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FREE FLAP MONITORING

Using Tissue Oxygen Measurement and Positron Emission Tomography

by

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To my family

Cover: Parametric blood flow image and transmission PET image of a breast area with regions of interest drawn on the breast reconstruction flap and on the contralateral healthy breast on the first postoperative day.

Abstract

Aleksi Schrey. **Free flap monitoring using tissue oxygen measurement and positron emission tomography**. From the Department of Otorhinolaryngology - Head and Neck Surgery, University of Turku, Finland.
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Aims: This study was carried out to evaluate the feasibility of two different methods to determine free flap perfusion in cancer patients undergoing major reconstructive surgery. The hypotheses was that low perfusion in the flap is associated with flap complications.

Patients and methods: Between August 2002 and June 2008 at the Department of Otorhinolaryngology – Head and Neck Surgery, Department of Surgery, and at the PET Centre, Turku, 30 consecutive patients with 32 free flaps were included in this study. The perfusion of the free microvascular flaps was assessed with positron emission tomography (PET) and radioactive water ($[^{15}O]$ H_2O) in 40 radiowater injections in 33 PET studies. Furthermore, 24 free flaps were monitored with a continuous tissue oxygen measurement using flexible polarographic catheters for an average of three postoperative days.

Results: Of the 17 patients operated on for head and neck (HN) cancer and reconstructed with 18 free flaps, three re-operations were carried out due to poor tissue oxygenation as indicated by $p_{ti}O_2$ monitoring results and three other patients were reoperated on for postoperative hematomas in the operated area. Blood perfusion assessed with PET (BF_{PET}) was above 2.0 mL / min / 100 g in all flaps and a low flap-to-muscle BF_{PET} ratio appeared to correlate with poor survival of the flap. Survival in this group of HN cancer patients was 9.0 months (median, range 2.4-34.2) after a median follow-up of 11.9 months (range 1.0-61.0 months). Seven HN patients of this group are alive without any sign of recurrence and one patient has died of other causes.

All of the 13 breast reconstruction patients included in the study are alive and free of disease at a median follow-up time of 27.4 months (range 13.9-35.7 months). Re-explorations were carried out in three patients due data provided by $p_{ti}O_2$ monitoring and one re-exploration was avoided on the basis of adequate blood perfusion assessed with PET. Two patients had donor-site morbidity and 3 patients had partial flap necrosis or fat necrosis. There were no total flap losses.

Conclusions: $P_{ti}O_2$ monitoring is a feasible method of free flap monitoring when flap temperature is monitored and maintained close to the core temperature. When other monitoring methods give controversial results or are unavailable, $[^{15}O]$ H_2O PET technique is feasible in the evaluation of the perfusion of the newly reconstructed free flaps.

Key words: free flap, microsurgery, oxygen partial pressure, perfusion, positron emission tomography, postoperative monitoring

Tiivistelmä

Aleksi Schrey.
Vapaiden kudossiirteiden seuranta kudoshappiosapaineen ja positroniemissiotomografian avulla. Korva-, nenä- ja kurkkutautioppi**,** Turun Yliopisto, Turku**.**
Annales Universitatis Turkuensis, Medica-Odontologica, 2009, Turku.

Tavoite: Väitöskirjatyön tarkoituksena oli tutkia kahden erityyppisen seurantamenetelmän sopivuutta vapaiden kudossiirteiden verenkierron arviointiin syöpäleikkauksen jälkeen. Vapaan kudossiirteen matalan verenvirtauksen oletettiin olevan yhteydessä kudossiirteisiin liittyviin komplikaatioihin.

Aineisto ja menetelmät: Aikavälillä elokuusta 2002 kesäkuuhun 2008 kerättiin 30 perättäistä potilasta, joille tehtiin syöpäleikkaukseen liittyen yhteensä 32 vapaata kudossiirrettä. Vapaiden mikrovaskulaaristen kudossiirteiden perfuusiota tutkittiin positroniemissiotomografialla (PET) ja lievästi radioaktiivisella vedellä ([^{15}O] H_2O) 40 radiovesi-injektiolla 33 PET-tutkimuksessa. Lisäksi 24 vapaata kudossiirrettä seurattiin kolmen päivän ajan jatkuvalla kudoshappimittauksella ($p_{ti}O_2$) käyttäen joustavia polarografiseen menetelmään perustuvia antureita.

Tulokset: Seitsemästätoista pään ja kaulan alueen (HN) syöpäpotilaasta kolme uusintaleikkausta tehtiin $p_{ti}O_2$-mittaustulosten perusteella ja kolme muuta uusintaleikkausta tehtiin leikkausalueen hematooman takia. PET-kuvauksella saatu perfuusio oli kaikissa pään ja kaulan alueen siirteissä yli 2.0 mL / min / 100 g. Matala siirre-lihas suhde korreloi siirteen huonoon menestymiseen. Tähän tutkimukseen osallistuneiden HN-syöpäpotilaiden mediaanielossaolo on 9.0 kk (vaihteluväli 2.4-34.2) 11.9 kuukauden seuranta-ajan jälkeen (mediaani, vaihteluväli 1.0-61.0 kk). Yksi tutkimukseen osallistuneista HN-potilaista on menehtynyt muusta syystä ja seitsemän potilaista on elossa taudista vapaina.

Kaikki tutkimukseen osallistuneet 13 rintarekonstruktiopotilasta ovat elossa ilman merkkejä taudin uusimisesta 27.4 kuukauden (mediaani, vaihteluväli 13.9-35.7 kk) seuranta-ajan jälkeen. Uusintaleikkauksia tehtiin kolmelle potilaalle perustuen $p_{ti}O_2$-mittaustuloksiin ja toisaalta yksi uusintaleikkaus vältettiin PET-kuvauksen antaman riittävän perfuusiotuloksen perusteella. Kahdella potilaalla oli siirteen ottokohdassa morbiditeettia ja 3 potilaalla esiintyi joko osittaista siirteen ihonekroosia tai rasvanekroosia. Yhtään siirrettä ei menetetty tässä tutkimuksessa kokonaan.

Johtopäätökset: $P_{ti}O_2$-monitorointi on käyttökelpoinen vapaiden kudossiirteiden seurantamenetelmä, kun samalla seurataan kudossiirteen lämpötilaa ja pyritään pitämään se lähellä normaalia kehon lämpötilaa. Kun muut seurantamentelmät antavat ristiriitaisia tuloksia tai niitä ei ole lainkaan saatavilla, [^{15}O] H_2O-PET menetelmää voidaan käyttää kudossiirteen verenkierron arviointiin.

Avainsanat: happiosapaine, positroniemissiotomografia, postoperatiivinen monitorointi, rekonstruktiivinen kirurgia, vapaa monikudossiirre

Table of contents

Abbreviations and definitions

Abbreviations

$[^{15}O]\ CO_2$	oxygen-15 labeled carbon dioxide
$[^{15}O]\ H_2O$	oxygen-15 labeled water
^{99m}Tc	technetium
^{133}Xe	xenon
AIF	arterial input function
ALT	anterolateral thigh flap
ATBF	adipose tissue blood flow
ATP	adenosine triphosphate
BC	breast carcinoma
BMI	body mass index
BF	blood flow
BF_{PET}	blood perfusion
BP	blood pressure
Bq	becquerel
CO_2	carbon dioxide
CT	computed tomography
DIEP	deep inferior epigastric perforator
DP	diastolic (blood) pressure
EDRF	endothelial-derived relaxing factor
FCD	functional capillary density
FDG	fluorodeoxyglucose
FOV	field of view
Gy	gray
H_2O_2	hydrogen peroxide
HG	heterogeneity
HN	head and neck
HNSCC	head and neck squamous cell carcinoma
ICU	intensive care unit
IFM	intravital fluorescence microscopy
IMA	internal mammary artery
LD	latissimus dorsi
LDF	laser Doppler flowmetry
MAP	mean arterial pressure
mmHg	millimeters of mercury
MS	muscle-sparing
MRI	magnetic resonance imaging
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate
NIRS	near-infrared spectroscopy
O_2	oxygen
O_2^-	superoxide
OC	oral cavity
OH^-	hydroxyl ion
OPS	orthogonal polarization spectral

OSEM	ordered subset expection maximisation
PET	positron emission tomography
PFL	partial flap loss
PO	postoperative
pO_2	oxygen partial pressure
p_aO_2	oxygen partial pressure in the alveoli
$p_{tc}O_2$	transcutaneous oxygen partial pressure
$p_{ti}O_2$	oxygen partial pressure in tissue
RFF	radial forearm flap
RM	retromolar
ROI	region of interest
RT	radiotherapy
SD	standard deviation
SP	systolic (blood) pressure
SPET	single photon emission tomography
SUV	standardized uptake value
Sv	Sievert
TDA	thoracodorsal artery
TRAM	transverse rectus abdominis musculocutaneous
UICC	International Union against Cancer
XO	xanthine oxidase

Definitions

Blood flow	amount of blood streaming through a vessel, tissue or organ in a given period of time (mL / min)
Dynamic scan	scan acquired over a given time period with multiple frames
Free flap	a free transfer of living tissue to a distant site connected to the new site by attaching donor and recipient blood vessels
Blood perfusion	amount of blood streaming through a volume of tissue or an organ per time (mL blood / min / mL tissue)
Pixel	two dimensional element of images
Plane	one aspect of an image´s matrix, indicating where the image is located relative to the patient´s position
Voxel	three dimensional volume element, representing a value on a regular grid in a three dimensional space (mL)

List of original publications

This thesis is based on the following original publications. The publications are referred to in the text by Roman numerals I-V.

I **Schrey AR, Aitasalo KM, Kinnunen IA, Laaksonen MS, Parkkola RK, Taittonen MT, Grénman RA, Minn HR.** Functional evaluation of microvascular free flaps with positron emission tomography. Journal of Plastic, Reconstructive & Aesthetic Surgery 2006; 59: 158-165.

II **Schrey AR, Kinnunen IA, Grénman RA, Minn HR, Aitasalo KM.** Monitoring microvascular free flaps with tissue oxygen measurement and PET. Eur Arch Otorhinolaryngol. 2008 Jul;265 Suppl 1:S105-13.

III **Schrey A, Niemi T, Kinnunen I, Minn H, Vahlberg T, Kalliokoski K, Suominen E, Grénman R, Aitasalo K.** The limitations of tissue-oxygen measurement and positron emission tomography as additional methods for postoperative breast reconstruction free-flap monitoring. Journal of Plastic, Reconstructive & Aesthetic Surgery. doi:10.1016/j.bjps.2008.09.029 (in press)

IV **Schrey AR, Kalliokoski K, Kinnunen I, Minn H, Grénman R, Vahlberg T, Niemi T, Suominen E, Aitasalo K.** Perfusion in Free Breast Reconstruction Flap Zones. *(submitted)*

V **Schrey AR, Kinnunen I, Vahlberg T, Minn H, Grénman R, Taittonen M, Aitasalo K.** Blood pressure and free flap oxygenation in head and neck cancer patients. (*submitted)*

1. Introduction

The first experimental free flap operation was reported by Krizek in 1965 (Krizek et al. 1965). Six years later, in 1971, Antia and Buch performed the first free microvascular flap operation in a patient (Antia and Buch 1971). A posttraumatic facial defect was reconstructed using a lower abdominal flap, which unfortunately became partially necrotic. McLean and Buncke reported the first completely successful microvascular transfer in clinical practice; they used an omental flap for filling a large scalp defect (McLean and Buncke 1972). Daniel and Taylor were the first to introduce the free skin flap (groin flap) in reconstructive surgery (Daniel and Taylor 1973). In 1974 Harii reported the successful use of different types of free microvascular flaps (Harii et al. 1974). Since these times, free tissue transfers that employ microvascular techniques have become an established and reliable method in the field of reconstructive surgery (Disa et al. 1997, Hidalgo et al. 1998).

With advanced microsurgical techniques and the development of more reliable flaps, free-tissue transfer has become a routine procedure in reconstructive surgery. However, postoperative circulatory failure warranting immediate re-exploration and restoration of adequate anastomosis patency still occurs in up to 28 % of patients even in the most experienced hands (Aitasalo et al. 1997, Hidalgo et al. 1998, Khouri et al. 1998, Shaari et al. 1998, Nieminen et al. 1999, Finical et al. 2001, Schultze-Mosgau et al. 2002). On the other hand, flap viability has improved significantly since the introduction of free microvascular reconstructions in the late 1960s, and the currently overall final success rates of greater than 95 % are being reported (Khouri et al. 1998, Disa et al. 1999, Wei et al. 2001). Free flaps that include an artery are more reliable than flaps relying on venous reconstructions and are thus used more often, albeit at the expense of greater donor morbidity (Tan et al. 2009).

Early detection of circulatory compromise allows timely re-exploration of the vascular anastomoses and salvage of a failing flap before irreversible no-reflow occurs (May et al. 1978). Salvage rates exceeding 50 % have been reported (Wei et al. 2001, Top et al. 2006). The time interval between the onset of circulatory compromise and salvage surgery is crucial for the outcome of microvascular flap surgery (Biemer 1981, Stack et al. 2003, Genden et al. 2004).

