row, upon which the DIEP flap was raised (i.e. medial, lateral or medial and lateral row), on Zone IV perfusion between the clamping interventions was assessed using a repeated measures ANOVA.

The perforator row did not have any statistically significant effect on Zone IV skin perfusion between clamping interventions as assessed by LDI (p=0.652). This was also true of Zone IV skin (p=0.753) and fat (p=0.081) perfusion as assessed by ICG angiography.

4.3.7 Effect of Perforator Number

The effect of number of perforators upon which the DIEP flap was raised on Zone IV perfusion between the clamping interventions was assessed using a repeated measures ANOVA.

The number of perforators did not have any statistically significant effect on Zone IV skin perfusion between clamping interventions as assessed by LDI (p=0.678). This was also true of Zone IV skin (p=0.695) and fat (p=0.079) perfusion as assessed by ICG angiography.

4.3.8 Sub-analysis excluding Patient 6

Total flap loss occurred in Patient 6 of our cohort. A separate sub-analysis was undertaken using a repeated measures ANOVA after exclusion of this patient.

There was still a statistically significant difference observed in LDI assessed skin perfusion (p=0.021) between the clamping interventions. Bonferroni correction pairwise comparison tests between the traditional flap and the clamping interventions showed that the only significant difference was observed when both the SIEA and SIEV were unclamped (p=0.0156).

There was still no statistically significant differences in ICG assessed Zone IV skin (p=0.161) or fat (p=0.055) perfusion between clamping interventions.

4.3.9 Sub-analysis excluding Patients 9 and 10

Patient 9 and 10 within this study did not have an SIEA. A separate sub-analysis was undertaken using a repeated measures ANOVA after exclusion of these patients.

There was still a statistically significant difference observed in LDI assessed skin perfusion (p=0.001) between the clamping interventions. Bonferroni correction pairwise comparison

tests between the traditional flap and the clamping interventions showed that the only significant difference was observed when both the SIEA and SIEV were unclamped(p=0.0018).

There was still no statistically significant differences in ICG assessed Zone IV skin (p=0.791) or fat (p=0.159) perfusion between clamping interventions.

4.4 Discussion

Our results show that there is a trend towards increasing Zone IV skin and fat perfusion in DIEP flaps when the flap is augmented using the contralateral superficial vascular system. The largest observed increase to Zone IV perfusion is when both the SIEA and SIEV are used in combination to augment the flap. However, this was only statistically significant in Zone IV skin perfusion as assessed by LDI (p=0.0045).

4.4.1 Augmentation with the SIEA, SIEA or Both

There are very few human in-vivo studies assessing the effects of contralateral arterial or venous augmentation of abdominal flaps however there are a multitude of experimental studies looking at the importance of venous drainage or arterial inflow in rat abdominal flaps.

Miles et al in 1997 published their findings comparing the effects of different venous interventions carried out upon 40 rat pedicled abdominal flaps. In this study their intervention groups consisted of a simple pedicled flap; a pedicled flap with additional contralateral venous augmentation, a venous only pedicled flap and an arterialised venous flap. They found that in the 25 flaps suitable for analysis at the end of their study that the only significant increase in flap survivability was found in flaps with additional contralateral venous augmentation[164]. Hallock and Rice in 2005 compared a traditional rat DIEP flap model versus DIEP flap models augmented with the contralateral vein and found that there was a statistically significant increased flap viability in the venous augmented group[163].

Chang et al in 2004 analysed the effects of various arterial augmentation procedures on 40 rats. They found that the augmentation of rat abdominal flaps with the contralateral arterial system led to a statistically significant improvement in flap survival[162].

Several experimental studies have looked at whether arterial or venous augmentation is most important in terms of flap survival. In an early study in 1982 by Nakayama et al investigating the effects of arterial input or venous drainage upon distal necrosis in abdominal skin flaps raised on 45 rats. They individually compared the effects of arterial or venous augmentation to the survivability of the distal portion of these skin flaps. They found that arterial augmentation seemed to be the most crucial in determining flap survivability. Despite not looking at arterial and venous augmentation in combination they still recommended that where possible both additional venous drainage and arterial input be used where possible[165].

In a study very similar to our own from 1994 Ueda et al divided 32 rat TRAM type abdominal flaps into four cohorts[166]. Cohort one had a flap augmented with the contralateral SIEA and SIEV; cohort two had augmentation with the SIEA only; cohort three SIEV augmentation only and cohort 4 a standard ipsilateral based flap. They found that cohort one and two had complete flap survivability in comparison to the others. In cohort two there was significant flap oedema post-operatively but this settled by day 4. They suggested that both an artery and vein should be utilised to augment flaps where possible but believed the arterial input to be most important. Similarly in 2009 Yamamoto et al assessed the importance of the contralateral SIEA/SIEV in a rat DIEP flap model[167]. They separated 30 rats into three cohorts; SIEA/SIEV both divided; SIEA intact/SIEV divided and SIEA divided/SIEV intact. They assessed overall flap survival area and also pressure within the venous system. Despite large variation amongst the individual rat flaps they found a statistically significant increase in flap area survival occurred in the rats with the SIEA intact.

Our in-vivo perfusion results complement the experimental results found by Ueda's and Yamamoto's research groups[166,167] although our only significant finding in terms of increased perfusion was when both the contralateral SIEA and SIEV were present.

Despite the fact only the LDI results reached statistical significance when the flaps were augmented using the contralateral SIEA and SIEV there were large differences in median perfusion observed between the traditional flap and flaps augmented with the SIEA alone and the SIEA+SIEV in both the LDI and ICG angiography groups; this could indicate that these increases in perfusion could be clinically relevant and the reason they failed to reach statistical significance was due to the power of our pilot study. The SIEV augmentation intervention alone seemed to have little effect on skin and fat perfusion.

4.4.1.1 Variability of the SIEA

During our study we found the SIEA to be completely absent in two (16.7%) of our patients (patients 9 and 10). This absence rate is similar to the 13% found by Stern and Nahai in 1995 in their series of 31 patients but lower than the 35% quoted by Taylor and Daniel in 1975 in their original anatomical dissections of 100 cadavers[66] or the 51% as quoted in-vivo by Chevray in 2004 on their experience of 47 consecutive abdominal flap breast reconstructions[65]. Given that absence of the SIEA may represent altered flap vascular anatomy a separate sub-analysis of our results was undertaken excluding patient 9 and 10. However, this still showed that there was only statistically significant effect on Zone IV Skin perfusion assessed by LDI when both the SIEA and SIEV were used to augment the flap.

Patient 4 in our study cohort had large outlying perfusion values as assessed by ICG angiography when the flap was arterially augmented using the contralateral SIEA. This is particularly interesting secondary to the odd vascular anatomy encountered in dissecting this patients contralateral SIE vascular system. Both the SIEA and SIEV in this patient were found to be sub-Scarpa in nature. We could find no other descriptions in the literature of these vessels noted to be in the sub-Scarpa plane and the influence of such vessels in perfusion of DIEP flaps is unknown. The large increase in perfusion as shown in the ICG angiography scans could indeed be due to increased arterial inflow to Zone IV or could be due to venous pooling secondary to poor vascular draining of the area by a SIEV in the sub-Scarpa plane. Unfortunately the LDI malfunctioned during this patients scanning sequence and we did not have these scans to correlate our ICG angiography findings with.

4.4.1.2 Other factors affecting patient outcome

One flap loss occurred in Patient 6 of our study cohort. This flap showed some intra-operative evidence of venous congestion upon initial raising and even prior to detachment from the abdomen. Upon detachment and transfer of this flap; despite multiple additional venous augmentation procedures (contralateral SIEV to distal IMV augmentation and then interposition vein graft from ipsilateral SIEV to secondary DIEV venae comitantes) the flap failed secondary to worsening venous congestion and the flap required debridement 5 days post operatively. Given the intra-operative findings of venous congestion and subsequent flap loss it may represent different intra-flap vascular anatomy and as such a separate sub-analysis of our results was undertaken excluding patient 6. This still showed

that there was only statistically significant effect on Zone IV Skin perfusion assessed by LDI when both the SIEA and SIEV were used to augment the flap.

4.4.2 Model validation and potential confounders

4.4.2.1 Zone IV selection

Whilst controversy remains over which area of the DIEP flap should be designated Zone II or Zone III there is global consensus on which area is Zone IV. Zone IV is the area of the flap which is subject to the worst perfusion and resultant complications of venous congestion; fat necrosis and partial flap loss. As a result of the doubtful viability and also the fact that Zone IV can be difficult to incorporate geometrically into a reconstruction whilst trying to recreate a suitable breast shape, many surgeons would routinely discard this section of the flap and we accept that this is the least useful portion of the flap clinically. However, in terms of translational research, Zone IV is most likely to resemble a failing flap model and thus the results of vascular augmentation may be of particular interest to the microsurgical community as a whole as this may give evidence for the use of vascular augmentation procedures to salvage failing flaps. We believed that the effect of any vascular augmentation procedure is likely to be most obvious in Zone IV. Additionally Zone IV is also routinely discarded by the senior operating surgeon involved in this study and in terms of ethical accountability any potential consequences to the survivability of Zone IV secondary to our interventions (i.e. clamping of SIE vascular system) or any unexpected intra-operative findings to this area of the flap during our study would have no influence to our patients' normal standard of care. We accept that it would have been favourable to have analysed all Zones of the flap during this research study; however it would have significantly added to each patients length of anaesthesia and operation and we could not ethically justify the additional time required. Longer operating times have been associated with increased risks of post-operative complications[218]. The research performed in this study was performed during a routine twenty minute interval, undertaken after the flap was initially raised, to allow time for any areas of potential vascular concern within the flap to identify themselves and allow a natural comfort break as per the operating surgeons' standard practice. Thus our study did not lengthen the patients' anaesthesia or operation time.

4.4.2.2 Clamping Intervention

In this procedure five minutes was allowed to elapse after any clamping or unclamping intervention prior to taking any scanning measurements to allow reactive hyperaemia to settle. This was decided upon following review of the evidence from a previous study by Tollan et al which has shown that a period of five minutes is an adequate amount of time to allow reactive hyperaemia to settle following clamping and unclamping of vasculature in DIEP flaps[211].