For instance, vasospasm, decreased distal perfusion pressure, release of local vasoconstrictive substances or depletion of vasodilator substances, and production of oxygen-derived free radicals are mechanisms causing microvascular problems in the flap circulation (Walkinshaw et al. 1988, Mounsey et al. 1992). Several vasoactive drugs have been used both topically and systemically to improve flap survival, but the results have not been universally successful (Carroll and Esclamado 2000). Various interventions, such as flap cooling, and ischemic preconditioning, have also been intensely studied (Tsai et al. 1982, Restifo and Thomson 1998, Dhar and Taylor 1999). Flap failure is disastrous to the patient and resource demanding to the society. Flap failure is most often due to vascular reasons and an improved understanding of flap

hemodynamics and how it responds to ischemia are thus of central importance for attempts to minimize morbidity.

To minimize the incidence of flap failure early detection of postoperative flap ischemia is necessary for timely re-exploration (Disa et al. 1999). More than 60 % of microsurgeons routinely utilize some adjuvant monitoring device to aid clinical assessment of the flap (Hirigoyen et al. 1995, Jallali et al. 2005). However, no single monitoring technique described in the literature has fulfilled all requirements to be universally applicable for this purpose (Heden et al. 1985, Jones 1988, Udesen et al. 2000, Yuen and Feng 2000a, Kamolz et al. 2002, Setala et al. 2004, Hölzle et al. 2006, Repez et al. 2008). The ideal monitoring method would be non-invasive, reliable, continuous, accurate, and easy to use even for the inexperienced personnel, inexpensive, and provide real-time information. Although a multitude of monitoring methods has been introduced, clinical observation is still the gold standard of flap assessment, despite its inherent problems (Neligan 1993, Hirigoyen et al. 1995).

In the present work, the vitality of free flaps was studied in 30 patients operated on for head and neck cancer (I-II, V) or breast cancer (III-IV). Positron emission tomography (PET) with oxygen-15 labeled water ([^{15}O] H_2O) was used in 33 scannings to assess quantitative blood flow or perfusion of the newly reconstructed area. Tissue oxygenation was evaluated with a polarographic catheter in 24 patients. Both methods were used altogether in 24 patients.

2. Review of the literature

2.1 Anatomy, physiology, and regulation of circulation

2.1.1. Functional anatomy of circulation

The heart is the muscular organ of the circulatory system that constantly pumps blood throughout the body. The blood streams through two anatomically separate vascular pumps. The pulmonary circulation conveys deoxygenated blood from the right ventricle to the lungs via pulmonary arteries, arterioles and capillary network. The blood is oxygenated in the capillary network around the alveoli of the lungs and then progresses to the left side of the heart via the pulmonary venules and veins to the left atrium. The systemic circulation delivers this oxygenated blood from the left ventricle thoughout the body through arteries, arterioles and capillaries and returns it through venules, veins and finally through the inferior and superior vena cavae to the right side of the heart (Opie 1998) (Figure 1).

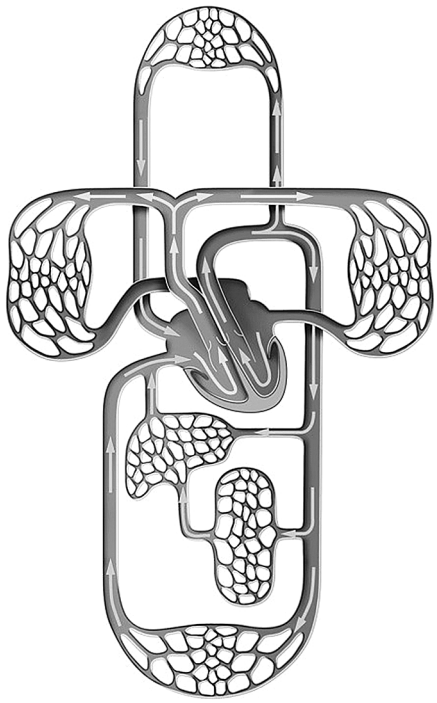

Figure 1. A schematic illustration of the human circulation (reprinted with permission from Dorling Kindersley Limited) (Harvey 1628).

Although the blood vessels in each flap tissue have their own specific characteristics, some general principles of vascular function apply to all parts of the circulation of the

body. The arteries transport blood under high pressure to the target tissues, and the walls of the large arteries with rapid blood flow are thick. The end branches of the arterial system, the arterioles, act as control conduits through which blood is released into the capillary beds. Arterioles have muscular walls that can close the vessels completely or dilate them several fold. Thus, arterioles can alter blood flow to the capillary network in response to the needs of the tissue in an efficient way. Capillaries consist of a single layer of endothelial cells, and the thin walls allow exchange of nutrients, hormones, electrolytes, and other substances between the blood and the interstitial fluid. About 84 per cent of the entire blood volume of the body is in the systemic circulation, and 16 per cent in heart and lungs. Of the 84 per cent in the systemic circulation, 64 per cent is in the veins, 13 per cent in the arteries, and 7 per cent in the systemic arterioles and capillaries. The heart contains 7 per cent of the blood, and the pulmonary vessels, 9 per cent. Veins act as a reservoir, and venous tone is important for maintenance of the return of blood to the heart, e.g. in severe hemorrhage, when sympathetic stimulation causes venoconstriction (Guyton and Hall 2006).

2.1.1.1. Cutaneous perfusion

The first referred publication of blood flow to the skin was in the 17th century by William Harvey (Harvey 1628). Tomsa described the subdermal and dermal plexuses of the skin in 1873 (Tomsa 1873), and Spateholz the direct and indirect perforators to the skin (Spateholz 1893). In 1889, Manchot detailed the cutaneous blood supply and identified distinct areas of the skin which were separately perfused by source vessel (Manchot 1889). His work formed the basis of the studies of Salmon, who found that in reconstructive surgery a flap must include an arterial pedicle (Salmon 1936a, Salmon 1936b). Our knowledge of cutaneous perfusion has since the 1930s remained relatively unchanged. Nowadays, the vasculature of the skin and subcutis is believed to consist of five vascular plexuses (Figure 2). The most superficial is the subepidermal plexus, beneath which run the dermal, subdermal, subcutaneous, and the fascial plexuses. Each plexus consists of a horizontal, fine meshwork of interconnecting vessels. The plexuses have a huge capacity for distributing blood flow to the skin and subcutis. The dermal plexus has muscular arteriolar vessels and is the main thermoregulatory system, while the subdermal plexus has thin-walled capillaries and is the main site for nutrient exchange. The blood flow to the vascular plexuses of the skin and subcutis is supplied through the perforator arteries, which arise from source arteries below the deep cutaneous fascia (Blondeel et al. 2006).

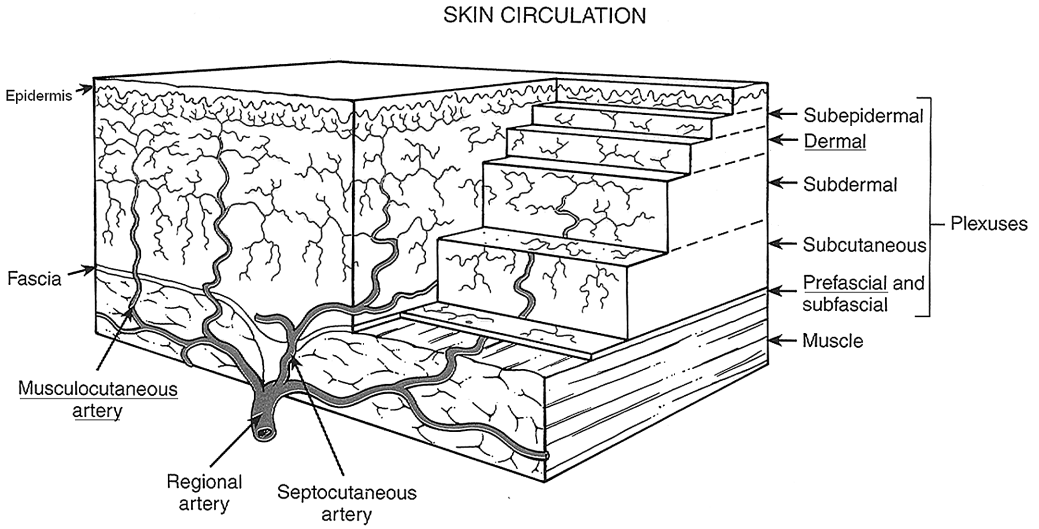

Figure 2. A schematic representation of the vascular structure of the skin and subcutis. The important structures for flap blood flow such as dermal and prefascial plexuses are underlined as welll as the musculocutaneous artery. Modified with permission (Mathes and Nahai 1997).

2.1.2. Physiology of circulation regulating blood flow in free flaps

Cardiac output is defined as the volume of blood pumped by the heart over time. In healthy adults with a body weight of 70 kg the cardiac output is approximately 5 liters per min at rest (Berne and Levy 1997). During maximal exercise it may rise 7-fold (Ekblom and Hermansen 1968, Guyton and Hall 2006). The most important factor determining cardiac output is the heart rate. The other three factors, which influence the stroke volume and thereby cardiac output, are preload, afterload, and myocardial contractile state (Opie 1998).

The definition of blood flow is the amount of blood streaming through a certain point in the circulation in a given period of time. Blood flow is determined by two factors in all intact living tissues: (1) the pressure difference of the blood between the two measuring points of a vessel, and (2) the vascular resistance impeding blood flow through the vessel.

Mathematically, blood flow is described by Darcy's law (which can be viewed as the fluid equivalent of Ohm's law) and approximately by Poiseuille equation.
BF can be calculated by:

$$\mathbf{BF = \Delta P/R} \qquad \text{(equation 1)}$$

In the above equation 1, BF is blood flow (L/min), ΔP is the pressure difference between the two measuring points of the vessel (mmHg), and R is the vascular resistance (mmHg/L/min) (McKee et al. 1982, Sasmor et al. 1992, Guyton and Hall

2006). Vascular resistance is thus inversely related to blood flow, and is an important regulator of the blood flow in a flap (Sasmor et al. 1992).

The diameter of the vessel is very important for its ability to conduct blood. The blood flow near the wall of the vessel flows quite slowly, whereas the velocity of blood in the middle of the vessel is more rapid (laminar flow). Thus, in a vessel with a small diameter, almost all the blood is near the wall, and the proportion of rapidly flowing blood in the center of the vessel is small. The blood flow rate is directly proportional to the fourth power of the radius of the vessel (Poiseuille's law):

$$\mathbf{BF = \pi \Delta P r^4 / 8 \eta L} \qquad \text{(equation 2)}$$

In equation 2, BF is the rate of blood flow (mL/min), ΔP is the pressure difference between the measuring points of the vessel (mmHg), r is the radius of the vessel (m), L is the length of the vessel (m), η is the viscosity of the blood (kg/s/m), and π is the ratio of perimeter to the vessel radius (approximately 3.1416). Therefore, in addition to vascular resistance the diameters of blood vessels substantially regulate perfusion of the flap.

Blood viscosity is an important variable in Poiseuille's law. If all other factors stay constant, the higher the viscosity of the blood, the lower the flow is in a vessel. The viscosity of blood of healthy persons (with a hematocrit 0.40) is about three times greater than that of water. The high number of suspended red cells is the main determinant of blood viscosity (Guyton and Hall 2006). Nitric oxide mediated vasodilation may also contribute to perfusion, at least in conditions of extreme hemodilution (Tsai et al. 2005).

Perfusion increases immediately after skeletal muscle denervation, which causes arteriolar vasodilatation and increased capillary perfusion (Chen et al. 1991, Chen et al. 1992, Wang et al. 1995). It is known that the perfusion of free flaps increases two weeks to three months postoperatively, as assessed by Doppler ultrasound measurements (Salmi et al. 1995a). Denervation of a cutaneous flap decreases its vascular resistance (Finseth and Cutting 1978, McKee et al. 1982). Experimentally it has been shown that (Erni et al. 1999) blood flow increases in skin island flaps after sympathectomy, which is most likely due to vasodilation caused by a lack of sympathetic neural regulation in the second and third order arterioles, which have the highest density of adrenergic innervation and contribute more than any other microvessels to vascular resistance and flow regulation. Wang (Wang et al. 1995) used the vessel diameter for representing changes in vascular resistance in innervated and denervated cremaster muscle with intravital microscopy. The major limitation of this study was that local blood flow of the cremaster muscle was not measured. Walkinshaw (Walkinshaw et al. 1988) showed that the recovery of perfusion after artery and vein anastomosis in a free rat groin flap was not affected by changes in the external vascular diameter of the pedicle. Their data may indicate that perfusion is not totally determined by the diameter of pedicle vessels, but by some other factors downstream from anastomosis.