4.4.2.3 Data Sampling and Analysis

During sampling and analysis of the LDI and ICG angiography scan images for each patient; defined regions of interest were selected within each flap (Zone IV) in order to obtain the perfusion values within this area. For each separate patient scan (assessing interventions) this region of interest had to be individually selected. Whilst care was taken to select the same region of interest for each patient scan; this user-defined area can be considered arbitrary in nature. In an effort to reduce bias several precautions were taken. Flaps were divided and marked on the operating table into the classical Hartrampf Zones of Perfusion prior to the commencement of the operation and these markings can be visualised on both the LDI and ICG angiography scan images whilst undergoing analysis essentially acting as a reference point upon which to select the region of interest. Only Zone IV of the flap was scanned during each intervention process and thus the area to select as the region of interest was minimised. All patient scans were undertaken and analysed using the same method by the same observer, with training and previous experience in both the scan capture and analysis. The median (LDI) and mean (ICG) scan results for each area of interest were utilised rather than individual points of reference and thus any minor inaccuracies in terms of area selected are unlikely to influence the overall results obtained significantly. Despite these precautions the specified location and area encompassed by the "region of interest" are therefore accepted as a potential confounder.

4.4.2.4 Choice of medial or lateral row perforator

Six patients in our study had DIEP flaps based upon medial row perforators; four upon lateral row perforators and two had flaps based upon both medial and lateral row perforators. Perforator selection in this study was based clinically upon what appeared to be the largest dominant perforating vessel offering easiest dissection with minimal collateral damage. Studies have suggested that medial row perforators cross the midline

supplying greater perfusion to Zone IV of DIEP flaps whereas lateral row perforators do not[76,81,219,220]. In this study we were only assessing the effect on flap Zone IV perfusion pre and post vascular augmentation. Therefore flaps based on medial or lateral row perforators are unlikely to affect the perfusion differences observed during our clamping and unclamping interventions as each patients (traditional) flap was essentially acting as a control. A separate sub-analysis of our results was also undertaken and the perforator row did not have any statistically significant effect on the observed results. However baseline Zone IV perfusion may be affected based upon medial or lateral row perforators and we accept this as a potential confounder.

4.4.2.5 Perforator number

Seven patients in our study had DIEP flaps based upon a solitary perforator whereas five had flaps based upon two perforators. Number of perforators chosen in this study was based upon several factors including non-apparent dominance of a solitary perforator; proximity of the perforators to one other and the ease of dissection minimising collateral damage. There are various debates in the published literature with regards to the number of perforators upon which to base a DIEP flap. Some authors advocate the use of solitary perforators believing they provide more reliable perfusion[168] or fewer complications[53] whereas others believe that flaps based upon multiple perforators reduce complication rates such as fat necrosis[221-223]. Others failed to find any difference or believe that perforator number has no effect on the perfusion or complication rate but instead that selecting the dominant perforator from the appropriate row has the most important influence[50,94,224,225]. As with perforator row selection irrespective of perforator number in our study cohort we were only assessing the difference between each individual patients Zone IV perfusion pre and post vascular augmentation and this is unlikely to be affected by number of perforators. A separate sub-analysis of our results was also undertaken and perforator number did not have any statistically significant effect upon the observed results. However baseline Zone IV perfusion may be affected may be affected by number of perforators and we accept this as a potential confounder.

4.4.2.6 Temperature

Patient core temperature was only recorded twice during the 30 minute research scanning process. As such only two measurements were available to compare for each patient. Whilst these measurements appeared stable for each patient ideally these should have been

recorded prior to each individual scan. Thus inadequate patient temperature recording is accepted as a design limitation and potential confounder.

4.4.2.7 Control Patient

No control patient, not undergoing any clamping intervention but undergoing the same number of scans to that of the research patient cohort, was included in this study. Since patients' own flaps were being used as a control (i.e. traditional flap versus vascular augmetation intervention) we did not believe an additional control patient would add much in terms of validity to the experiment as both the clamping interventions and scanning processes have been validated in previous studies[212,226]. In a similar fashion we did not feel it beneficial to scan individual patients abdomens prior to raising the flap as a control for "normal values" as it has been shown in the literature that upon raising a flap the blood supply grossly changes in comparison to pre-operative values[227].

4.4.2.8 Performance Bias

All flap dissection and raising in this study was by a single senior Consultant Plastic Surgeon with significant experience in the technique. Mr Mackay's failure rate for free DIEP flap breast reconstruction is less than 2% over the past five year period; well below the quoted acceptable range of less than 5%[90] for free tissue transfer and in keeping with the 1.95% quoted in the National Mastectomy and Breast Reconstruction Audit[228]. All flaps were raised as per his standard protocol and given his career experience in the technique performance bias is likely to be minimal but we accept it as a potential confounder.

4.4.3 Conclusion

In conclusion from the findings of our initial pilot study we believe that vascular augmentation of DIEP flaps using the contralateral SIE vascular system leads to an increase in Zone IV perfusion. Our results show that the biggest increase in Zone IV perfusion occurs when flaps are augmented using both the SIEA and SIEV in combination. Therefore from our preliminary work we would suggest that when Zone IV of a DIEP flap is to be incorporated into the reconstruction rather than being discarded that both components of the SIE vascular system (SIEA and SIEV) are used to augment the flap.

Chapter 5: The effects of vascular augmentation on abdominal flap Zone I / IV perfusion and main pedicle arterial blood flow in an experimental animal model

5.1 Introduction

In 2001 Oskar et al were the first to describe an experimental animal model upon which to raise perforator flaps[214]. In their study they successfully raised abdominal flaps in Sprague Dawley rats based upon solitary musculocutaneous perforators and found that these flaps had similar areas of survival to traditionally used random pattern pedicled flaps raised in rats. They proposed that the rat was a reliable experimental model for further research into perforator flaps. This model was further validated subsequently by Ozkan et al in 2006[229].

Whilst the vascular supply to the lower abdominal tissue is similar in rats and humans there are several differences which must be taken into account when using the rat abdominal perforator flap model. In humans the dominant blood supply to the lower abdominal tissue comes from the caudal DIE vessels (cf. 1.5) whereas in rats the dominant supply comes from the cranial Deep Superior Epigastric (DSE) vessels[230,231]. (See Figure 5.1)

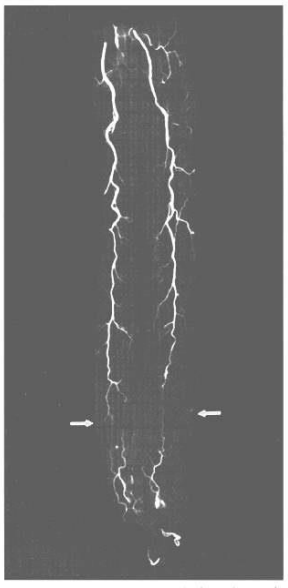

Figure 5.1: Contrast injection study in Sprague Dawley rat showing dominance of cranial (DSE) vessels
Image reproduced with permission from Wolters Kluwer Health, Inc. (Hallock & Rice 1995)[230]

Thus any perforator flaps based upon the lower abdominal tissue in rats must take account of this cranial dominance and factor it in to any research studies using the model opting

instead to base the perforator flaps on the DSE vessels. Another major anatomical difference is that unlike the defined medial and lateral rows branching from the deep source vessels in humans, there is no such defined branching pattern in the deep vessels of rats[230]. As such it is not possible to perform research based upon medial or lateral rows in the rat abdominal model.

Despite these anatomical vascular differences since the description by Oskar et al[214] the rat abdominal perforator flap model as an analogue to human abdominal perforator flaps has become widely accepted and established in plastic surgery research[94,163,167,168].

Whilst our own study (cf. Chapter 4) suggests that vascular augmentation of the DIEP flap will lead to a greater perfusion of Zone IV, the concern would be that the addition of another supplementary pedicle could potentially lead to a decreased main pedicle blood flow and overall flap perfusion in a similar way to that occurring when more than one perforator is utilised as found by Douglas et al[168]. There were no studies addressing this concern in the literature.

The aim of this experimental study was to assess in-vivo, using a rat abdominal flap model as an analogue to a DIEP flap, the effects on Zone I / IV perfusion and on main pedicle arterial blood flow by vascular augmentation using the contralateral vascular system.

5.2 Methods

In a randomised fashion (cf. 3.2.5) left or right islanded abdominal flaps were raised in 12 Sprague Dawley rats so that the flap was obtaining its vascular supply from a pedicle containing the cranial most perforating artery and vein from the DSEA and DSEV (main pedicle) on one side of the flap and the contralateral DSEA and DSEV pedicle (vascular augment pedicle) containing only the caudal most perforating artery and vein (cf. 3.2.9). Flaps were divided into Hartrampf's four Zones of perfusion (cf. 1.6.4). The vascular augment pedicle was clamped and unclamped and the resultant effects on Zone I and Zone IV perfusion assessed using LDF flowmetry (cf. 3.2.7.2). The effect of the vascular augment intervention on blood flow through the main pedicle was also assessed using transit-time ultrasound flowmetry (cf. 3.2.8.3).

5.3 Results

5.3.1 Animals

Twelve consecutive male outbred Sprague Dawley rats; obtained from Harlan UK Ltd., Bicester, Oxfordshire, UK; were used in this research carried out between 31/01/2013 to 04/03/2013. Seven animals (58%) were randomised to have a flap based upon the right side and the five upon the left (42%). The average weight of the animals used in this study was 344grams.

A summary of animal demographics and mean intra-operative measurements are shown in Table 5.1.

Animal	Weight (grams)	Flap side	Oxygen saturation (%)	Heart rate (bpm)	Core temperature (°C)
1	343	Right	98.5	382	37.2
2	351	Left	98	390	37.7
3	335	Right	99	386	37.7
4	343	Left	98	333	37.9
5	353	Right	97	380	37.6
6	377	Left	98	428	38.0
7	366	Left	97.5	376	37.5
8	386	Left	98	367	37.8
9	300	Right	99.5	381	37.3
10	314	Right	99	349	37.3
11	323	Right	99	344	37.0
12	333	Right	96	368	37.2

Table 5.1: Summary of pre and peri-operative characteristics

5.3.2 Perfusion

5.3.2.1 Zone I vs Zone IV perfusion

Perfusion values obtained for Zone I and Zone IV in the traditional flap model and in the vascular augmented flap model are shown in Table 5.2 and Figure 5.2.

Animal	Perfusion (flux)	
	Zone I	Zone IV
1	27.5	18.1
2	36.1	12.9
3	51.7	13.9
4	31.7	20.1
5	23.3	16.9
6	89	37.5
7	22.9	7.7
8	91.7	5.0
9	97.7	23.4
10	37.9	16.2
11	24.9	17.5
12	58.9	21.9
Median	37	17.2

Table 5.2: Mean Zone I/IV perfusion of the traditional flaps

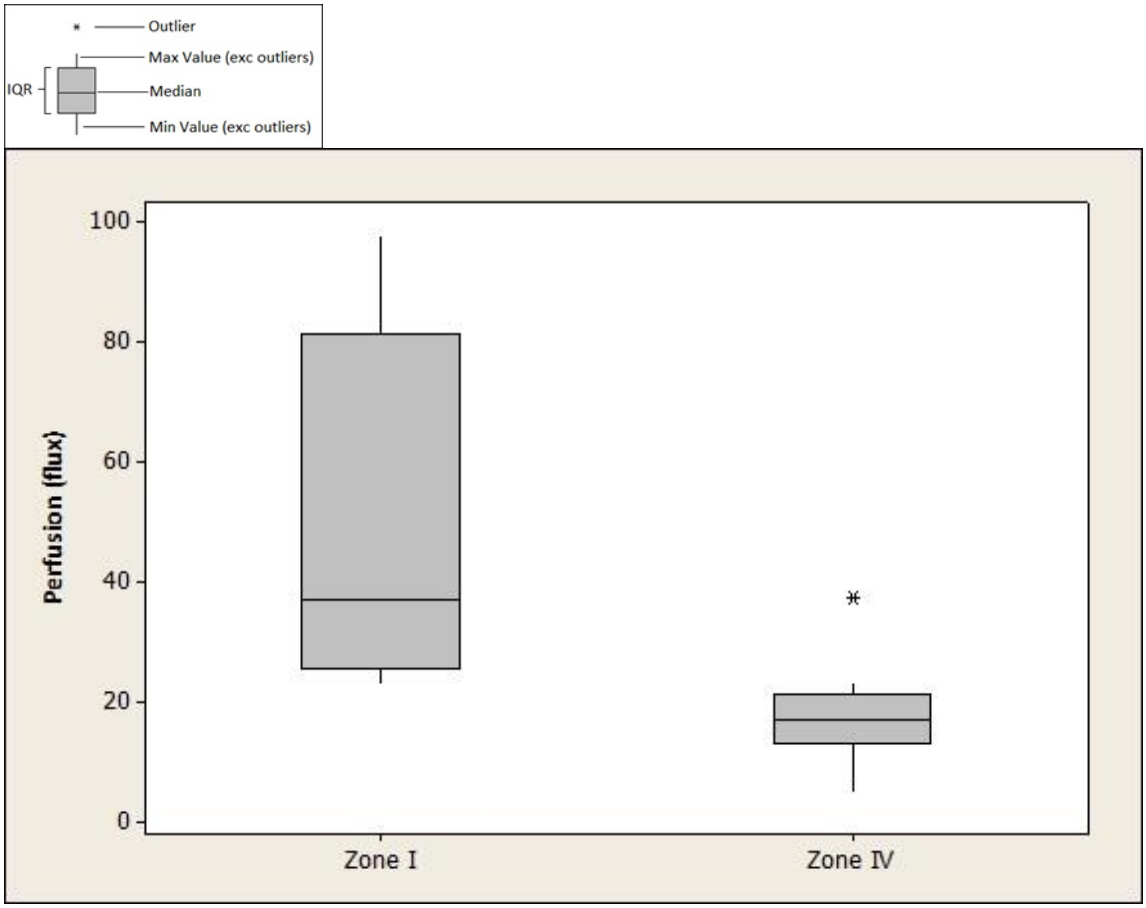

Figure 5.2: Box and whisker plot of median Zone I versus Zone IV perfusion in a traditional flap
In a traditional flap Zone I (37.0) perfusion is significantly higher than Zone IV (17.2) perfusion (p<0.01).

It is obvious from the figure above that there is a greater degree of perfusion in Zone I in comparison to Zone IV.

The effects of vascular augmentation on abdominal flap Zone I vs Zone IV perfusion are shown in Table 5.3 and Figure 5.3.

	Perfusion (flux)	
Animal	Zone I	Zone IV
1	16.5	19.5
2	34.9	13.4
3	45.0	23.8
4	26.8	21.0
5	19.5	28.8
6	74.1	35.0
7	14.9	14.9
8	64.7	12.8
9	43.2	49.5
10	58.8	62.4
11	20.5	24.8
12	44.7	32.1
Median	39.1	24.3

Table 5.3: Mean Zone I/IV perfusion of vascular augmented flaps

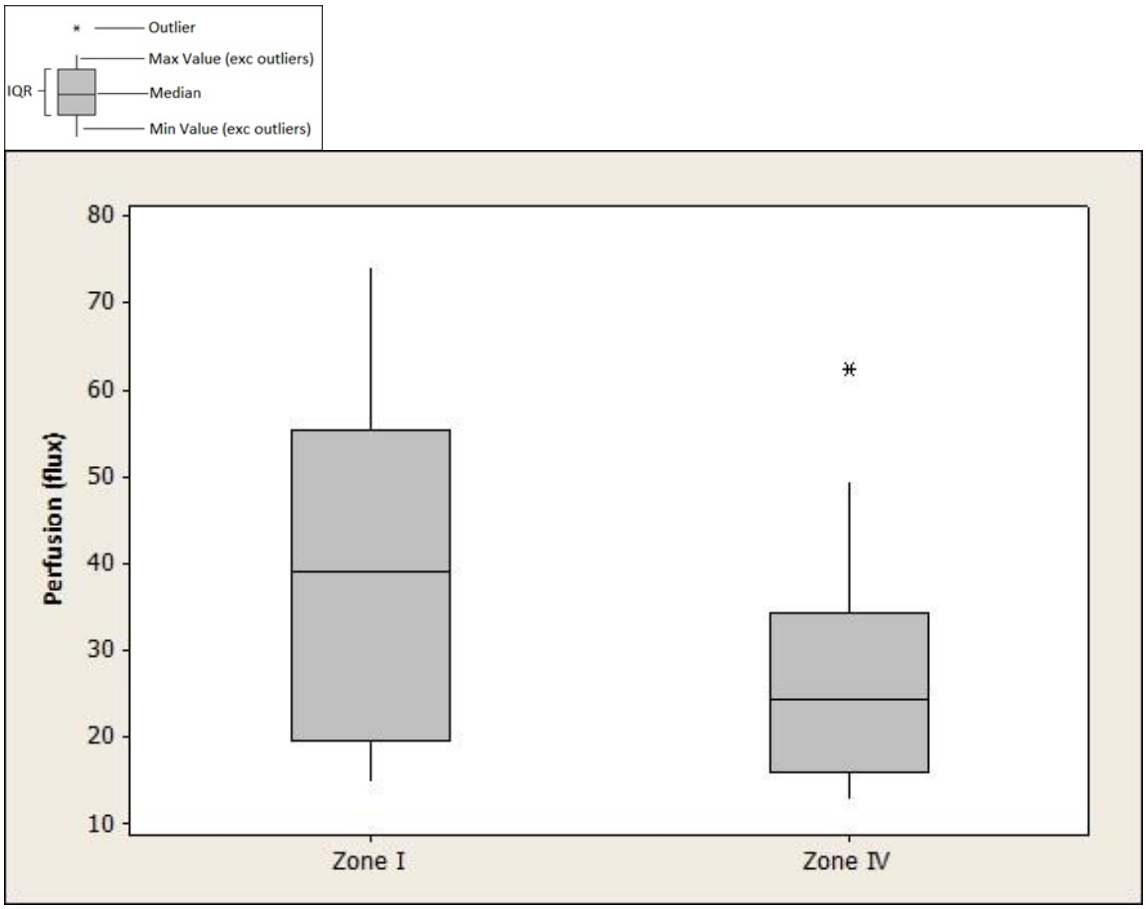

Figure 5.3: Box and whisker plot of median Zone I versus Zone IV perfusion in the vascular augmented flaps.
In a vascular augmented flap Zone I (39.1) perfusion is higher than Zone IV (24.3) perfusion although this does not reach significance (p>0.05).

There is still greater perfusion in Zone I than Zone IV in a vascular augmented flap however the observed difference between the Zones is not as apparent as in the traditional flap.

The differences in median perfusion between Zone I and Zone IV are summarised in Table 5.4.

Type of Flap	Median Zone I perfusion (flux)	Median Zone IV perfusion (flux)	Difference between Zone I and Zone IV (flux)	*p* value
Traditional Flap	37	17.2	19.8	0.003
Vascular Augmented Flap	39.1	24.3	14.8	0.168

Table 5.4: Zone I vs Zone IV perfusion

In the traditional flap model Zone IV has a statistically significant poorer degree of perfusion in comparison to Zone I (p=0.003). In the vascular augmented flap model whilst

there still appears to be a greater degree of perfusion in Zone I when compared to Zone IV however this difference is no longer statistically significant (p=0.168).

5.3.2.2 Traditional Flap vs Vascular Augmented Flap

Perfusion values in Zone I and Zone IV in the traditional flap model and the vascular augmented flap model area summarised in Table 5.5 and Figure 5.4.