Blood pressure (BP) is the force exerted by circulating blood on the walls of blood vessels and constitutes one of the principal vital signs of the living human being. The pressure of the circulating blood decreases as blood moves through the arteries, arterioles, capillaries, and veins; the term blood pressure generally refers to arterial pressure, i.e., the pressure in the larger arteries. For each heartbeat, the blood pressure varies between zenith (systolic) and nadir (diastolic blood pressure). The systolic pressure peaks in the arteries near the beginning of the cardiac cycle at the time of ventricular contraction of the heart. The diastolic blood pressure is the minimum pressure in the arteries, which occurs near the end of the cardiac cycle when the ventricles are filled with blood. The mean arterial pressure (MAP) is a term used to describe the average blood pressure. It is defined as the average arterial pressure (mmHg) during a single cardiac cycle.

$$\mathbf{MAP = (CO \times SVR) + CVP} \qquad \text{(equation 3)}$$

In equation 3, CO is cardiac output (L/min), SVR is systemic vascular resistance (mmHg/L/min), and CVP is central venous pressure (mmHg). CVP is usually small and can be neglected in this formula.

At normal resting, MAP can be approximated (MAP_a) using the more easily measured systolic and diastolic pressures, SP and DP:

$$\mathbf{MAP_a = DP + 1/3\ (SP - DP)} \qquad \text{(equation 4)}$$

At high heart rates MAP_a approaches the arithmetic mean of the systolic and diastolic pressures because of the change in shape of the arterial pressure pulse. The configuration of the pulse wave form is known to change with sympathetic stimulation and the duration of diastole to decrease (as heart rate increases) during exercise. MAP is considered to be the perfusion pressure of the organs in the body. MAP greater than 60 mmHg, is assumed to sustain the perfusion of the organs of the average person under most conditions. If the MAP falls significantly below 60 mmHg for any appreciable amount of time, the end organ will become ischemic (Rowell 1993).

The terms blood flow and perfusion are often used as synonyms, although are not quite the same. The term flap blood perfusion (BF_{PET}) is used to describe flap tissue perfusion (mL blood / min / volume of tissue) in this thesis and the papers it is based on, although the terminology may be somewhat confusing in the literature.

2.1.3. Local control of blood flow in free flaps

Changes in perfusion pressure elicit changes in vascular resistance in the teleological attempt of the tissue to maintain a constant level of oxygen delivery. These changes are elicited to allow constant blood flow and are called autoregulation, which is the ability of each tissue to control its own local blood flow in relation to its metabolic

needs. Autoregulation is one of the fundamental principles of circulatory function (Johnson 1986, Berne and Levy 1997).

Metabolically active tissues, e.g. active muscles need much more blood than e.g. muscles at rest. Arterial blood pressure is a determinant of the number of capillaries with red blood cell transit: it decreased when the major supply artery to the tissue is occluded or vasoconstricted by an oversupply of oxygen (Lindbom and Arfors 1985). However, under regular conditions the heart cannot increase its output by more than four- to seven-fold and it is not possible to increase the blood flow everywhere in the body when a particular tissue demands more blood flow. In an intact living organ, specific local vascular effects are provided by the neural control of the circulation to regulate tissue perfusion. Moreover, the microvessels of each organ continuously monitor the tissue needs for oxygen and other nutrients, as well as the accumulation of tissue waste products such as carbon dioxide. Although sympathetic innervation regulating vascular responsiveness of tissues is abolished by microsurgery, local and circulating factors still persist that control vascular responses (Seaber 1987, Pang 1990, Pang et al. 1993, Banbury et al. 1999, Guyton and Hall 2006).

Vascular smooth muscle can be stimulated to contract by multiple types of signals: e.g. neural signals, muscle stretching, and hormonal stimulation. Experimentally it has been found that proximal sympathectomy of a muscle flap with somatic denervation leads to increased capillary perfusion and hyperreactivity to vasoactive substances (Banbury et al. 1999). Another experimental work with rats (Richards et al. 1985) showed that microcirculatory blood flow responds to catecholamine in denervated tissue and that ischemia aggravates vasospasm that has been induced by intra-arterial norepinephrine. In vitro papaverine elicits a concentration-dependent relaxation of rabbit carotid artery rings precontracted with norepinephrine (Gherardini et al. 1998). A totally isolated artery preparation contracts when norepinephrine is added, and relaxed by acetylcholine. Endothelial cells are apparently needed for the relaxation (Furchgott 1983). Intraluminal papaverine increases the lumen size of the isolated human internal mammary artery (IMA) (Hillier et al. 1992).

The endothelium is a single layer of cells of the innermost vascular wall. It is a source of vasoactive substances that can cause contraction or relaxation of vascular smooth muscle, i.e., endothelium-mediated control of flow. These substances include relaxing factors, e.g., nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor, and the contracting factor endothelin (Berne and Levy 1997).

According to the study by Holtz (Holtz et al. 1984) the changes in vessel diameter that follow changes in blood flow are endothelium-dependent. On the other hand, Hillier (Hillier et al. 1992) concluded that the use of vasoactive agents like acetylcholine or bradykinin elicits vascular smooth muscle relaxation only in the presence of an intact endothelium. Other vasodilators, such as sodium nitroprusside or glyceryl trinitrate, are not endothelium-dependent, but cause relaxation by acting directly on the smooth muscle cells (Busse et al. 1985, Hillier et al. 1992). Vascular endothelial cells secrete endothelins. In the healthy human body, endothelin-1 is a vasodilator at low doses but

a vasoconstrictor causing strong and long-lasting vasoconstriction at large doses (Samuelson et al. 1992, Tuominen et al. 1997). Furchgott (Furchgott 1983) and Busse (Busse et al. 1985) have studied the vascular endothelium and its ability to release vasoactive substances. Endothelial-derived relaxing factor (EDRF) composed either entirely of nitric oxide or of a mixture of substances, is a vasodilating substance. In addition to the relaxation of the arterial wall, EDRF also increases the dimensions of the upstream large arterial vessels (Furchgott 1983). This dilation appears to be an effect of vasodilator substances that diffuse into the precapillary sphincters, metarterioles, and arterioles. The state of tissue nutrition is more or less proportional to the number of precapillary sphincters that are open at any specific moment. The precapillary sphincters open and close cyclically several times per minute, and the duration of the open phase depends on the metabolic needs of the tissues. This phenomenon is named vasomotion (Guyton and Hall 2006).

The vasodilating substances appear to be released from the tissue mainly by hypoxia. Decreased availability of oxygen can cause both adenosine and lactic acid release in the tissue. During skeletal muscle ischemia, tissue levels of adenosine triphosphate (ATP) decrease substantially (Kerrigan and Stotland 1993). Exogenous adenosine appears to protect against ischemia-reperfusion injury (Lee and Lineaweaver 1996), maybe since it is a potent vasodilator. Adenosine also inhibits neutrophil-mediated cellular injury after prolonged ischemia and inhibits neutrophil-mediated free radical production (Ely and Berne 1992, Lee and Lineaweaver 1996). Adenosine limits the release of various inflammatory mediators, interferes with neutrophil-endothelial cell adhesion, and activates intracellular antioxidant systems. Adenosine has thus multilevel protective effects in the setting of ischemia-reperfusion injury (Bouma et al. 1997). Clinically, pretreatment with adenosine in the setting of limb transplantation or microvascular tissue transfer may prove protective against ischemia-reperfusion injury (Carroll and Esclamado 2000), although recent studies have suggested the opposite to be the case (Ulusal et al. 2006).

The regulation of local blood flow in a flap is explained by two basic theories, when either the rate of tissue metabolism or the availability of oxygen changes: 1) The metabolic theory proposes that reduced arterial inflow causes a decreased rate of washout of aerobic vasodilator metabolites, such as carbon dioxide, adenosine phosphate compounds, histamine, potassium ions, hydrogen ions, and glucose. Reduced arterial inflow reduces further the tissue O_2 pressure and this causes typically subsequent vasodilation. 2) The myogenic mechanism theory postulates that the arterioles or resistance vessels respond by vasoconstriction to a stimulus of intravascular pressure elevation and that this increases tension and stretching of smooth muscle cells (Johnson 1986, Guyton and Hall 2006). Both theories explain local blood flow regulation of a free flap in relation to the metabolic needs of the tissues and a combination of these two mechanisms is probably the most accurate explanation of local blood flow regulation (Morff and Granger 1982).

2.1.4. Perfusion heterogeneity in free flaps

Perfusion heterogeneity is defined as the degree of uneven distribution of blood flow among blood vessels or regions of different tissues. The exchange of small particles such as oxygen in capillaries is largely dependent on perfusion, which may be distributed spatially uniformly or not (Duling and Damon 1987). The efficacy of tissue oxygenation and oxygen delivery to the free flaps is greatly influenced by this spatial flow heterogeneity (Piiper and Haab 1991). Moreover, there is great variation of perfusion also over time. The physiological significance of this phenomenon called temporal heterogeneity is unclear.

2.1.4.1. Perfusion zones of lower abdominal flaps (TRAM, DIEP)

A breast reconstruction flap from the lower abdomen was first described by Scheflan and Dinner in their studies on the unipedicled transverse rectus abdominis musculocutaneous (TRAM) flap. The abdominal flap was divided into four equal parts and numbered according to the clinical impression of perfusion. Their first series consisted of 16 patients who underwent this TRAM operation (Scheflan and Dinner 1983b, Scheflan and Dinner 1983a). The numbering of the flap zones was based on the tenet of a centrally perfused skin ellipse and a declining perfusion of the peripheral ends. The idea was that the perfusion zones immediately adjacent to the territory of the vascular pedicle have better perfusion than zones farther away. Arguments failed, however, to explain why the adjacent perfusion zone on the contralateral side (zone II) was considered to have better perfusion than the bordering perfusion zone on the ipsilateral side (zone III) (Figure 3B). These perfusion zones became better known after the publication by Hartrampf (Hartrampf et al. 1982) (Figure 3A).

Nowadays, however, the inferior, rather than the superior, epigastric pedicle plays the dominant role in the transfer of lower abdominal tissue for autologous breast reconstructions. The free muscle-sparing TRAM flap and, more recently, the free deep inferior epigastric perforator (DIEP) flap both depend on the stronger inferior epigastric pedicle but are nourished by fewer musculocutaneous perforators, as opposed to Hartrampf's pedicled TRAM flap (Dinner et al. 1983, Holm et al. 2006) (Figure 3B).

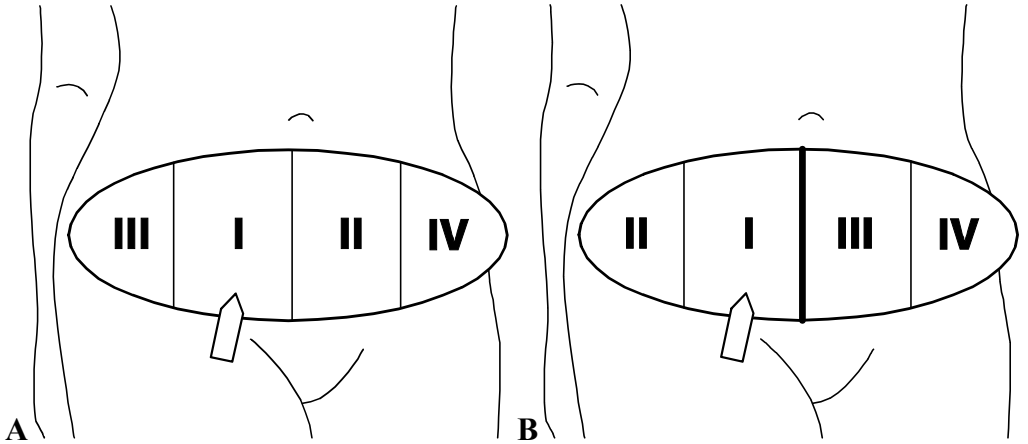

Figure 3. **A** Conventional perfusion zones of lower abdominal flap (Hartrampf et al. 1982). **B** Zones of lower abdominal flap suggested by Holm (Holm et al. 2006). I = zone I presenting the region of the abdominal flap closest to the vascular pedicle of the flap; II = zone II; III = zone III; IV = zone IV, which is usually discarded from the abdominal flap in reconstructions.

Breast reconstruction is an important part of the treatment of breast cancer patients. Although many patients are treated with breast conservation, mastectomy is still an important treatment modality (Granzow 2006). Breast reconstruction with autogenous tissue is the gold standard for a natural looking and natural feeling restoration of the female breast (Howard and Mehrara 2005). Over the years, the free transverse rectus abdominis myocutaneous (TRAM) flap has been developed into more sophisticated flaps, such as the muscle-sparing (MS-1, and MS-2) TRAM flap and the deep inferior epigastric perforator (DIEP, MS-3) flap. These flaps minimize donor-site morbidity since less muscle and less anterior rectus fascia are needed (Figure 4) (Kroll et al. 1995, Blondeel et al. 1997, Nahabedian et al. 2002a). However, this attempt to limit donor site morbidity may affect the blood circulation of the flap. In a 10-year retrospective review of 758 DIEP flaps (Gill et al. 2004), 6 % of patients required reoperations because of flap related problems. Partial flap loss (PFL) occurred in 2.5 % while total flap loss occurred in less than 1 % of all cases. Problems with the vein or venous anastomoses were almost eight times more frequent than problems with the artery or arterial anastomoses. The frequency of fat necrosis is 13 % of flaps (Gill et al. 2004, Granzow 2006). There appears to be an increased incidence of fat necrosis in DIEP flaps compared to free transverse rectus abdominis muscle (TRAM) flaps (Kroll 2000, Gill et al. 2004), which may be caused by venous problems. The experience of the microsurgeon and limiting the use of DIEP flaps to patients with at least one sufficiently large perforator in each flap reduces the complication rate significantly (Busic and Das-Gupta 2006).