	Zone I Perfusion (flux)		Zone IV Perfusion (flux)	
Animal	Traditional Flap	Vascular Augmented Flap	Traditional Flap	Vascular Augmented Flap
1	27.5	16.5	18.1	19.5
2	36.1	34.9	12.9	13.4
3	51.7	45	13.9	23.8
4	31.7	26.8	20.1	21
5	23.3	19.5	16.9	28.8
6	89	74.1	37.5	35
7	22.9	14.9	7.7	14.9
8	91.7	64.7	5	12.8
9	97.7	43.2	23.4	49.5
10	37.9	58.8	16.2	62.4
11	24.9	20.5	17.5	24.8
12	58.9	44.7	21.9	32.1
Median	37	39.1	17.2	24.3

Table 5.5: Mean Zone I and Zone IV perfusion for traditional flaps versus vascular augmented flaps

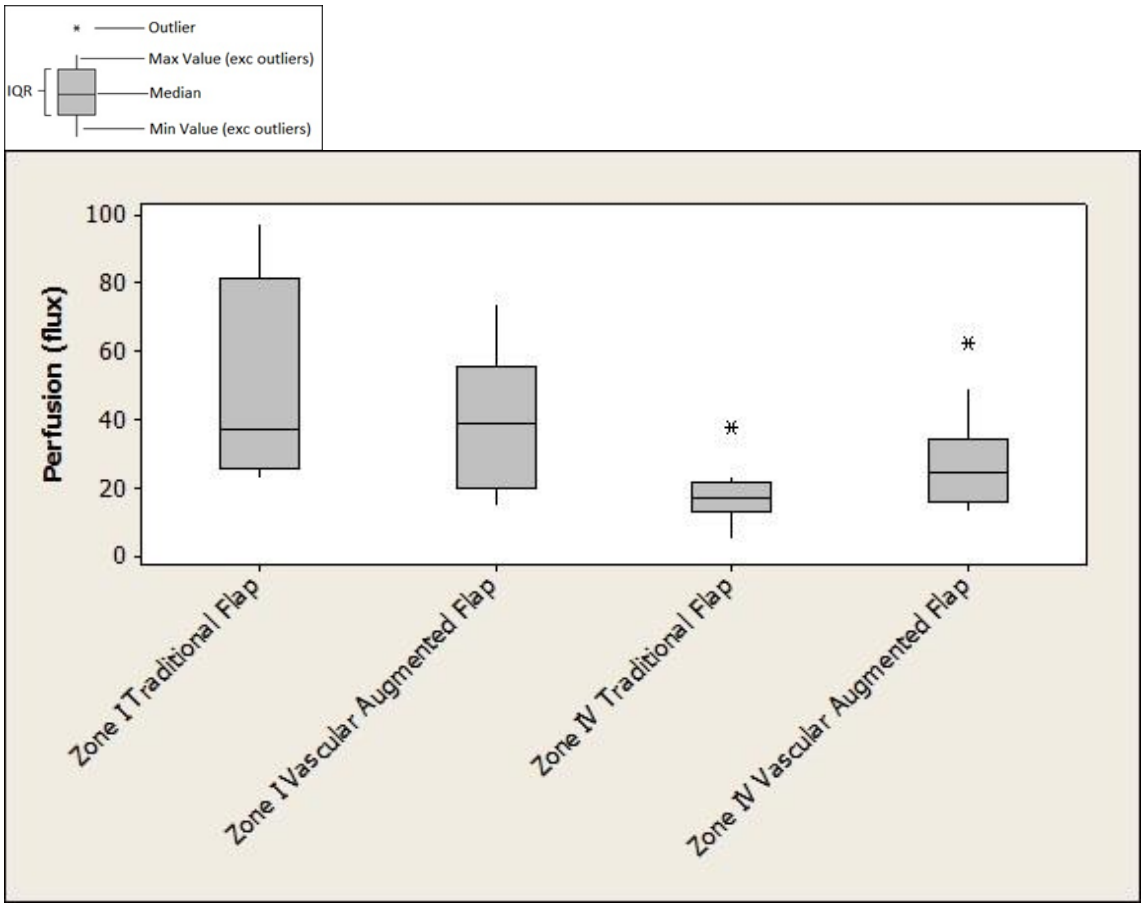

Figure 5.4: Box and whisker plot of median LDF perfusion in Zone I and Zone IV for traditional and vascular augmented flaps
Vascular Augmentation of the traditional flap significantly improves Zone I (p<0.05) and to a greater degree Zone IV (p<0.01) perfusion.

It can be observed from the box and whisker plot that there is an increase in Zone IV perfusion upon vascular augmentation of the flap.

Traditional versus vascular augmented flap differences in both Zones are summarised in Table 5.6.

Flap Zone	Traditional flap perfusion (flux)	Vascular Augmented flap perfusion (flux)	Difference in perfusion traditional vs vascular augmentation (flux)	*p* value
I	37	39.1	+2.1	0.025
IV	17.2	24.3	+7.1	0.007

Table 5.6: Traditional versus vascular augmented flap

From our results it is evident that vascular augmentation of the abdominal flap leads to a statistically significant increase in in Zone IV perfusion (p=0.007). The spread of data was wide but there also appears to be an increase in Zone I perfusion, although to a much smaller degree. (p=0.025).

5.3.3 Flow

Flow values observed through the main pedicle DSEA in the traditional flap and the vascular augmented flap for each animal are shown in Table 5.7 and Figure 5.5.

	Flow ml/min	
Animal	**Traditional Flap**	**Vascular Augmented Flap**
1	0.25	0.23
2	0.33	0.3
3	0.48	0.43
4	0.29	0.2
5	0.3	0.15
6	0.34	0.29
7	0.42	0.37
8	0.37	0.36
9	0.45	0.31
10	0.48	0.45
11	0.29	0.25
12	0.79	0.65
Median	0.37	0.31

Table 5.7: Main DSEA flow in the traditional flap model compared to the vascular augmented flap model

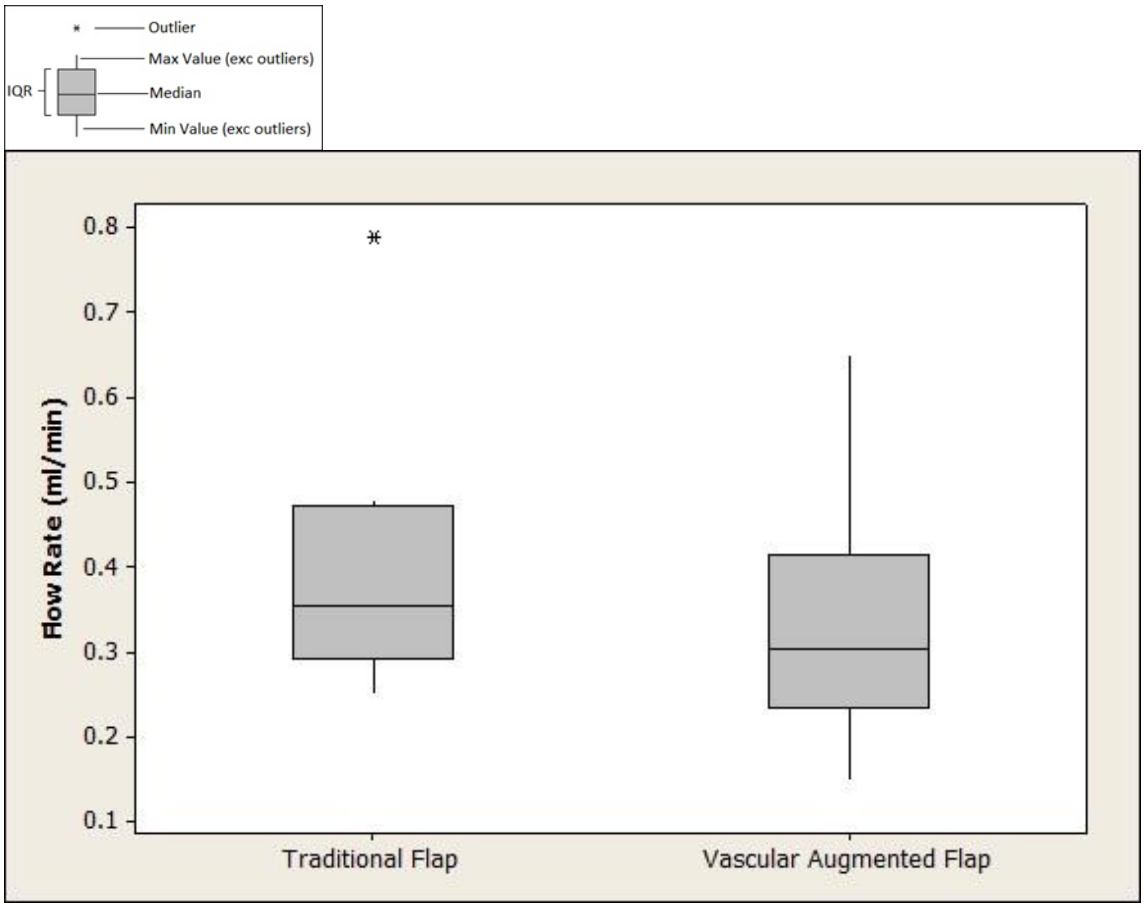

Figure 5.5: Box and whisker plot of median main pedicle blood flow rate in traditional versus vascular augmented flaps
In comparison to the flow observed in a Traditional flap (0.37ml/min) the flow in a vascular augmented flap (0.31ml/mins) is significantly lower (p<0.01).

It is shown that there is great inter-animal variability for the rats used in this study with regards to flow but there appears to be a decrease in flow through the main pedicle when vascular augmentation of the abdominal flap is performed.

The effects of vascular augmentation on arterial blood flow through the main pedicle of the abdominal flap are summarised in Table 5.8.

Type of Flap	Flow (ml/min)
Traditional Flap	0.37
Vascular Augmented Flap	0.31
Difference in flow	-0.06
p value	0.003

Table 5.8: Effect of abdominal flap vascular augmentation on main pedicle flow

From our results it has been shown that there is a statistically significant reduction in flow through the main DSEA when vascular augmentation of the abdominal flap is performed. (p=0.003)

5.3.3.1 Pulsatility Index

The pulsatility index was also calculated for each of the animals in this study and is shown in Table 5.9 and Figure 5.6.

	Pulsatility Index	
Animal	Traditional Flap	Vascular Augmented Flap
1	4.58	4.27
2	3.65	3.40
3	6.11	2.86
4	3.89	7.31
5	0.81	10.48
6	4.73	9.81
7	2.84	2.35
8	3.74	2.71
9	0.30	0.46
10	1.16	1.13
11	3.49	3.65
12	1.87	1.55
Median	3.49	2.86

Table 5.9: Pulsatility Index for traditional and vascular augmented flaps

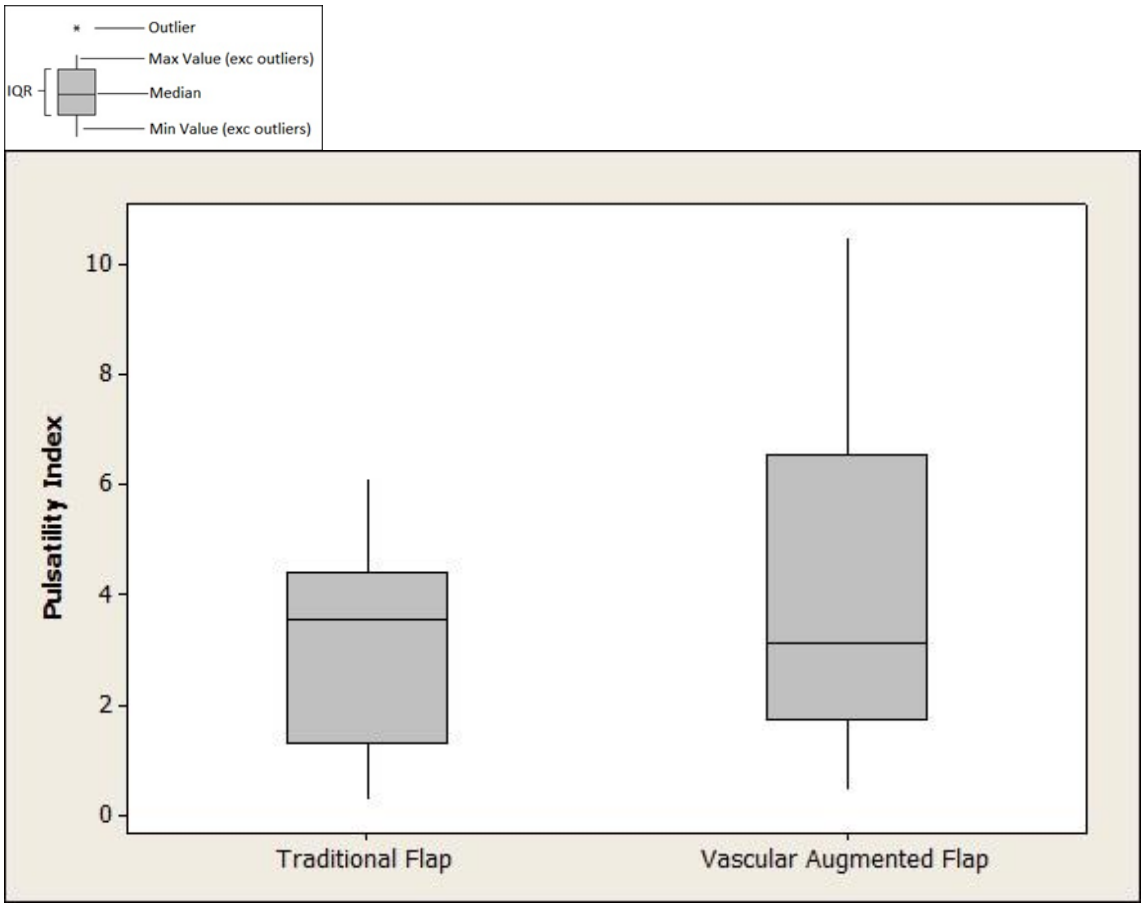

Figure 5.6: Box and whisker plot of median Pulsatility Index in traditional flap model vs vascular augmented flap model
There is wide inter animal variability but there appears to be a higher pulsatility index in a traditional flap (3.49) compared to a vascular augmented flap (2.86) although this failed to reach significance (p>0.05).

The above box and whisker plot shows that the data is widely spread but that there is a decrease in median Pulsatility Index.

The effects of vascular augmentation of the abdominal flap on Pulsatility Index are summarised in Table 5.10.

Type of Flap	Median Pulsatility Index
Traditional Flap	3.49
Vascular Augmented Flap	2.86
Difference	0.63
p value	0.969

Table 5.10: Effect of vascular augmentation of abdominal flap on Pulsatility Index

There is decrease in Pulsatility Index following vascular augmentation of the flap; however this difference failed to reach statistical significance. (p=0.969)

5.4 Discussion

The results from our study show that in a traditional perforator abdominal flap and a vascular augmented flap, perfusion was higher in Zone I when compared to Zone IV, however this difference was only found to be statistically significant in the traditional flap model. (p=0.003 vs p=0.168). Perfusion in Zone IV was increased significantly upon vascular augmentation of the flaps (p=0.007) and the same was also found in Zone I (p=0.025). There was also a significant reduction in main pedicle arterial flow when vascular augmentation of the flap was performed (p=0.003) whilst the pulsatility index decreased.

Our significant findings of increased perfusion as assessed by LDF in Zone I in comparison to Zone IV helps to validate the rat perforator abdominal flap model used in this study. Our animal study correlates with our human study in that vascular augmentation of flaps leads to an overall increase in Zone IV perfusion. Zone I perfusion was also found to have increased however there was found to be a reduction in main pedicle arterial flow and pulsatility index.

5.4.1 Model Validation and Potential Confounders

5.4.1.1 Selection of Zone I and Zone IV

Zone I represents the area of best perfusion within a flap and Zone IV the area of worst perfusion. This has been well shown in both human studies[73-75,212] and in rat models[94,226]. It was for this reason that these zones were selected to compare in this study. Whilst it would have been ideal to have measured the perfusion in all Zones of the rat perforator abdominal flap model simultaneously this would not have been possible secondary to the small size of the flaps. Despite using the smallest LDF probe it was incredibly difficult during this study to site even two probes on the flaps concurrently. In order to measure all Zones it would have required that 2-3 separate measurement periods be undertaken and this would have led to less robust results as recording of the perfusion between Zones during exactly the same interval, such as that measured in this study, could not have been undertaken.

5.4.1.2 Perfusion

Of the few studies using LDF to assess rat abdominal skin flap perfusion[94,230,232,233] only a recent animal model experiment by Douglas utilised the same LDF system and software as

that used in our study to allow comparison[226]. In this study perforator abdominal flaps were raised in 20 Wistar rats and the effects of perforator number and location on perfusion in Zones I-IV were being assessed. Douglas found that mean Zone I perfusion (34.55units) was higher than Zone IV perfusion (17.85) for flaps based on a cranial perforator. These results correlate well with our findings that mean Zone I perfusion (49.44) is greater than mean Zone IV perfusion (17.85) for similarly raised flaps. The slightly higher perfusion values found in our study may be secondary to the difference in strain of rat used (Sprague Dawley versus Wistar) and that our rats had a higher average weight (344grams versus 322grams). Despite the use of different LDF systems Hallock and Rice in an experimental rat study using Sprague Dawley rats in 2004 also found Zone IV perfusion to be significantly lower than that of Zone I further corroborating our findings[94].

5.4.1.3 Flow and Pulsatility Index

In a study by Rickard et al in 2009 investigating the development of a model to assess microvascular anastomotic techniques in vessels with size mismatch, flow rates through the femoral arteries of Wistar rats were assessed in the same fashion to that used in this study[234]. Flow rates of 2.39m1/min and 2.882ml/min are quoted which are significantly greater than those found in our study. However, given that the femoral vessel was being assessed in this study it is not surprising that the flow rates are much greater as the diameter of the femoral artery is much larger than that of the superior epigastric.

In another study by Douglas assessing the effect of varying the number and location of perforators on main pedicle arterial flow in Wistar rats, mean flow values of 0.21(range 0.09-0.4)ml/min are quoted for flaps raised on a cranial perforator[168,226]. This is much lower than the mean 0.40 (range 0.15-0.65)ml/min found in our cohort. However, there was a great degree of variability between animals in both studies and again the difference between strains in the rats used in each study and the larger weight of our rats could account for the differences observed.

Only flow through the main pedicle DSEA was assessed during this study. In the animals used in this study we found these vessels to be approximately 0.25-0.35mm in diameter. A previous study assessing the microvascular transfer of free rectus abdominus myocutaneous flaps in Sprague Dawley rats by Zhang et al has previously quoted that the mean vessel diameter of the DSEA was 0.4-0.5mm[235]. However the rats used in this study were much larger than our own weighting between 400-500grams and this could account

for the observed difference. Whilst the microcirculation transit-time flow probes used in this study can detect flow rates in vessels of 0.25-0.5mm we could not position 2 concurrently on both the artery and the vein due to the size of the probes without placing undue tension on the vessels. We also found detection of flow within the vein to be extremely difficult and as such only arterial flow rate was measured and we accept this as a potential confounder.

We found that vascular augmentation of the rat abdominal flaps led to a significant decrease in main arterial pedicle blood flow. A similar finding was found by Douglas, in the aforementioned study, when assessing the total number of perforators upon which to base a flap[168]. She found that by increasing the number of perforators used to supply a flap there was a decrease in main pedicle flow.

5.4.1.4 Perforator Selection

In all flaps raised in our animal cohort the ipsilateral most cranial perforator and the contralateral most caudal perforator were selected upon which to base the flaps and perform the study. The reasoning behind this choice of perforator configuration was in terms of translational research as it is likely to be the most useful in terms of potential vascular augmentation of flaps. By having such a perforator configuration it allows the maximal pedicle length available (without the use of interposition grafts) to subsequently augment a flap. For example if this configuration was chosen in a human subject the entire length of the ipsilateral DIEA and DIEV distal to the first perforator (caudal in humans) would be available to potentially use as afferent vessels to augment the flap. Conversely the entire length of the contralateral DIEA and DIEV from proximal to the last perforator (cranial in humans) to their origin would be available as recipient/efferent vessels upon which to perform vascular augmentation of the flap.

5.4.1.5 Genetic Variability of Animal

All rats used in this study were male out-bred Sprague Dawley rats. Sprague Dawley rats are the most commonly used out-bred strain of rat in experimental research[236] and have been used extensively in abdominal flap models[94,162,163,214,229,230]. We accept the genetic variability of the out-bred strain as a potential confounder however our justification for utilising this strain was that they tend to be of a larger size and thus any experiments requiring meticulous dissection of the small perforating blood vessels more feasible and less prone to complications.

5.4.1.6 Performance bias

A single surgeon with some previous microsurgical experience was responsible for performing all procedures and measuring the results. However as exposure/experience was gained in rat abdominal perforator flap dissection and raising it is feasible that flaps were raised quicker and more efficiently in the latter animals in the cohort and we accept this as a potential confounder. Potential consequences of efficient flap raising could be that those animals in the latter part of our study would have higher rates of flow and perfusion secondary to shorter anaesthetic time to reach that point. However given that we were comparing the difference in perfusion and flow between the traditional flap (essentially control) against the vascular augmented flap on each individual rat it is unlikely that this will have affected our results.

5.4.1.7 Temperature

Temperature is known to influence cutaneous blood flow as shown by Bircher et al[208] and also the transit-time ultrasonic probes used to measure flow are calibrated to operate at approximately 37°C. As such room temperature in this experiment was kept constant between 21-24.5°C and the animal temperature continuously monitored by specialist animal technicians and attempts to maintain core temperature made at 37-38°C using an ambient heat source. Mean temperature for all animals was stable and kept within this range. However we accept that temperature can be a confounding factor.