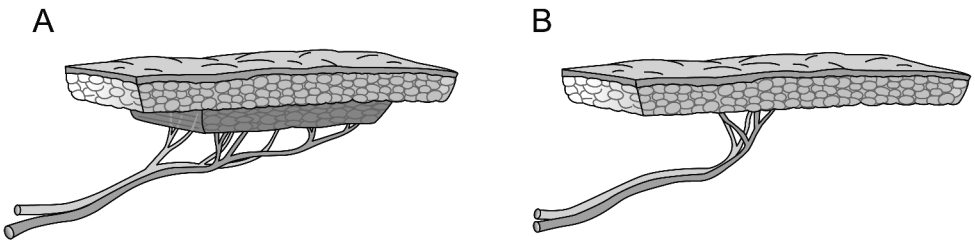

Figure 4. A Schematic drawing of free TRAM flap showing multiple perforators with muscle included with the flap. **B** Schematic drawing of DIEP flap with no muscle and fewer perforators supplying blood to the flap.

There have been several studies on the conventional division of the zones of the lower abdominal flap (Hartrampf et al. 1982) (Figure 3A). Yamaguchi (Yamaguchi et al. 2004) used indocyanine green dye fluorescence videoangiography analysis to assess blood supply and suggested that the results may have predictive value for preservation of viability of the unipedicled TRAM flap. Their method confirmed that there is an individual pattern of the flap perfusion or "perfusion map", where zone II is not always perfused as well as zone III (Yamaguchi et al. 2004). Kim presented later similar findings in pedicled TRAM flaps (Kim et al. 2007). The incidence of fat necrosis in zone II is significantly higher than in zone III. The weight of the mastectomy specimen, and the relative amount of zone II tissue included in the flap, correlate positively with the incidence of fat necrosis. This implies that zone II is perfused proportionately less well than zone III (Kim et al. 2007). Ohjimi studied 14 free TRAM flaps in 12 patients with ex vivo intraoperative angiography and found that the cephalic half of zone II and all of zone IV are poorly perfused and prone to skin or fat necrosis (Ohjimi et al. 2005). Takeishi reported similar results with DIEP flaps (Takeishi et al. 2008).

Lorenzetti observed that the blood flow rate in the IMA is initially much higher (5-fold) than in the thoracodorsal artery (TDA) but that it returns to the same level as initially in the deep inferior epigastric vessel. This indicates that the IMA does not guarantee better inflow to the flap than does the TDA. Indeed, it seems that a free TRAM flap, which is a musculocutaneous flap with little muscle and a large adipose tissue component, cannot accept blood at a rate exceeding 10-20 mL per min and that the flow is independent of the recipient artery flow. Thus, even a low-flow recipient artery is a suitable vessel for free TRAM flaps (Lorenzetti et al. 2001a, Lorenzetti et al. 2001b).

The standard human adipose tissue blood flow (ATBF)value is about 2.8 mL/min/100g (Williams and Leggett 1989). This value is based on ^{133}Xe washout measurements (Larsen et al. 1966) of the local flow of a very limited region of adipose tissue. ATBF has also been determined by a whole body kinetic model with a highly lipid soluble solute (Lesser and Deutsch 1967). Ardilouze used the ^{133}Xe washout method and found that the subcutaneous ATBF of the anterior abdominal wall is greater at the superior level than inferiorly (4.4±0.3 vs. 3.8±0.2 mL/min/100 g, $p = 0.005$). They did

not detect any differences in ATBF between the right and the left sides at either level (Ardilouze et al. 2004).

Holm (Holm et al. 2006) performed a prospective clinical study and found that the intraoperative perfusion of zone III in the elevated deep inferior epigastric perforator (DIEP) flap is superior in terms of median perfusion index to zone II (47 % and 25 %, respectively, $p = 0.001$). In a study by Hallock (Hallock 2001) on 13 free TRAM flaps the mean blood flow assessed with laser Doppler flowmetry (LDF) diminished significantly in an orderly fashion: the flow in zone I was highest and in zone IV lowest. The comparison of recordings of a laser Doppler flowmeter for different areas is difficult, because the results are expressed in relative values and may vary considerably.

In a retrospective analysis of 185 reconstructed breasts in 175 patients with free microvascular TRAM flaps, the overall surgical success rate was 99 % suggesting that microsurgery using free TRAM flaps is reliable (Nieminen et al. 1999).

Although the overall surgical failure rates are relatively low when breasts are reconstructed with free microvascular flaps, the postoperative monitoring of BF_{PET} and vitality in free flap surgery is of utmost importance to allow early recognition of circulatory problems. On the other hand, the monitoring technique must be reliable, if it is to be effective with regard to final success and patient outcome, which includes a minimum of re-explorations.

2.1.5. Oxygen transport to the free flap during breathing of room air

Oxygen is transported from the inhaled air to each cell and extracellular matrix in the body. Transport follows the principle that gases move from an area of higher concentration (pressure) to areas of lower concentration (pressure). If there is a mixture of gases in a container, the pressure of each gas i.e. partial pressure, is equal to the pressure that each gas would produce if it occupied the container alone (Guyton and Hall 2006).

The air around us has a total pressure of 760 mmHg at sea level. Air consists of 21 % oxygen, 78 % nitrogen and small quantities of CO_2, argon and helium. The pressure exerted by the main two gases individually, when added together, equals the total surrounding pressure or atmospheric pressure. The partial pressure of oxygen (pO_2) of dry air at sea level is therefore 159 mmHg (21/100 x 760 = 159). However by the time the inspired air reaches the trachea it has been warmed and humidified by the upper respiratory tract. The humidity is formed by water vapor which exerts a pressure. At 37°C the water vapor pressure in the trachea is 47 mmHg. Taking the water vapor pressure into account, the pO_2 in the trachea when breathing air is (760-47) x 21/100 = 150 mmHg. By the time oxygen reaches the alveoli the pO_2 has fallen to about 100 mmHg. This is because the pO_2 of the gas in the alveoli (p_aO_2) is a balance between

the removal of oxygen by the pulmonary capillaries and its continuous supply by alveolar ventilation (Guyton and Hall 2006).

Blood returning to the heart from the tissues has a low pO_2 (40 mmHg) and travels to the lungs via the pulmonary arteries. The pulmonary arteries divide into pulmonary capillaries, which surround the alveoli. Oxygen diffuses from the high pressure in the alveoli (100 mmHg) to the area of lower pressure of the blood in the pulmonary capillaries (40 mmHg). Oxygenated blood moves into the pulmonary veins which return to the left side of the heart to be pumped to the systemic tissues. In a 'perfect lung' the pO_2 of pulmonary venous blood would be equal to the pO_2 in the alveolus (p_aO_2). Three factors may cause the pO_2 in the pulmonary veins to be less than the p_aO_2: ventilation / perfusion mismatch, shunting, and slow diffusion (Guyton and Hall 2006).

Oxygen is carried in the blood mostly bound to hemoglobin, and in a minor degree physically dissolved in plasma; at normal pO_2 only 3 mL of oxygen will be dissolved in a liter of plasma. Each gram of fully saturated hemoglobin can carry 1.34 mL of oxygen. Therefore, every liter of blood with a Hb concentration of 150 g/L can carry about 200 mL of oxygen, if the hemoglobin is 100 % saturated (Guyton and Hall 2006).

If the pO_2 of oxygen in arterial blood is increased significantly (by breathing 100 % oxygen) then a small amount of extra oxygen will dissolve in the plasma (at a rate of 0.003 mL O_2 / 100mL of blood / mmHg pO_2) but there will normally be no significant increase in the amount carried by hemoglobin, which is already >95 % saturated with oxygen. Adequacy of oxygen delivery to the tissues depends on three factors: hemoglobin concentration, cardiac output and oxygenation (Guyton and Hall 2006).

Oxygen moves down the pressure or concentration gradient from a relatively high level in the air to the levels in the respiratory tract and then to alveolar gas, the arterial blood, capillaries and finally the extracellular matrix and the cell. The pO_2 reaches its nadir (4-20 mmHg) in the mitochondria (Conley et al. 2007). This decrease in pO_2 from air to mitochondria is known as the oxygen cascade, and the size of any one step in the cascade may be increased under pathological circumstances and may result in hypoxia.

The quantity of oxygen made available to the body is known as the oxygen delivery. Oxygen delivery is directly proportional to the cardiac output and the arterial oxygen content, i.e., 5,000 mL blood / min x 200 mL O_2 / 1,000 mL blood = 1,000mL O_2 / min.

$$\mathbf{DO_2 = CO \times Hb_C \times 1.34 \times SaO_2} \qquad \text{(equation 5)}$$

In equation 5, DO_2 represents the oxygen delivery (mL/min), CO is the cardiac output in liters per minute, and Hb_C is the concentration of hemoglobin in grams per a liter of blood, 1.34 is the amount of oxygen in milliliters per fully saturated hemoglobin gram, and SaO_2 is the percentage of hemoglobin O_2 saturation.

A low cardiac output, a low hemoglobin concentration, or low hemoglobin O_2 saturation will result in an inadequate delivery of oxygen, unless a compensatory change occurs in one of the other factors. Alternatively, if oxygen delivery falls relative to oxygen consumption, the tissues extract more oxygen from the hemoglobin. A reduction in the saturation of mixed venous blood below 70 % cannot be compensated for by increased oxygen extraction, and thus results in anaerobic metabolism and lactic acidosis (Guyton and Hall 2006).

2.2. Clinical methods to assess flap blood flow

Accurate assessment of free flap perfusion is a challenge to the microsurgeon and nursing staff. A plenitude of techniques for monitoring flap blood flow has been developed. To be able to minimize flap failure due to circulatory impairment, a reliable diagnostic method that gives early warning signals is needed. Although different methods to assess flap blood flow are available, there is still no single method widely accepted for clinical use. The ideal technique of monitoring would be straightforward and any data collected for the postoperative monitoring of flap blood flow should be unambiguous and easy to interpret, it should satisfy the criteria proposed by Creech and Miller (Creech and Miller 1975). According to Jones, the ideal monitoring method should be noninvasive, reliable, objectively repeatable, promptly reactive to perfusion changes, appropriate for continuous monitoring in all kinds of free tissue transfers, usable also by unskilled, and not too expensive (Jones 1984).

In addition to conventional clinical monitoring, various methods of monitoring have been developed and used. Photopletysmography, laser Doppler, and ultrasonic Doppler, microdialysis, near-infrared spectroscopy, dynamic CT, and MRI, have all been used (Hutchinson 1993, Machens et al. 1994, Thorniley et al. 1998, Yuen and Feng 2000a, Zhang et al. 2001, Edsander-Nord et al. 2002, Gaggl et al. 2002, Hölzle et al. 2006). None of these methods has become a gold standard.

Most surgical complications after tissue transfer surgery are related to vascular thrombosis, which usually occurs within 3 days of surgery. However, late thrombosis can occur and is often associated with a local infection or mechanical compression of the vascular pedicle. Therefore, nursing staff taking care of the microsurgical patients routinely monitor all free flaps e.g. hourly for the first 24 postoperative hours, then every 2 hours for the next 1 to 2 days, and thereafter every 4 to 8 hours until discharge (Evans and Evans 2007).

The procedures of postoperative free flap monitoring by clinical means are often unreliable. Tissue color, turgor, temperature, capillary refill, and bleeding time are subjective variables that may be dependent on room-light, temperature, and the

experience of the observer. Many monitoring methods are based on relative measuring results and cannot be directly compared interindividually.

The intricacies of flap microcirculation are frequently difficult to assess despite all examination techniques available today. Especially if the free flap is buried during surgery direct observation is not possible. External Doppler probe, endoscopy, monitoring island flap, or even leaving the wound temporarily open, might be used to monitor buried flaps.

Quantitative measurements of BF have been made with various tracers for over a century (Wootton 1982). Initially these tracers included non-radioactive dyes which have later widely been replaced by radioactive tracers. Two distinguished classes of method are a) measurement of total organ blood flow, and b) measurement of organ perfusion (ie. blood flow per unit volume, or unit mass of tissue).

2.2.1. Tissue oxygen tension ($p_{ti}O_2$)

Tissue oxygen tension ($p_{ti}O_2$) is the directly measured local partial pressure of oxygen in a specific tissue, e.g. flap tissue. It may be thought of as the local expression of global DO_2 (equation 5) in a particular tissue. Tissue oxygen tension is the balance between oxygen perfusion and oxygen consumption in the tissue at a given time. In tissues with stable oxygen consumption, $p_{ti}O_2$ reflects tissue perfusion more precisely than traditional clinical indices such as MAP, CO, urine output, skin temperature, or skin capillary refill. Tissue oxygen tension may be measured in any tissue of interest, but values in peripheral subcutaneous tissue are the most widely reported (Ragheb and Buggy 2004).