5.4.1.8 Intra-observer error

All results were analysed by the same individual, to reduce inter-observer error; however the possibility of intra-observer error remains and we accept this as a potential confounder.

5.4.2 Conclusion

In conclusion from the findings of our study it has been shown that vascular augmentation of the rat abdominal perforator flap using the contralateral pedicle leads to an increase in Zone IV perfusion however this also coincides with a decrease in main pedicle arterial flow. Further studies in this area are required to assess if these findings correlate in human subjects.

Chapter 6: The use of real-time digital video in the assessment of post-operative outcomes of breast reconstruction.

6.1 Introduction

Standardised pre and post-operative photographs are still the most commonly used method of recording breast reconstruction outcomes. Whilst this method is readily available and reproducible it is inherently flawed by the fact that the images obtained are static and two dimensional. These images only provide limited information to the assessor and it is difficult to ascertain the degree of volume associated with the reconstruction.

Recently more complex methods of assessment utilising three-dimensional capture technology coupled with computer assessment software allowing objective measurement of volume have been described[202,203]. However these assessment methods have largely been utilised for research purposes as they require specialist software, expertise and /or equipment not routinely available in most breast or plastic surgery units.

A major advantage in terms of free tissue transfer breast reconstruction over implant based reconstruction is that the reconstruction is more likely to feel and to move like a normal breast.

In 2005 a study by Gui et al evaluating post-operative outcome methods after immediate breast reconstruction they specifically comment upon the point that standardised photography failed to convey the impact of movement upon breast reconstruction and felt that better assessment may be made utilising video clips, but noted that there were no such studies comparing the use of still photographs with video footage[187].

The aim of this study was to assess whether standardised real-time digital video footage was a valid assessment tool in assessing post-operative outcomes and to compare its use against standardised static photography.

6.2 Methods

35 patients post breast reconstruction underwent photography (cf. 3.3.6), digital video capture (cf. 3.3.7) and completed Breast Cancer Treatment Outcomes Scale (BCTOS) questionnaires (cf. 3.3.5). The photographs/video clips were randomised and shown to a 21

member panel. Opinions on aesthetic aspects of the reconstruction were assessed using the BCTOS and Harris scale (cf. 3.3.9).

Panel inter-rater agreement and patient-panel correlation was assessed using Kendall's Coefficient of Concordance and Spearman's rank correlation tests respectively (cf. 3.3.10).

6.3 Results

35 patients were recruited to this study carried out between 06/08/2012 and 30/01/2013. A summary of patient demographics and type of surgery are shown in Table 6.1.

Age in years	
Mean	52
Standard Deviation	±7
Range	35-67
Side of reconstruction, n (%)	
Left	21 (60%)
Right	14 (40%)
Type of reconstruction, n (%)	
Implant	3 (9%)
Latissimus dorsi (LD) flap plus implant	5 (14%)
Extended Latissimus Dorsi (LD) flap	15 (43%)
Free tissue transfer (abdominal flaps)	12 (34%)

Table 6.1: Summary of patient demographics and type of surgery

All 35 patients underwent the photography and video capture processes uneventfully. Unfortunately one set of patient photographs were corrupted during the transfer process from camera to computer and as such there were only 34 patients included in the photography assessment process. All 35 digital video clips were available for assessment.

The average file storage size of each photo and video set were 0.38 megabytes and 14.9 megabytes respectively. Each video clip ranged approximately (Range 140-194 seconds)

6.3.1 Inter-rater Agreement

A summary of the total panel inter-rater agreement scores are shown in Table 6.2.

Aspect of cosmesis assessed	Photograph Assessment Score	Video Assessment Score
Overall	0.507	0.548
Size	0.514	0.514
Shape	0.486	0.505
Elevation	0.514	0.514

p<0.001 for all quoted values

Table 6.2: Total panel inter-rater agreement scores

Overall there was a "moderate" degree of inter-rater agreement amongst the panel in all categories. However there was a slightly greater degree of panel inter-rater reliability using video assessment in comparison to photographs to assess the overall cosmesis of the reconstruction (0.548 vs 0.507) and to assess shape (0.505 vs 0.486). There was no difference in levels of panel inter-rater agreement between the two methods in terms of assessing size and elevation (0.514 vs 0.514).

A summary of individual panel type inter-rater agreement scores are shown in Table 6.3.

Panel Composition	Overall Cosmesis		Size		Shape		Elevation	
	Photographs	Video	Photographs	Video	Photographs	Video	Photographs	Video
Plastic Surgery	0.655	0.64	0.637	0.678	0.545	0.6	0.475	0.565
General Surgery	0.552	0.563	0.564	0.51	0.516	0.48	0.418	0.443
Non-Surgical	0.496	0.574	0.5	0.529	0.518	0.584	0.384	0.401

p <0.001 for all quoted values

Table 6.3: Inter-rater agreement based on panel composition

On dividing the panel members into their respective cohorts (plastic surgery, general surgery and non-surgical) the highest degree of inter-rater agreement (moderate-strong) occurs amongst the plastic surgery panel utilising both photographs and video images. The plastic surgery panel members showed a slightly lower level of agreement using video footage to assess the "overall" quality of the reconstruction (0.655 vs 0.640) but actually agreed more using video to assess the individual components of the reconstruction (size, shape and elevation).

6.3.2 Patient/Panel Correlation

The correlation between panel scores and patient self-assessment scores are shown in Table 6.4.

Panel Composition	Photographs	*p* -value	Video	*p* - value
Plastic Surgery	0.217	0.204	0.282	0.091
General Surgery	0.357	0.032	0.466	0.004
Non-Surgical	0.083	0.629	0.346	0.036
Total	0.281	0.097	0.311	0.061

Table 6.4: Correlation between patient and panel scores as assessed using photographs versus video

Upon comparing the patient self-scores in comparison to the median panel scores obtained using still photographs and video assessment, the correlation was generally low for both assessment methods. However, a greater degree of correlation exists between the patient and the panel when using video footage as the assessment material (0.311 vs 0.281). The General Surgery panel showed the greatest degree of correlation with the patients' own views using both photography and video footage to assess. The non-surgical panel showed the least degree of correlation using the still photographs as the assessment medium but showed a significant increase in correlation when using the video footage (0.083 vs 0.346).

6.3.3 Assessment Panel Preference

All 21 members (100%) of the panel were of the opinion that video clip footage was more useful in terms of assessing the post-operative outcome of breast reconstruction and that it yielded more information than the still photograph images.

6.4 Discussion

The total panel results from this study show that; whilst marginal, video is equivalent or better than still photographs in terms of inter-rater reliability. The Plastic surgeon sub-division of the panel had the highest degree of inter-rater reliability and this may reflect their experience in terms of assessing reconstruction outcomes.

Given that patient satisfaction is the goal in any form of reconstruction it is of importance to have an assessment tool which more accurately correlates with patient self-evaluation. Our results show that digital video footage assessment gives significantly better correlation between patient views and panel views in comparison to still photograph assessment. This again could be due to the fact that it offers a greater degree of information with regards to the reconstruction (such as mobility) which is not apparent on static photographs. It is also apparent that the greatest degree of correlation between patient perception and sub-panel

(in both photograph and video clip assessment) occurred with the General Breast Surgeons. This is interesting and may be worth further investigation.

All 21 members of the assessment panel felt that video footage yielded a greater amount of information upon which to base their assessment. It was readily apparent throughout the assessment process that the video clips were capturing information which would not be apparent on standardised photographs. In particular these included significant "muscle twitching" of innervated Latissimus Dorsi flaps whilst moving the arm and also the relative absence of reconstruction movement in patients with implant only based reconstructions.

Many published articles analysing ordinal data such as the results obtained in this study have focussed on utilising Kappa scores to assess inter-rater reliability[189,190,198-200]. These kappa scores however only measure the level of "absolute agreement" between raters and fail to take into account the magnitude of difference observed using ordinal data. The Kendall Coefficient of Concordance is a better measure of agreement for ordinal responses as it calculates the consistency amongst raters by also taking the ranking of the scores into account[237]. Furthermore several of the papers have changed their assessment scales by dichotomising categories of the scale to obtain higher kappa scores which may invariably skew the results[198,200].

The model demonstration video which was created for the purposes of this study proved very effective in terms of providing a standardised routine for the patients to emulate. One concern raised after the completion of this study was that each digital video clip was too long at between 140-194 seconds. This was in part due to the fact that the original demonstration script duplicated each of the movements twice. It is feasible that the length of the video clips can easily be reduced to half the duration by omitting the duplication of each of these movements and creating a more streamlined instructional video. This would confer several benefits in that; the video capture process would be quicker; assessment time shorter and the file size of all video clips would be significantly reduced.

Breasts are not static by nature and move to a huge degree through the effects of gravity and bodily motion. In an effort to achieve symmetry (the goal of breast reconstruction) the natural breast motion should not be overlooked. In the post-operative assessment process there is no way to judge this on standardised photographs. Real-time digital video capture offers a suitable replacement to the outdated standardised photographs commonly used in current practice. These could easily be incorporated into both the pre and post-operative

assessment process in most modern plastic surgery departments as an adjunct to standardised photographs.

Furthermore using the protocol in this study the same static images obtained via photography can easily be acquired from the video clips using a "screen-grab" type technique thereby reducing the need for patients to undergo two different capture processes and completely replacing standardised photography.

When Gui et al originally theorised that video footage may provide a more accurate assessment than static photographs alone they foresaw several problems in terms of specialist equipment, cost, storage space and standardisation[187]. Since then technology, software and storage media have advanced significantly as well as becoming cheaper. As a result these issues are no longer as constraining as previously highlighted. Whilst the average file size associated with the digital video clips in this study (14.9 megabytes) was 39.2 times greater than that of the digital photograph files (0.38megabytes) it is still relatively small in comparison to the capacity of most modern storage media which often have gigabytes or terabytes of space.

Through the use of real time digital video capture a huge amount of information can be obtained in a short period of time and the results available for subsequent review and analysis.