The effect of absolute pressure on blood oxygenation is determined by Henry's law, which states that the volume of a gas dissolved in a liquid is proportional to its partial pressure:

$$\mathbf{C = KP_x} \qquad \text{(equation 6)}$$

where C is the concentration of gas dissolved in the liquid (mol/L), K is the solubility constant (mol/L atm), and P_x is the partial pressure of the gas on the liquid (atm). Because the concentration of oxygen in blood is determined by its partial pressure, there is an infinite set of absolute pressures and gas mixture ratios that result in the same concentration of dissolved oxygen. An example is oxygen breathing by scuba divers: the partial pressure of air oxygen increases proportionally to the absolute pressure. Therefore, to maintain a constant amount of oxygen dissolved in the blood, the percentage of oxygen must be reduced. To avoid oxygen toxicity during deep dives, the amount of oxygen inspired has to be reduced to < 1 % (West 1990).

Tissue oxygen tension can be measured by a probe that consists of a silver anode and a platinum cathode. The resulting electrical current is directly proportional to the

number of oxygen molecules reduced at the cathode. Since Hunt (Hunt 1964) described a new method of determining tissue oxygen tension, several clinical applications using this method have been introduced (Niinikoski and Hunt 1972, Aitasalo and Aro 1986, Hirigoyen et al. 1997, Kamolz et al. 2002).

Previously, transcutaneous oxygen tension ($p_{tc}O_2$) was used experimentally and clinically for determining the microcirculation of cutaneous tissue (Booth 1975, Pollitzer et al. 1979, Achauer et al. 1980, Serafin et al. 1981, Tuominen et al. 1992). Tuominen found that laser Doppler flowmetry is a more sensitive technique than transcutaneous flap oxygen tension to monitor hemodynamic changes during TRAM flap operations (Tuominen et al. 1992).

Wechselberger reported promising results using this system in 17 different free microvascular flap reconstructions, and found two true hemodynamic problems and one false alarm in a buried flap, as the probe was misplaced (Wechselberger et al. 1997). Kamolz monitored the oxygen tension of 60 free tissue flaps with Licox® system and reported all hemodynamic failures necessitating revision (Kamolz et al. 2002). Raittinen reported hemodynamic failures necessitating revision with an overall flap survival rate of 97.2 % in a series of 37 head and neck microvascular reconstructions; they monitored $p_{ti}O_2$ with the Licox® system. They found three false positive alarms and no false negative results. (Raittinen et al. 2005)

Fixation of the $p_{ti}O_2$ probe into the flap is important in order to achieve reliable results. In the Licox® CC1.2 polarographic probe, which is often used in flap $p_{ti}O_2$ measurement, the first 15 mm from the tip of the probe is a safe area for anchoring the probe with a suture. Local tissue pO_2 values are averaged over a 5 mm-long oxygen sensitive area, which is the cylindrical outer surface of the probe, situated 18 mm distal of the catheter tip. If the flap is in the oral cavity, it is important to pass the probe through the skin below the mandible to prevent the patient from inadvertently biting the probe (Raittinen et al. 2005).

The polarographic microcatheter (0.47 mm in diameter, Figure 5) carries a 5-mm oxygen sensor tip, from which it integrates the local oxygen tension values over the adjacent tissue area (Wechselberger et al. 1997). According to the movements within the organ structure, the effective sample area of the catheter in the tissue varies between 10 mm^2 and 30 mm^2. The single use probes are provided sterile and they retain their calibration characteristics (sensitivity and drift) within a narrow range for at least 7 days after implantation (Machens et al. 1994, Gopinath et al. 1999, Sarrafzadeh et al. 2003).

The Licox® probe is called Revoxode (Figure 5). With a Revoxode, as with a regular Clark type polarographic cell, the oxygen diffuses from the tissue through a thin (70 μm) polyethylene wall of the catheter tube into its inner electrolyte chamber. In this chamber, O_2 is transformed to hydroxyl (OH^-) ions at a negatively polarized noble metal electrode, the polarographic cathode. The cathode can be seen near the probe tip as a gold-colored wire-end. The anode of the circuit is at a distance of approximately

30 mm from the rear of the electrolyte chamber. The current from the O_2 reduction is the raw signal of the sensor. The reactions in a Revoxode are reversible and no insoluble layers are irreversibly deposited on the electrodes. Additionally, the Revoxode can be reset to its starting condition electronically. This process is comparable to charging an accumulator. The proximal end of the probe is closed by an electrical cable connection, which is attached to a digital, bedside monitor displaying real-time $p_{ti}O_2$ values every 20 seconds (Licox® Medical Systems and Neurosciences. 2002).

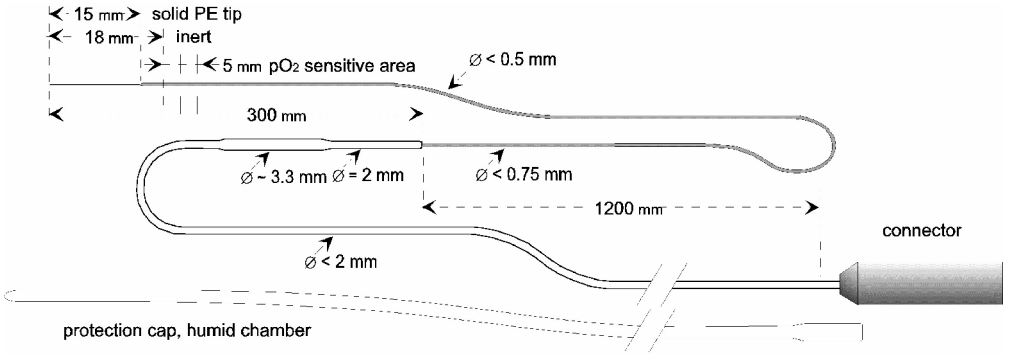

Figure 5. Schematic figure of the Licox® CC1.2 polarographic $p_{ti}O_2$ catheter probe. Reprinted with permission from the GMS (*Gesellschaft für Medizinische Sondenteknik*, mbH).

$P_{ti}O_2$ depends on the oxygen uptake of tissue cells and the transport rate of the microcirculation. Normal in situ $p_{ti}O_2$ in brain tissue is around 25 mmHg, in muscle 25-35 mmHg, and in dermal connective tissue 40-80 mmHg. High readings immediately after surgery are reported during the probe calibration and following post-occlusive hyperemia. Within a few hours, $p_{ti}O_2$ stabilizes and reaches a steady level. (Licox® Medical Systems and Neurosciences. 2002) Although the readings vary somewhat individually, the trend seems to be the same in all cases. These findings are similar to those reported by Kamolz and Raittinen (Kamolz et al. 2002, Raittinen et al. 2005). A sudden drop, or an obvious change in the level of the $p_{ti}O_2$ curve, predicts in a reliable way circulation problems of the flap. Postoperative anxiety and nursing procedures, e.g. suctioning or moving the patient, cause artifact fluctuations in the $p_{ti}O_2$ curve. This can cause false alarms (Figure 6). Mild sedation, correction of the patient's position and administration of supplemental oxygen, the oxygen challenge test, all rapidly improve tissue oxygenation. In case of an authentic circulatory problem, the $p_{ti}O_2$ readings decline, without being effected by these procedures.

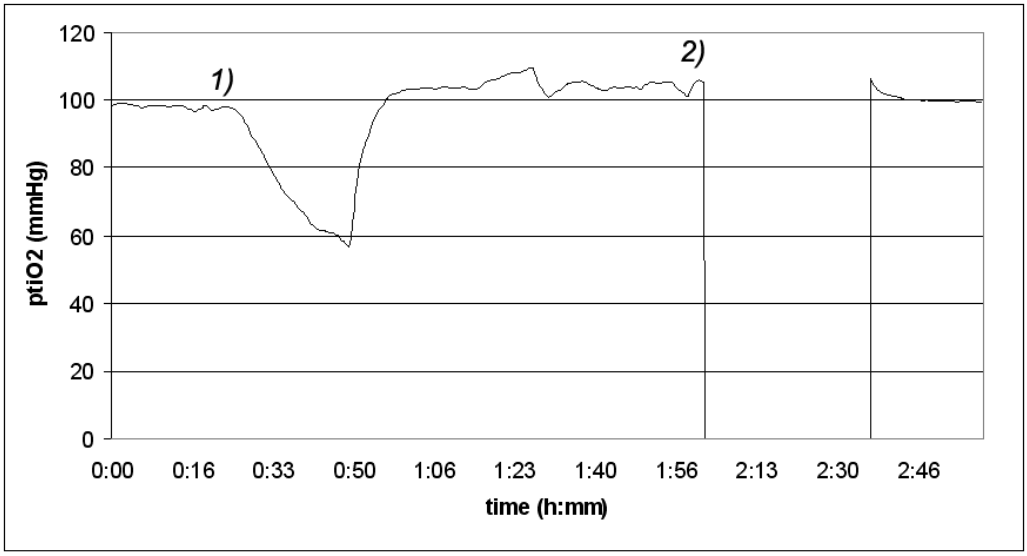

Figure 6. Sample of $p_{ti}O_2$ measurement of a free HN reconstruction flap. **1)** $P_{ti}O_2$ reading reaction (artifact) to the suction of secretions of the patient, with the $p_{ti}O_2$ probe fixed to the intraoral flap. **2)** Short circuit of the Licox® $p_{ti}O_2$ measurement system by a monopolar electrocoagulator used to control a tracheostomy bleeding in the intensive care unit. A "cold boot" of the Licox® system corrected the measurement level.

The polarographic method was first introduced in the 1960s (Hunt 1964). In the early 1990s, a commercially available method of polarographic measurement of tissue oxygenation was presented (Fleckenstein 1990, Kallinowski et al. 1990), and since then, many studies on human tumor oxygenation have been done. This technique has been regarded as the gold standard for the measurement of hypoxia (Stone et al. 1993). Disadvantages of the technique include the fact that necrosis cannot be differentiated from viable cells, nor can acute and chronic hypoxia be separated. The use of probes is limited to superficial tissues that can be reached with the probe (Hirigoyen et al. 1995, Hirigoyen et al. 1997, Kamolz et al. 2002, Raittinen et al. 2005).

2.2.2. Laser Doppler flowmetry (LDF)

Laser Doppler measures the phase shift of the frequency of light reflected from moving objects within the microcirculation of any flap, which can be converted to an electrical signal that provides a light-emitting diode display proportional to tissue blood volume and velocity, from which blood flow can be calculated (Place et al. 1996). The sampling volume of each probe is small (~ 0.1 mm^3) and thus the measurements mainly reflect microcirculatory flow, but variations in perfusion over time can be measured. However, absolute measures of perfusion are not possible (Brenman et al. 1990, Place et al. 1996, Yuen and Feng 2000b). In all applications of LDF, a laser beam is brought to impinge on the surface of the tissue volume under study. The laser light reaches the probe tip via an optic fiber and penetrates the surrounding tissue area. The frequency

shift of the light scattered back from moving red blood cells (Doppler effect) is given by equation 7:

$$\Delta f = v f_o / c \qquad \text{(equation 7)}$$

where Δf is the magnitude of the frequency shift (1/s), v is the velocity of the source with respect to the observer (m/s), c is the velocity of the carrier wave (m/s), and f_o is the unshifted frequency (1/s). Static objects do not cause such change. The shifted backscattered light is collected by the photodetectors where it is processed and amplified. In capillary blood flow applications the velocity is around 10^{-3} m/s (Stucker et al. 1996). Thus, the Doppler shift of laser light is relatively small. Such minor frequency shifts are difficult to measure directly and consequently the frequency-shifted light is combined with non-shifted light to extract the information of interest (Öberg 1990).

The amount of backscattered laser light is presented as a laser Doppler flux reading, which is an arbitrary measure, proportional to the concentration and velocity of moving red blood cells under the probe. The laser Doppler flux reading cannot be described by any physiological definition, such as the actual number of cells flowing through a spesific volume of tissue during a given time period. Thus, the laser Doppler flux readings must be compared in relation to each other (Öberg 1990) and are not comparable interindividually.

The measuring depth of LDF is the depth below the tissue surface to which approximately two-thirds of the surface light penetrates and returns to the tissue surface. This measuring depth depends on tissue properties, e.g., wavelength of the laser light ultilized, the density and the structure of the capillary beds, and the distance between the fibers in the probe. In well-perfused organs, such as the kidney and the liver, the measuring depth of LDF is less than one millimeter, while in the intestine the measuring depth can be several millimeters. The longer the wavelength, the deeper the penetration is into the tissue. If the blood supply to a measured region is occluded, the measuring depth will increase, since the lack of blood permits more passage of light (Öberg 1990).

In microvascular surgery, Jones and Mayou (Jones and Mayou 1982) first reported the use of LDF for monitoring free flaps in patients. They found that increased postoperative blood flow indicated satisfactory free flap survival, while a reduction in flow is a warning signal of thrombosis formation. Since this preliminary report, LDF has become a widely used monitoring technique of free flaps (Tuominen et al. 1992, Machens et al. 1994, Yuen and Feng 2000a). Although LDF is generally considered to be a good monitoring technique of free flaps, there is also scepticism about the reliability of LDF (Hickerson et al. 1990). Falsely elevated measurements can be caused by vibration, motion of the probe, location of the probe over a large vessel, or extreme variation in the hematocrit value. The differentiation between arterial and venous blood flow disturbances is difficult with LDF (Machens et al. 1994) although in cases of venous occlusion there is typically a less abrupt decline in LDF flux readings

(Fischer et al. 1983). Heller (Heller et al. 2001) reported that LDF is a valuable tool for the postoperative monitoring of free flaps and that its use significantly improves flap salvage rates once artifacts are ruled out.