6.4.1 Potential Confounders

6.4.1.1 Nipple Reconstruction / Areolar Complex Tattooing

In order to ensure that patients had completed all oncological, adjuvant and major reconstructive procedures it was decided to recruit those patients attending for areolar complex tattooing. Areolar complex tattooing is also usually only performed in patients once their reconstructions have had time to settle (i.e. scars to heal and the effects of ptosis to become apparent). By choosing these patients the aim was also to reduce inconvenience as they were already planning to attend for their procedure and standard photographs and the video process would only add approximately 15minutes onto their appointment.

Patients from a variety of consultants within the unit were included and due to differences within consultants routine practice there was variability as to whether patients had nipple reconstruction or not, i.e. some consultants prefer to tattoo prior to nipple reconstruction

and some consultants prefer to tattoo after nipple reconstruction. Also some patients included in this study had already had previous tattooing procedures carried out and were attending to have the previous tattoo augmented and as a result already had some element of nipple areolar complex during the photo and video process.

Whilst this introduced a degree of variability into our patient sampling it was unlikely to interfere with statistical comparisons or validation of the technique of real time digital video capture. The comparisons were made only between the panel assessment scores for each individual patient individually and no comparisons were made between scores for different patients. However we do accept this degree of variability could be considered as a potential confounder.

6.4.1.2 Types of Breast Reconstruction

Patients included in this study varied upon which type of breast reconstruction they had (cf. 1.3). As such there was a degree of variability introduced into our patient sampling. However, no comparison was made between panel assessment scores given to different reconstructions. Panel member assessment scores were only compared against each other for each individual patients' photographs and video footage to assess for concordance. Whilst it may be an interesting prospect to compare the varying reconstructive techniques utilising real time digital video footage, the purpose of this study was only to validate it as a technique.

6.4.1.3 Symmetrising Surgery

Some patients included in our cohort had contralateral symmetrising surgery whilst others did not. This led to variability in our sample with occasional large size/shape/elevation mismatches between the reconstructed breast and the contralateral breast. However given that the purpose of the study was to assess inter-rater agreement between panel members and no comparison was made between patients it would be unlikely to have any influence on the statistical results obtained (it would be expected that if a large size mismatch existed, panel members assessment scores would reflect this and inter-rater agreement can be measured.)

6.4.2 Conclusion

From our initial findings we have demonstrated that real time digital video is a valid method of capturing patient reconstructions for later analysis. Coupled with panel review it provides a method of assessing post-operative outcomes of breast reconstruction and has advantages in comparison to still photographs in terms of inter-rater agreement and correlation with patient self-assessment.

Chapter 7: Discussion

Breast cancer is the commonest type of cancer to affect women in the United Kingdom; accounting for 30% of all new cases of female cancer[238]. Surgical treatment is the current mainstay of breast cancer management, although this is often augmented by chemotherapy, endocrine therapy and radiotherapy. A large multidisciplinary team approach is used in the individual assessment, management and follow-up of every breast cancer patient.

Surgical treatment options vary dependent upon the size, location, histopathological type, number, receptor status and grade of the tumour(s) present within the breast tissue. Other factors such as genetic risk, patient age, patient health and patient choice also play important roles in the choice of surgical treatment. The surgical options can be broadly divided into breast conserving surgery, where only the tumour is removed and a portion (often the majority) of the patient's own breast tissue is conserved; and mastectomy which involves the complete removal of the breast. Despite modern standard of care favouring breast conservation surgery in those patients with low grade cancers and new oncoplastic techniques allowing a larger number of cancers to be managed via breast conserving surgery it has been shown that greater than 16,000 mastectomies are still required in the United Kingdom each year[228].

Current joint national guidelines state that, disease permitting, all females due to undergo mastectomy for breast cancer should be offered breast reconstruction surgery[239]. The National Mastectomy Audit and Breast Reconstruction audit published in 2011 showed that 21% of women undergoing mastectomy opted to have an immediate reconstruction with a further 11% undergoing delayed reconstruction[228]. The audit highlighted several key points and issues associated with breast reconstruction. Firstly breast reconstruction procedures are safe with few complications requiring emergency transfer to intensive or high dependency care (<1%) and low levels of mortality (<0.3%). Women opting for breast reconstruction had greater levels of physical, emotional and sexual well-being than those undergoing mastectomy alone. Yet despite these factors only 21% of women who underwent mastectomy had immediate breast reconstruction and the findings of the audit suggested that some patients may not have been offered the option of reconstruction based upon geographical location or due to the clinician involved in their care feeling they were not adequate candidates for a variety of reasons. Previous concerns that immediate breast reconstruction may be detrimental to patient outcomes have been shown to be false[240,241] and there is no significant difference in survival rates between patients undergoing

immediate or delayed reconstructions[242-244]. Subsequent to the audit findings, joint national guidelines ("Oncoplastic breast surgery – a guide to good clinical practice) were released in 2012 stipulating that all patients requiring mastectomy be offered immediate breast reconstruction and the relative and absolute contra-indications to immediate breast reconstructions were defined to raise awareness and provide clarity for both breast cancer patients and the clinicians involved in their care[239]. As a result of these guidelines and the fact that the incidence of breast cancer in the UK is increasing, whilst the associated mortality rate is decreasing[238], then it is likely that the number of breast reconstruction procedures performed will also increase.

Another interesting point raised by the audit was that out of the common reconstructions available to patients, implant only, pedicled autologous LD flap +/- implant or free tissue transfer, those patients undergoing autologous reconstructions in the form of pedicled or free flap had higher outcome scores than those undergoing implant based reconstructions. In the delayed setting free tissue transfer outcome scores were the highest out of all reconstructions[228]. These findings agree with evidence in the literature which suggests that patients undergoing autologous tissue transfer reconstruction have higher levels of satisfaction than those undergoing implant based reconstruction[24-26,31,32] and that in the longer term these patients may be happier from an aesthetic point of view with their reconstructions[24,26]. Despite this the audit showed that the majority of patients undergoing immediate reconstruction had implant based reconstructions and alluded to the fact that access to autologous tissue reconstruction may be limited to some patients (particularly in the immediate setting)[228]. This may have been due to several factors including, lack of specialist services in the region offering autologous based reconstructions, funding concerns, lack of theatre time available to accommodate the increased duration of autologous reconstructive procedures and logistical difficulties in terms of the co-ordination between the various surgical teams involved in autologous tissue reconstruction. Irrespective of services offered within each region the joint national guidelines have recommended that all reconstructive options be discussed with each patient[239]. Recent evidence has also been published suggesting that whilst implant based reconstruction may be slightly cheaper initially that in the long-term autologous based reconstructions are more cost-effective[245,246]. With emphasis on development of oncoplastic breast services, increasing evidence base as to the benefit of autologous tissue reconstruction and experience in perforator based flaps, then it is likely that the number of autologous based breast reconstructions and free abdominal flaps being performed will also increase.

The other significant point highlighted by the audit was that involvement of patients throughout their cancer treatment and reconstructive journey is paramount to ensuring the best quality of care[228]. This includes providing adequate information to facilitate their informed contribution to the decision making process. In this respect PROMs are one method of assessing the care we are providing. However difficulties exist in using PROMs to assess post-operative aesthetic results as we know these outcome measures are influenced by a variety of different factors. It was recommended that, as a minimum standardised photographs should be taken as an objective cosmetic outcome measure and that satisfaction with cosmetic outcome should be assessed by PROMs at three and eighteen months.

As discussed the number of autologous based breast reconstruction procedures, in particular free tissue transfer, is likely to increase in current practice. Therefore it is important that every effort be made to ensure that these procedures are safe with low rates of complications and are able to fulfil the reconstructive goal in terms of size, shape and texture. Furthermore due to deficiencies in the use of patient reported outcome measures in the aesthetic assessment of post-operative results other accurate assessment tools are required in order to be able to compare post-operative results. Research into the field of free tissue breast transfer and post-operative breast reconstruction assessment is required to ensure the best of care is available to each patient.

7.1 Summary of results

The research contained within this thesis addresses some of the concerns discussed above.

It was recognised that vascular complications associated with free DIEP flaps can occur and are most likely to occur in areas of the flap furthest from the perforating vessels. Additional vascular augmentation procedures to improve perfusion to the DIEP flap have been described, however the evidence for their use is limited; confined to case or small series based studies. We undertook a pilot study using LDI and ICG angiography to objectively assess the effects of vascular augmentation procedures on Zone IV perfusion of DIEP flaps using the contralateral SIE vascular system. Our findings suggest that there does appear to be a trend towards increasing perfusion to Zone IV skin and fat when the flap is augmented using the SIEA and SIEV. SIEV augmentation alone seemed to have the least effect on perfusion to Zone IV which is interesting since most DIEP flap problems seem to occur as a result of venous congestion. Whilst SIEV augmentation alone may help

venous drainage in some failing flaps it was apparent that this augmentation alone would not improve overall Zone IV perfusion. SIEA augmentation led a much greater degree of perfusion however SIEA augmentation alone did not lead to statistically significant results. The only statistically significant increase in perfusion found in this study was when both the SIEA and SIEV in combination were used to augment the flap. However this was only a pilot study with a small cohort number and given the large difference in median perfusion observed upon SIEA augmentation alone it is likely that this may be clinically relevant and the failure to reach statistical significance could be due to the power considerations of the study. The results obtained from this pilot study could be used to make power calculations to assess the sample size required for a larger study. We accept that experimental nature of this study and that the arbitrary values obtained via LDI and ICG angiography are very difficult to interpret in terms of translational research as these values differ widely between individual patients and upon the type of machine or software used. As such no exact values of perfusion, like those contained within this study, correlating with flap viability have been published in the literature. In our study given the fact that Zone IV was discarded immediately after the research measurement process we also accept that the follow-up is inadequate to assess the effects of vascular augmentation on fat necrosis or overall outcome of reconstruction.

From our human study it was apparent that vascular augmentation of DIEP flaps led to an increase in Zone IV perfusion however we wished to investigate the effect that this augmentation procedure may have on Zone I perfusion and main pedicle blood flow. An experimental animal based study raising abdominal perforator flaps on rats was undertaken and the effect of vascular augmentation on these flaps utilising the contralateral pedicle and a caudal perforator was assessed. The results from our animal study corroborated our human study findings in that vascular augmentation does indeed significantly increase Zone IV perfusion. It also increased Zone I perfusion to a smaller degree. However the findings of our study showed that whilst vascular augmentation of the rat abdominal flaps raised perfusion it decreased blood flow through the main arterial pedicle. The effect that this decrease upon main pedicle flow has on overall flap viability is uncertain and as discussed previously flap flow/perfusion is a complex process with multiple interdependent variables (cf. 1.2.3). It may be that this decrease in main pedicle flow is a transient process or with little effect on skin/fat perfusion and we accept that the follow up in this experimental study is inadequate to assess these points.