2.2.3. Doppler ultrasound

Doppler ultrasound is a non-invasive, real-time technique applied for many clinical purposes to provide information on blood circulation (Scoutt et al. 1990). It is possible to evaluate the velocity and direction of blood flow by recording the reflected ultrasound waves (Scoutt et al. 1990, Salmi et al. 1995a, Lorenzetti et al. 1999) not only in large vessels but also in small ones with a diameter of less than 2 mm (Hutchinson 1993, Giesswein et al. 1994). This technique can also be used to investigate vessel patency (Scoutt et al. 1990), and to evaluate vascular resistance (Taylor and Holland 1990). In free flap surgery, Doppler ultrasound has been used to identify the recipient artery and vein (Giesswein et al. 1994, Lorenzetti et al. 1999), and to measure blood flow velocities after surgery (Salmi et al. 1995a, Lorenzetti et al. 1999).

However, the measurements can be performed only in relatively superficial regions, within reach of the ultrasound probe on the skin, and quantitative measurements are not available. The results reflect mainly blood flow in larger vessels and not in the microvasculature, since flow volume and velocity in capillaries are too low to be detected (Vaupel et al. 1989a, Delorme and Knopp 1998). However, new measurement techniques can discern somewhat lower velocities, as well (Sohn et al. 1997). Conventional Doppler imagining is based on the estimation of the mean Doppler frequency shift. Power Doppler imagining is a method based on estimating the integrated Doppler power spectrum (Rubin et al. 1994).

The biggest drawbacks of this method include the inability to measure regional perfusion, the requirement of an experienced operator, and the availability of costly ultrasound device. Moreover, Doppler ultrasound can only measure velocity of moving particles in the blood stream, e.g. moving red blood cells.

2.2.4. Microdialysis

In microdialysis, a probe with a semi-permeable membrane is inserted into the tissue and transport of molecules across the membrane is measured (Radegran 1999). The microdialysis probe mimics the function of a blood capillary (Figure 7).

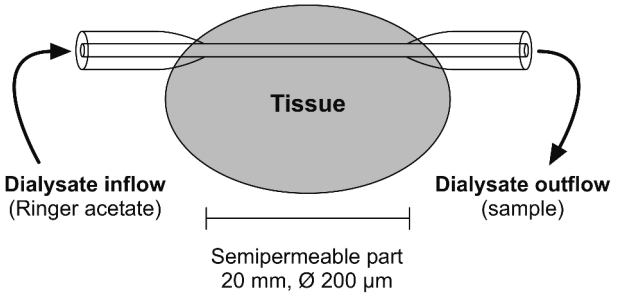

Figure 7. Schematic illustration of a microdialysis catheter inserted through tissue.

The microdialysis catheter (e.g. CMA 60 Microdialysis Catheter, CMA Microdialysis AB, Stockholm, Sweden, Figure 8A) consists of a dialysis tube (30 × 0.6 mm, 20,000 molecular weight cut-off) that is glued to the end of a double-lumen catheter. The inlet tube of the catheter is connected to a battery-powered microinfusion pump (e.g. CMA 106 Microdialysis Pump, Figure 8B), and continuously perfused with a sterile isotonic Ringer solution at a flow rate of 0.3 microliters per minute. The solution flows in the outer cannula inside the dialysis membrane to the distal end of the catheter (Figure 7). At the tip of the microdialysis catheter there is a permeable membrane where equilibration with extracellular substances, such as glucose, takes place. The equilibrated solution (the dialysate) flows in a retrograde direction and leaves the catheter through the inner cannula, and aliquots are collected in microvials. The vials are placed in a microdialysis analyzer, where the dialysate is analyzed bedside. The results can be viewed as a graph on a computer screen, and metabolic trends can be viewed. (Lonnroth and Smith 1990, Arner and Bolinder 1991, Ungerstedt 1991, Edsander-Nord et al. 2002)

A **B**

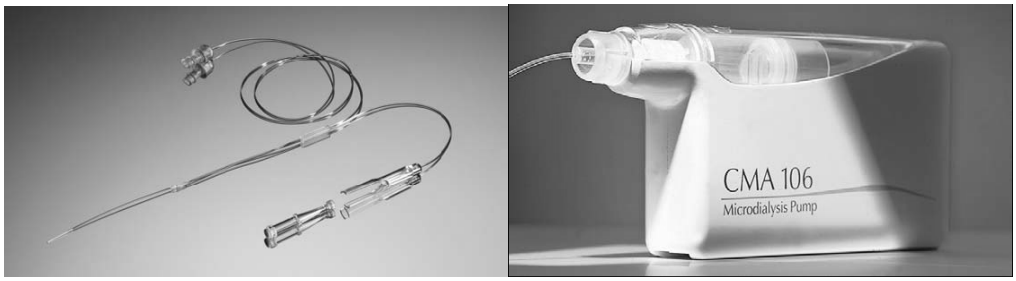

Figure 8. **A** Microdialysis catheter and **B** a microdialysis pump. Reprinted with permission of CMA.

Earlier microdialysis studies (Rojdmark et al. 1998, Udesen et al. 2000) have shown that glucose, lactate, and glycerol concentrations change in a characteristic way during ischemia. These results were verified by Edsander-Nord (Edsander-Nord et al. 2002). Ischemic signs are indicated by decreasing glucose and pyruvate concentrations and increasing lactate and glycerol concentrations and thus the lactate-to-pyruvate ratio increases during ischemia (Edsander-Nord et al. 2002, Jyränki et al. 2006). Nowak

monitored tissue metabolism in the transplanted liver using microdialysis (Nowak et al. 2002).

Microdialysis is reportedly a clinically feasible and sensitive monitoring method for all kinds of microvascular flaps, especially for those in which clinical observation is difficult or impossible (Jyränki et al. 2006, Setala et al. 2006), e.g. buried flaps.

2.2.5. Near-infrared spectroscopy (NIRS)

Near-infrared spectroscopy (NIRS) is a spectroscopic method which uses the near-infrared region of the electromagnetic spectrum (from about 700 to 2,500 nm). This method is based on detecting light attenuation from dye (indocyanine green) infused into the tissue or from circulating oxygen. NIRS allows continuous non-invasive monitoring of concentration changes occurring in oxy- and deoxy-hemoglobin and oxidized cytochrome, the terminal enzyme of the respiratory chain and site of 90 % of oxygen consumption in the body (Jobsis 1974). Information is provided on changes in tissue oxygen supply and intracellular oxygen availability and utilization. The summation of oxy- and deoxy-hemoglobin reveals changes in total hemoglobin, reflecting changes in blood volume and providing an indirect indication of blood flow and perfusion. The oxygenation index may also be derived. This is the difference between oxy- and deoxy-hemoglobin, and reflects net changes in oxygenation independent of change in blood volume.

Selective light absorption by oxygen-dependent tissue chromophores, predominantly hemoglobin, results in reduced light intensity. Attenuated optical signal exiting the tissue is analyzed using spectrophotometric principles that relate light absorption to the tissue concentration of the chromophore (Wukitsch et al. 1988). Characteristic absorption spectra of oxygenated and deoxygenated hemoglobin allow the system to calculate concentration of both hemoglobin forms (Wray et al. 1988). NIRS measures hemoglobin content and oxygenation in all small-diameter vascular compartments of tissue (arterioles, venules and capillaries) and of vessels of larger caliber which absorb the light completely.

The depth of the tissue measured by spectrometry is directly proportional to the distance between the tips of the probes that emit and receive the signal. The mean depth of measurement is roughly half of this length. A probe with 25-mm spacing was used in a study by Repez which gave a mean depth of measurement around 12.5 mm (Repez et al. 2008). The values reported by NIRS monitoring reflect changes in the skin and subcutaneous fat tissues of cutaneous flaps. In these tissues NIRS parameters vary to a notable extent between individual patients. Any dislocation of probe position influences the measurement considerably (Stranc et al. 1998, Scheufler et al. 2004). However, Repez reported that by attachment of the probe to the skin via a disposable plastic shield, the probe can be temporarily disconnected when necessary and repositioned to exactly the same spot as long as the shield is left attached to the skin.

Parameters are expected to fluctuate to a certain degree also during prolonged observation. This inherent variability seriously obstructs any reasonable standardization of NIRS threshold values which would reliably indicate an imminent threat to flap. For this reason, it is recommended to observe the trends of NIRS parameter changes rather than absolute values (Repez et al. 2008).

As suggested previously (Irwin et al. 1995, Stranc et al. 1998, Scheufler et al. 2004, Payette et al. 2005, Hölzle et al. 2006) NIRS provides benefits over laser Doppler flowmetry and other current monitoring techniques. One of its main advantages when compared to other noninvasive modalities is the capacity of near-infrared light to penetrate to a considerable depth of tissue and to provide data on microcirculatory events occurring in a comparatively large volume of tissues. Conversely, laser Doppler flowmetry is limited to observing relatively superficial (1-2 mm) cutaneous circulatory phenomena susceptible to changes in local environment and microvascular heterogeneity (Sloan and Sasaki 1985). Many clinical monitoring devices fail to detect venous congestion which is reliably identified at early stages by NIRS (Repez et al. 2008). An experimental study on a pedicled flap model by Payette (Payette et al. 2005) showed that optical spectroscopy is consistently more reliable in detecting problems with arterial inflow than laser Doppler assessments. (Figure 9)

A rather important drawback of NIRS monitoring is cost. The price of the spectrometry system was approximately €35,000 in 2008 while the cost of the disposable shield of the probe was €18. These might prove unjustifiable for departments with a low volume of flap reconstructions (Repez et al. 2008).

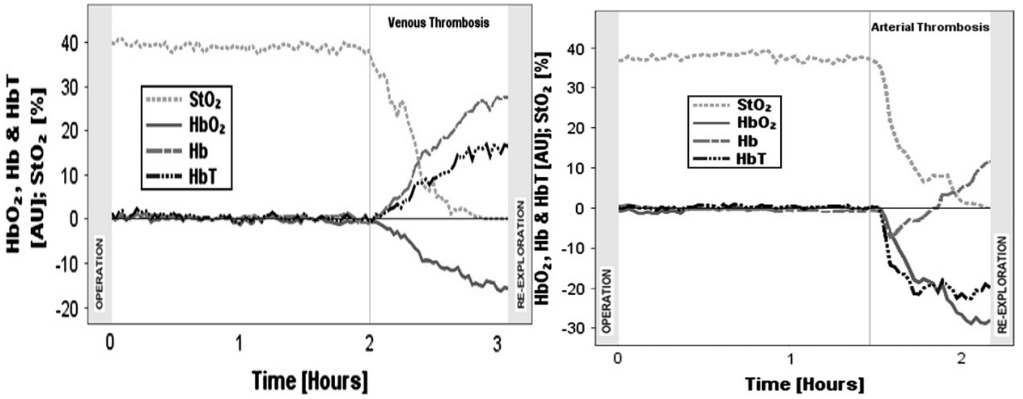

Figure 9. Characteristic changes in NIRS parameters due to postoperative venous (*on the left*) and arterial thrombosis (*on the right*). (Repez et al. 2008) Reprinted with permission from Elsevier.

2.2.6. Electromagnetic flowmetry

Quantitative blood flow measurements in milliliters per minute can be achieved by electromagnetic flowmetry (Banis et al. 1980). A blood vessel is placed between the poles of a strong magnet and electrodes are placed on the two sides of the vessel perpendicular to the magnetic lines of force. When blood flows through the vessel, an electrical voltage proportional to the rate of blood flow is generated between the two electrodes (Guyton and Hall 2006). Beekman studied the resolution of microvascular vasoconstriction using an electromagnetic perivascular flow sensor (Beekman et al. 1988). Although electromagnetic flowmetry is a method that provides immediate, continuous, and quantitative measurement of blood flow, high technical standards, exact probe placement, and the requirement for electrogel around the vessel reduce clinical applicability.

2.2.7. Intravital microscopy

Intravital microscopy is an old method to study microcirculation. It is based on direct visualization (Figure 10). Ichioka (Ichioka et al. 1998) studied the effects of amrinone on microcirculation. They measured the diameters of individual microvessels of rats for quantification of microcirculatory blood flow. Also videomicroscopy and direct microscopic measurements have been used to study the vasodilating capacity of pharmacological agents (Kerschner and Futran 1996).

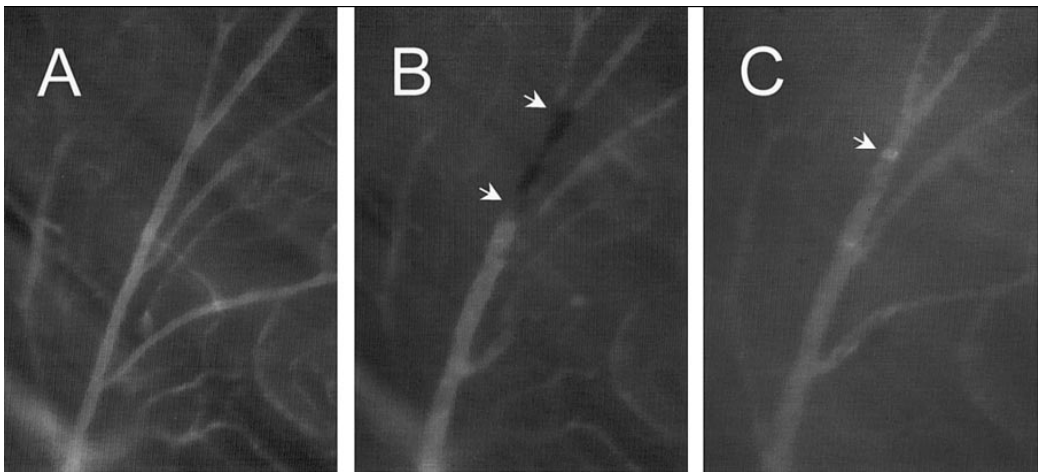

Figure 10. Intravital fluorescence microscopy of a thromboembolus arrested in a transverse arteriole in an osteomyocutaneous experimental flap. **A** The transverse arteriole was stained with fluorescein-labeled dextran immediately before embolization. **B** Thromboembolus arrested in the same arteriole visualized by negative contrast (arrows) using the blue filter combination and **C** by rhodamine staining of the platelets using the green filter combination. Magnification x 70. (Rucker et al. 2002) Reprinted with permission from Elsevier.

The major advantage of using intravital fluorescence microscopy for microcirculatory assessment is that the microscopic technique is direct and therefore allows in vivo visualization of individual microvessels (Menger and Lehr 1993). The method cannot be used to study buried flaps.

High-resolution microvascular pO_2 measurements have been made using phosphorescence quenching microscopy (Kerger et al. 1995). Oxygen measurements are based on the oxygen-dependent quenching of phosphorescence emitted by an albumin-bound metalloporphyrin complex after light excitation. It is independent on tissue dye concentration and optimal for detecting hypoxia, because the decay time is inversely proportional to the pO_2 level (Saldívar et al. 2003).

2.2.8. Single photon emission tomography

Single photon emission tomography (SPET) is a nuclear imaging technique as is PET. SPET uses tracers labeled with gamma emitting isotopes. Single photons are detected by a gamma camera instead of coincidence pairs, as in PET. SPET utilizes one-, two- or three-headed gammacameras rather than ring-detectors, as PET. Thus, SPET is less sensitive than PET, but less costly and more widely available, since it utilizes generator-based isotopes with long half-lives (e.g. ^{99m}Tc $T_{½}$ = 6 h) and on-line cyclotrons are not required. Since 1994, the addition of the coincidence detection mode to the detector system has enabled imaging of positron emitting tracers with gamma cameras as well (Ak et al. 2001).

Single photon emission computed tomography (SPECT) has been used in the evaluation of bone revascularization of microvascularized fibular grafts for mandibular reconstruction (Hervás et al. 2001). However, the perfusion results attained are only semiquantitative and thus absolute values are not available using this method.

2.2.9. Dynamic computed tomography

Dynamic computed tomography (CT) is an imaging method in which a rapid sequence of images is accrued while a contrast medium, which typically contains iodine, is injected intravenously to the patient. The spatial resolution of the images is high and the signal intensity is in linear relation to the concentration of iodine. As the tracer flows through an organ, there is a short period of time before venous outflow begins and at this moment the amount of tracer is the highest in the organ tissue. Perfusion can be calculated as the ratio of the slope of the tissue time density curve to the peak arterial density (Choyke et al. 2002). Numerical values have been calculated rather than plain ratios (Hermans et al. 1997, Brix et al. 1999, Hermans et al. 1999). Nevertheless, the concentrations cannot be exactly measured and thus the results should be regarded at the most as semiquantitative. Until recently, dynamic CT has been limited to a single study plane, and repeated measurements cannot be performed

until several hours later, when the contrast medium has been excreted. One disadvantage of this method is the relatively high radiation exposure.

Dynamic CT data correlate with the thermal washout data in tissues (Feldmann et al. 1992). Dynamic CT has been used to study perfusion in brain tumors (Jinkins and Sener 1991), head and neck cancer (Hermans et al. 1997, Brix et al. 1999, Hermans et al. 1999) and lymphomas, where it is associated with grade (low grade vs. intermediate or high-grade) (Dugdale et al. 1999). Dynamic CT is helpful in distinguishing benign from malignant lung nodules (Swensen et al. 2000). Increased arterial perfusion in the liver in dynamic CT studies is a predictor of hepatic metastases (Leggett et al. 1997, Platt et al. 1997). The maximum attenuation value in CT correlates positively with vascular endothelial growth factor staining of the tumor – thus, dynamic CT might also be a measure of tumor angiogenesis (Tateishi et al. 2002a, Tateishi et al. 2002b).

Gaggl studied perfusion-CT scans in 38 LD flaps in patients as predictors of postoperative ischemia. In these cases, clinical examination of the transplant and examination by means of O_2 -probes were either unsuccessful or impossible. They found that central malperfusion resulted in complete transplant loss in 2 cases. Peripheral malperfusion was seen in 6 cases, and these patients required localized resection and secondary wound closure. When no malperfusion was registered, healing was straightforward (Gaggl et al. 2002).

Among the recent-generation angiographic diagnostic techniques, multislice-CT has emerged as an outstanding noninvasive method. The use of multislice-CT angiography for preoperative DIEP flap evaluation has yielded promising results. This technique would allow not only to locate DIEP vessels but also to globally assess presurgical planning in noninvasive way. However, more patients will need to be evaluated in order to clarify the benefits of this technique (Alonso-Burgos et al. 2006).

2.2.10. Dynamic magnetic resonance imaging

Dynamic magnetic resonance imaging (MRI) can be used as a non-invasive method for assessing perfusion of a tissue. A concentrated bolus of a paramagnetic contrast agent needs to be administered to the patient. The contrast agent is usually a gadolinium chelate, gadopentetate dimeglumine. The signal produced by gadolinium depends not only on blood flow, but also on vessel permeability, since the contrast agent passes into the interstitial space in substantial amounts. Sequential images are acquired from the area of interest following the intravenous injection of the contrast agent. For moving structures, such as the lungs, breath-held imaging is required (Choyke et al. 2002). This method provides only relative, not absolute, quantification of perfusion (Cha et al. 2002).

Dynamic CT and dynamic MRI data correlate well with each other (Feldmann et al. 1993). Dynamic MRI data correspond also well with quantitative blood flow measured with [^{15}O] H_2O PET (Muramoto et al. 2002). However, the results of the MRI scan can

only be interpreted as semiquantitative. In addition, MRI scanners with enclosed magnetic coils are of limited space, which limits the usefulness in e.g. large patients, or anesthesized patients, or in patients otherwise in need of close monitoring.

2.2.11. Plethysmography

Plethysmography is one of the oldest methods to measure organ blood flow (Brodie and Russell 1905). It is based on measuring volume changes of the object, e.g. limb after venous outflow of the limb is occluded by a cuff.

Stack used reflectance photoplethysmography for monitoring 30 free flaps. One ischemic event (skin paddle loss only) occurred in this group of patients, which was detected by the monitor but not by a bedside duplex scanning of the vascular pedicle (Stack et al. 2003). On the other hand, new methods studied by Chuah are not dependent on venous occlusion and produce more consistent results with or without hyperemia (Chuah et al. 2004).

2.2.12. Other methods to assess flap perfusion

Temperature monitoring. Thermography is easy, inexpensive, and non-invasive. It has been used for mapping cutaneous perforators pre-, intra-, and postoperatively, and for monitoring the flaps bedside (Salmi et al. 1995c, Suominen and Asko-Seljavaara 1996). May described an implantable thermocouple probe for the diagnosis of arterial and venous thrombosis. The probes were fixed proximal and distal to the arterial anastomosis, thus registering an increasing temperature difference after arterial occlusion. Reoperation was indicated if there was a temperature difference of more than 0.30 °C for more than 1 h. (May et al. 1983) Furthermore, the adequacy of tissue perfusion can be assessed via surface temperature recordings (Leonard et al. 1982). On the other hand, Kaufman (Kaufman et al. 1987) reported in a small study that temperature monitoring is unreliable and nonreproducible.

In experimental studies, the **measurement of collected venous drainage** has been used to estimate flap tissue perfusion. Crabb (Crabb et al. 1985) determined flap blood flow gravimetrically as milliliters of blood per 5 min collected from the brachial vein.

Intraoperative ultrasonic transit-time flowmetery. Here, the probe uses two piezoelectric crystals transmitting a pulsed ultrasound beam. The two crystals are placed on the same side of the vessel and a metal reflector on the contralateral side. The time between transmission of the ultrasound beam from one crystal and its reception at the other is measured. The difference between the upstream and downstream transit times is proportional to the volume flow evaluated blood flow in the donor and recipient artery in free microvascular flaps using a transit-time

flowmeter and suitable probes fitting small 2 or 3 mm diameter vessels (Albäck et al. 1996, Lorenzetti et al. 2001c).

P**ulse oximetry** measures the percentage of oxygen saturation of hemoglobin. The principle is to transmit two separate wavelengths of light to distinguish between oxygenated and deoxygenated hemoglobin by light absorption differences. Menick successfully monitored postoperatively a revascularized free flap using pulse oxymetry on a cutaneous monitoring island flap in a latissimus dorsi muscle flap (Menick 1988). However, the movement of the sensor causes artifacts and the fixation of the probe to a flap is difficult (Machens et al. 1994).

Dilution techniques are the common methods to measure limb blood flow during exercise. Dilution techniques are based on the infusion of indicator into blood and thereafter measuring its rate of dilution. The indicators must be fully mixed with the blood and they must not be retained or metabolized. **Indo-cyanine green dye dilution and thermodilution** are two of the most widespread methods. In dye dilution, blood flow is measured by the continuous infusion of dye of a known concentration at a specific rate into the vessel (i.e. femoral artery). Simultaneously, the dye concentration is determined in the blood at the corresponding downstream vessel (i.e. femoral vein) and blood flow is calculated by a specific formula. In thermodilution, again, cold saline is infused instead of dye. The temperature of the blood before infusion, the temperature of the infused saline and the temperature of the blood and saline mixture during the steady state infusion are measured. A specific formula is used to calculate blood flow. (Radegran 1999) The thermodilution method can be used for repetitive measurements of blood flow in the tissue of interest because heat has no re-circulation problems, such as dye (Andersen and Saltin 1985, Kiens et al. 1993, Baron et al. 2000, Radegran and Saltin 2000). The drawbacks of the dilution methods include an invasive technique and the inability to measure regional blood flow.

Orthogonal polarization spectral (OPS) imaging allows direct visualization of the microcirculation bedside. OPS has been validated against intravital fluorescence microscopy (IFM) for microvascular measurements in experimental skin flaps. Quantitative analysis of functional capillary density (FCD) was feasible. Comparison of OPS imaging and IFM revealed a significant correlation of FCD values ($p < 0.001$) at all time points (Langer et al. 2001). OPS method is not directly suitable for monitoring of buried flaps (Verdant and de Backer 2005).

2.3. Positron emission tomography (PET)

2.3.1. Principles of PET

PET is a functional imaging method that enables in vivo measurement of physiological and biochemical processes noninvasively and quantitatively. The radionuclides used in PET have a nuclear imbalance, i.e. an excess of protons. To restore the stability of the

radioactive nucleus an extra proton is converted into a neutron and a positron is emitted. After travelling a short distance of 3-5 millimeters, the speed of the positron slows down and it collides with a nearby electron. Annihilation occurs, since positrons and electrons are antiparticles of each other. The mass of these particles is converted into energy in the form of two gamma rays, i.e. photons that travel in opposite directions.

The PET scanner detects the two simultaneous (coincident) gamma rays from the annihilation site by detectors that are arranged in a ring-shaped pattern around the patient. An image can be reconstructed after a sufficient number of coincident gamma rays (counts) have been detected. The distance that the positron travels before annihilation depends on its energy, which is specific to the radionuclide. The resolution of the PET image is determined by the design of the PET scanner, the radionuclide, and the processing of data during reconstruction. To achieve quantitative measures several corrections need to be applied, e.g., tissue attenuation, scattering gamma rays, random counts and dead time losses (Bacharach 1992).

There are several tracers available for PET studies. The compounds are labeled with a positron-emitting radionuclide and are injected into a patient and thus, numerous biological and pharmaceutical processes can be analyzed using a PET scanner both under physiological and pathological conditions. The most common PET-radionuclides have a short half-life. This moderates the radiation dose to the patient, which is comparable to a CT study of the abdomen, i.e. approximately 1-3 milliSieverts. Typical radionuclides used are fluorine (^{18}F), carbon (^{11}C), nitrogen (^{13}N) and, oxygen (^{15}0) with the respective half-lives 109.8 min, 20.4 min, 10.0 min and 2.05 min (Early 1995, Early and Landa 1995). The radiation dose has to be assessed in any PET study, and evaluated in detail when implementing new tracers. Critical organs receiving the highest radiation dose should also be defined to know the maximum amount of tracer that can be administered (Stabin et al. 1999).