It was identified that current methods of post-operative outcome assessment following breast reconstruction are flawed secondary to the fact that patient reported outcome assessment of aesthetic outcome has many additional confounding factors and also current photography and panel assessment methods have poor inter-rater agreement and patient correlation. Whilst newer techniques have been developed to attempt to provide more robust objective methods of assessment, the equipment / expertise required to utilise these are out-with those commonly available to the majority of Breast or Plastic Surgery units. Digital video recording equipment and medical illustration staff trained in filmography are available in the majority of Breast and Plastic Surgery units. We undertook a study to assess whether real-time digital video footage may be a valid assessment tool coupled with panel assessment and to compare its use to current standardised photography. Through this research we validated the use of real time digital video in the post-operative outcome assessment of breast reconstruction patients. We have devised a protocol outlining a simple, reproducible sequence of movements for patients to perform whilst being recorded to allow subjective assessment. Patients found this an easy intervention to comply with and the assessment panel universally agreed that it offered more information upon which to base an assessment. Furthermore our results suggest that not only is real-time digital video a valid option in post-operative outcome assessment but that it provides more consistent inter-rater agreement with regards to cosmesis and shape. It also has an increased level of correlation with patient satisfaction in comparison to conventional photography.

7.2 Potential Clinical Implications

Our research into the effects of vascular augmentation of DIEP flaps using the contralateral SIE vascular system suggests that this does increase Zone IV perfusion to the flap. This could potentially allow the inclusion of Zone IV if required such as when a larger skin paddle is needed in delayed reconstructions. Whilst additional anastomoses would increase the operative time for patients the ability to utilise larger more robust flaps flaps could reduce the need for subsequent revision procedures. This should be undertaken with caution however and we would not recommend routinely carrying out the vascular augmentation procedures as our findings also suggest that such procedures may have deleterious effects upon main pedicle arterial flow. Further in-vivo investigational work is required in this area.

The technique and use of real-time digital video capture in post-operative breast reconstruction outcome assessment has been validated by this research. From our

experience and the patients involved in our study it is an acceptable reproducible way to record clinical information. Real time digital video footage yields more information than standardised photographs and appears to be a superior to standardised photography in panel assessment. Given the readily available nature of digital video capture in modern practice coupled with cost effective storage media we believe that real time digital video footage could be a potential replacement for standardised photographs in the field of breast reconstruction outcome recording and assessment. Some may comment that still images may be required on occasion, for example to publish in a hard copy journal, but these images can easily be obtained from the real time digital video footage by pausing the playback of the recording at the desired time and obtaining a still image via screen capture.

7.3 Future Work

With regards to the vascular augmentation of DIEP flaps, even though these interventions are commonly undertaken in clinical practice as salvage procedures, we believe that further clinical studies are required. Prior to carrying out such vascular augmentation procedures pro-actively, studies should be undertaken to assess the potential deleterious effects it may have on the rest of the flap. We propose that a further in-vivo human study should be undertaken and the effects on main pedicle blood flow and Zone I-IV perfusion assessed after vascular augmentation of the flap.

With regards to the use of real time digital video in the post-operative outcome assessment of breast reconstruction further work could be undertaken to shorten the length of the video clips recorded by providing a more succinct sequence of movements for the patient to perform. This could easily be achieved by removing the repetition of movements at each stage from our established protocol and producing a new model demonstration for patients to copy. We believe that this would result in a video capture process of approximately the same time; or indeed shorter than the photography process which patients currently undergo. Once the shortened video sequence was revalidated a large scale study comparing the various breast reconstruction options (implant based or autologous) could be undertaken using video footage coupled with panel assessment and patient satisfaction.

Another interesting project to consider with regards to real time digital video would be whether the capture process could be fully automated. In our initial study patients found it easy to copy the model demonstration coupled with audio cues being played to them during the recording process. We believe that a set-up could easily be arranged in which a

patient could independently control their own recording process. Such a set-up would consist of a private room containing a digital video camera and a television monitor to play the model demonstration. A spot could be marked on the floor dictating where the patient should stand facing the camera and television screen. The patient would be provided with a a remote control to activate the model playback process. By linking the playback initiation with the commencement of the recording capture process the patient themselves can choose when to begin. An automated real-time digital video capture system like this to replace traditional photography could have huge benefits for both breast reconstruction patients and the healthcare system. An automated patient driven system would empower the patient allowing them to control one aspect of their reconstructive journey pre and post operatively. It would also be beneficial in terms of respect for patient privacy as they would not need to undress in front of an additional member of staff (i.e. photographer). There would also be cost saving implications of such a system as it is feasible that any private room (i.e. clinic room) could be utilised to carry out the capture process in comparison to a dedicated photography studio. Also it would reduce the need for photography staff and chaperones (if required).

Future studies could also investigate the possibility of four dimensional breast reconstruction capture and assessment. This could be achieved by incorporating a real time capturing component into the three-dimensional process already described by Henseler et al.[202,203] This could provide extremely detailed objective measurements at the same time as assessing the effects of mobility. Whilst it is unlikely that such a system could be used in current clinical practice it could provide a useful research tool.

Appendices

Appendix A

Repeated Measure ANOVA: LDI zone IV skin perfusion
Measurement versus Intervention, Patient

```
Factor        Type    Levels  Values
Intervention  fixed          4  Art/Vein Supercharged, Arterial Supercharged,
                                Traditional Flap, Venous Supercharged
Patient       random        11  2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12


Analysis of Variance for Measurement, using Adjusted SS for Tests

Source        DF   Seq SS   Adj SS  Adj MS      F      P
Intervention   3   1505.8   1612.2   537.4   5.07  0.008
Patient       10  25773.1  25773.1  2577.3  24.33  0.000
Error         23   2436.3   2436.3   105.9
Total         36  29715.2


S = 10.2921   R-Sq = 91.80%   R-Sq(adj) = 87.17%


Unusual Observations for Measurement

Obs  Measurement      Fit  SE Fit  Residual  St Resid
 15       57.000   57.000  10.292    -0.000         * X
 33       79.000  102.000   7.633   -23.000     -3.33 R
 34      128.000  105.000   7.633    23.000      3.33 R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.
```

Figure A 1: Minitab output for repeated measures ANOVA LDI Zone IV Skin Perfusion

Repeated Measure ANOVA: ICG zone IV skin perfusion
Measurement versus Intervention, Patient

Factor	Type	Levels	Values
Intervention	fixed	4	Art/Vein Supercharged, Arterial Supercharged, Traditional Flap, Venous Supercharged
Patient	random	12	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12

Analysis of Variance for Measurement, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Intervention	3	2784	2063	688	0.43	0.734
Patient	11	31164	31164	2833	1.77	0.107
Error	29	46470	46470	1602		
Total	43	80418				

S = 40.0304 R-Sq = 42.21% R-Sq(adj) = 14.32%

Unusual Observations for Measurement

Obs	Measurement	Fit	SE Fit	Residual	St Resid
14	42.000	120.550	22.526	-78.550	-2.37 R
15	207.000	132.175	22.821	74.825	2.28 R
23	20.000	111.925	22.821	-91.925	-2.80 R

R denotes an observation with a large standardized residual.

Figure A 2: Minitab output for repeated measures ANOVA ICG Zone IV Skin Perfusion

Repeated Measure ANOVA: ICG zone IV fat perfusion
Measurement versus Intervention, Patient

Factor	Type	Levels	Values
Intervention	fixed	4	Art/Vein Supercharged, Arterial Supercharged, Traditional Flap, Venous Supercharged
Patient	random	12	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12

Analysis of Variance for Measurement, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Intervention	3	4932.9	3912.1	1304.0	2.04	0.130
Patient	11	6826.0	6826.0	620.5	0.97	0.493
Error	29	18545.1	18545.1	639.5		
Total	43	30304.0				

S = 25.2881 R-Sq = 38.80% R-Sq(adj) = 9.26%

Unusual Observations for Measurement

Obs	Measurement	Fit	SE Fit	Residual	St Resid
6	111.000	68.517	14.230	42.483	2.03 R
14	19.000	62.767	14.230	-43.767	-2.09 R
15	119.000	70.225	14.416	48.775	2.35 R
44	130.000	77.825	14.416	52.175	2.51 R

R denotes an observation with a large standardized residual.

Figure A 3: Minitab output for repeated measures ANOVA ICG Zone IV Fat Perfusion

Appendix B

Breast Reconstruction Questionnaire

NHS Greater Glasgow and Clyde

Trial number : [ ]

Patient Name _____

Patient Date of Birth ____/_____/_____

Date of Reconstruction ____/_____/_____ (if known)

Please delete as appropriate

Side of Reconstruction: Left / Right

Type of Reconstruction:
Implant based reconstruction
Latissimus Dorsi reconstruction
DIEP flap reconstruction
Other _____ (please specify if known)

We are interested in your evaluation of your physical appearance and functioning since your breast reconstruction surgery. Please rate the following items on this four-point scale, according to your evaluation at this point in time.

1 = no difference between reconstructed breast/area and untreated breast/area
2 = slight difference between reconstructed breast/area and untreated breast/area
3 = moderate difference between reconstructed breast/area and untreated breast/area
4 = large difference between reconstructed breast breast/area and untreated breast/area

___ 1. Breast size
___ 2. Breast texture (hardening)
___ 3. Arm heaviness
___ 4. Nipple appearance
___ 5. Shoulder movement
___ 6. Arm movement
___ 7. Breast pain
___ 8. Ability to lift objects
___ 9. Fit of shirt sleeve
___ 10. Breast tenderness
___ 11. Shoulder stiffness
___ 12. Breast shape
___ 13. Breast elevation (how high the breast is)
___ 14. Scar tissue
___ 15. Shoulder pain
___ 16. Arm pain
___ 17. Arm swelling
___ 18. Breast swelling
___ 19. Arm stiffness
___ 20. Fit of bra
___ 21. Breast sensitivity
___ 22. Fit of clothing

Designed & Produced by Medical Illustration Services Ref 253558-Questionnaire

Figure B 1: Patient breast reconstruction questionnaire form

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