2.3.2. Oxygen-15 labeled water

Oxygen-15 labeled water ([^{15}O] H_2O) is a chemically and metabolically inert tracer used to assess tissue perfusion. It is freely diffusible and has a short radioactive half-life ($T_{½}$ = 2.05 min or 123 s), allowing repeated measurements. The method is based on the difference between arterial blood activity and the activity in the tissue of interest. However, this difference diminishes in very high flow rates, and under these conditions the method becomes less reliable (Anderson and Price 2002). [^{15}O] H_2O is administered to the subject usually intravenously as an infusion or as a bolus. This tracer can be studied also by letting the subject inhale oxygen-15 labeled carbon dioxide ([^{15}O] CO_2), which is rapidly converted in the lungs to [^{15}O] water by carbonic anhydrase, producing arterial input of [^{15}O] water. By continuous inhalation a steady state of radioactivity can be reached in the tissues. By measuring the activity level in arterial blood during inhalation of [^{15}O] CO_2, quantitative values can be obtained.

Estimates of tissue uptake can be obtained roughly either by calculating the target-to-normal tissue ratio, or by measuring the standardized uptake value (SUV). The latter is the ratio of the measured radioactivity concentration to the estimated mean tracer concentration, assuming a uniform distribution throughout the entire body volume (Huang 2000). More accurate, quantitative results can be obtained with dynamic techniques or autoradiography (Bacharach et al. 2000, Anderson and Price 2002). A parametric, pixel-by-pixel-based model fitting method has been recently introduced (Lodge et al. 2000). On the other hand, quantitative values of BF_{PET} can be estimated directly from tissue curves obtained through [^{15}O] H_2O PET imaging. This suggests the possibility to enable completely non-invasive technique to assess tissue perfusion in patho-physiological studies (Kudomi et al. 2008).

2.3.3. Measuring perfusion in patients with [^{15}O] H_2O

PET and [^{15}O] H_2O allows the quantitative evaluation of the region of interest like no other method. Moreover, [^{15}O] H_2O is an inert tracer that permits non-invasive functional study of the region of interest.

According to the literature PET and [^{15}O] H_2O has not previously been used in the evaluation of microsurgical free flap BF_{PET}. There are, nevertheless, several feasible BF_{PET} studies with PET and [^{15}O] H_2O. PET and [^{15}O] H_2O have been most extensively used to study cerebral perfusion in humans. Extensive studies of muscle blood flow (Kalliokoski et al. 2002, Laaksonen et al. 2003) are available. Moreover, clinical results are available for measuring the blood flow in carcinomas of the head and neck, the breast, the liver, the lung, the gut, the prostate, and the pancreas (Kubo et al. 1991, Inaba 1992, Dimitrakopoulou-Strauss et al. 1998, Yamaguchi et al. 2000, Hoekstra et al. 2002, Slimani et al. 2008). Patients with Moyamoya's disease which is an extremely rare disorder characterized by progressive intracranial vascular stenoses of the circle of Willis resulting in successive ischemic events, have been studied with PET and [^{15}O] H_2O (Morimoto et al. 1999, Khan and Yonekawa 2005).

2.3.4. Other PET tracers for measurement of tissue perfusion

[^{13}N] ammonia has also been used to study tissue perfusion with PET. [^{13}N] ammonia is extracted from plasma and trapped in tissue in proportion to blood flow. However, metabolic factors influence the uptake, as well (Keiding et al. 2006). Therefore, quantitative assessment of blood flow with [^{13}N] ammonia is not feasible.

2.4. Flap complications

Flap failure occurs clinically with devastating consequences. Ablative tumor surgery is often followed by reconstructive surgery. Cancer patients are subjected to increased

systemic risk of thrombotic events, and may be at an even greater risk of local thrombosis. Thromboses compromise outcome. Siemionow reported that the first 3 days following microvascular procedures are the most critical ones for tissue survival (Siemionow et al. 1995). Complications prolong the median hospital stay of these patients by 7.5 days (Singh et al. 1999). In a study of 1,142 free flaps, the re-exploration rate was 9.9 % (113 flaps) with 63.7 % completely salvaged flaps. Of the re-explored flaps, 82.3 % presented with circulatory compromise within 24 hours, and 95.6 % within 72 hours (Chen et al. 2007).

In a large study of 493 free flaps the overall incidence of flap failure was 4.1 %. Reconstruction of an irradiated recipient site and the use of a skin-grafted muscle flap were the only statistically significant predictors of flap failure. Postoperative thrombosis requiring re-exploration occurred in 9.9 % of the flaps (Khouri et al. 1998). In the study of Aitasalo, 101 patients underwent microvascular reconstruction; the success rate was 88 % in irradiated tissue and 91 % in nonirradiated tissue (Aitasalo et al. 1997). Deutsch reported that the incidence of complications in patients with free fibular flaps is similar between irradiated and non-irradiated tissue (Deutsch et al. 1999). Schultze-Mosgau reported an overall success rate of 90 % in an analysis of 217 free flaps in 199 patients. After preoperative radiotherapy (RT), vascularization of the graft bed decreased progressively as a function of total dose and time after RT. They suggested that primary reconstruction should be performed after a time interval of 4 to 6 weeks after preoperative irradiation (Schultze-Mosgau et al. 2002). According to Lindholm preoperative hyperfractionated accelerated RT combined with surgery is a feasible treatment of HNSCC: it does not raise the incidence of serious late complications (Lindholm et al. 2006).

Hidalgo reported an overall success rate of 98 % in 716 consecutive free flaps used to treat cancer patients (Hidalgo et al. 1998). Suominen reported an overall flap failure rate of 9.3 % in a retrospective analysis of 75 consecutive free flap patients. The immediate vascular complication rate was no less than 22.7 %. Flap failure correlated with the rate of preoperative infections of the recipient site and with prolonged operation time (Suominen and Asko-Seljavaara 1995).

Finical reported high complication rates in free flaps in recurrent head and neck cancers in a 10-year retrospective review of 121 patients: 31 % required additional surgery for complications, 14 % total flap failures were reported, and 4 % of the patients died within 1 month of the reconstructive operation (Finical et al. 2001).

Sasmor suggests that the flap failure rate increases by recipient site in the following order: upper extremity, breast, head and neck, and lower extremity (Sasmor et al. 1992). Failure of a skin flap has been attributed to either intrinsic or extrinsic causes. The extrinsic factors include systemic conditions (e.g. infection, arteriosclerosis, hypotension, and malnutrition) and local causes (e.g. compression, tension, thrombosis of the anastomoses, kinking of a pedicle and inadequate nutrient blood flow within the skin flap) (Kerrigan and Daniel 1983).

2.4.1. Flap ischemia and reperfusion injury

Flap survival requires sufficient tissue perfusion. If this fails, hypoxia and waste products induce harmful effects to the flap. In free microvascular flaps, two types of ischemic injury are possible: distal ischemia or global ischemia. Global ischemia is a consequence of obstruction in arteries or veins of the pedicle. Distal ischemia might develop by inadequate flap design; the flap may be too large in relation to feeding vessels, which subjects the edges of the flap to ischemia. On the other hand, even with impeccable flap design and flawless surgical technique, partial or total flap ischemia leading to flap necrosis is possible. Therefore, an improved understanding of global flap hemodynamics and the response of the flap to compromised perfusion are fundamental for successful surgery (Kerrigan et al. 1984, Kerrigan and Stotland 1993).

The first ischemic episode in free flap operations occurs when the flap is elevated and disconnected from its original circulation and it continues until the moment when the circulation is re-established after proper vessel anastomosis. The maximum length of time that vital tissue can tolerate complete ischemia and yet remain viable once circulation is restored, is called the primary critical ischemia time (Kerrigan and Daniel 1982, Kerrigan et al. 1984, Zelt et al. 1986, He et al. 1997). Secondary ischemia occurs, if a postoperative complication reduces flap circulation. Compromised venous BF is more detrimental to flap survival than arterial ischemia (Kerrigan et al. 1994). The average primary and secondary critical ischemia times of cutaneous flaps are 13.1 hours (Kerrigan and Daniel 1982) and 7.2 hours (Kerrigan et al. 1984), respectively. Here, average means that 50 percent of the flaps are lost if this time is not kept. Thus, cutaneous flaps are more vulnerable to secondary ischemia than to primary ischemia. The primary and secondary critical ischemia times of musculocutaneous flaps are 9.1 hours and 11.3 hours, respectively (Zelt et al. 1986). Therefore, muscle tissue adapts better to secondary ischemia, possibly due to stimulated anaerobic metabolism. It is fair to state, however, that the difference in the tolerance of ischemia and in the response to ischemia between skin and myocutaneous flaps has not been confirmed. Picard-Ami found a statistically significant decrease in survival of both skin and myocutaneous flaps after 6 and 8 hours of ischemia, respectively (Picard-Ami et al. 1990). In practice, skin tolerates ischemia better than muscle tissue in a free flap.

Ischemia causes tissue damage and restoration of blood flow causes even more damage (Khalil et al. 2006). Anaerobic metabolism generates a variety of noxious substances during the period of ischemia (Figure 11). A flap subjected to global ischemia is damaged not only by the period of hypoxia, but also by the reperfusion period, when many of these substances are further metabolized, and toxic free radicals are generated. The production of the toxic substances can proceed for 24-48 hours after reestablishment of perfusion (Pang et al. 1993). These toxic free radicals may cause peroxidation of cellular and intracellular membranes and intracellular proteins, resulting in irreversible cell injury (Mounsey et al. 1992, Pang et al. 1993) (Figures 11-12). Exposure of endothelial cell membranes to free oxygen radicals results in increased permeability, leading to edema and hemorrhage, and to exposure of collagen

and basement membranes; this promotes platelet and granulocyte adhesion and initiates a cascade leading to microvascular thrombosis (Carroll and Esclamado 2000). Increased vascular resistance and decreased blood flow rates further enhance platelet adhesion and thrombosis in the flap (Sasmor et al. 1992). The extent of this phenomenon, called ischemia-induced reperfusion injury, is related to the duration of ischemia (Labbe et al. 1987). Ischemia-induced reperfusion injury is also associated with irreversible ATP depletion and excessive metabolite accumulation (Pang et al. 1993).

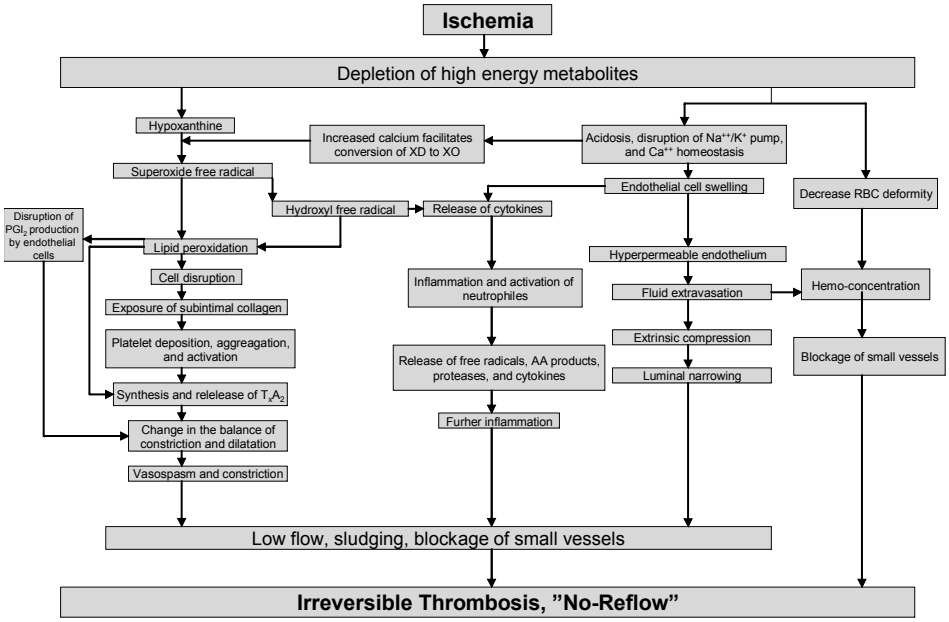

Figure 11. Proposed interconnected mechanisms of reflow. ATP = adenosine triphosphate; XD = xanthine dehydrogenase; XO = xanthine oxidase; Na^+ = sodium; K^+ = potassium; Ca^{2+} = calcium; RBC = red blood cell; PGI_2 = prostacyclin (prostaglandin I_2 [PGI_2]); T_xA_2 = thromboxane A_2. Modified with permission (Calhoun et al. 1999).

Prolonged ischemia induces stepwise catabolism of ATP to hypoxanthine, and xanthine oxidase activity increases (Im et al. 1989). Xanthine oxidase (XO) generates superoxide (O_2^-) by univalent reduction of molecular oxygen in the presence of hypoxanthine during reperfusion. Interaction of O_2^- with hydrogen peroxide (H_2O_2) in the presence of a transitional metal (e.g. iron) generates hydroxyl radicals (OH^-) which are extremely cytotoxic (Pang et al. 1993, Carroll and Esclamado 2000). The xanthine oxidase activity correlates well with the fate of free flaps (Im et al. 1989). Also other sources of oxy-radicals contribute to ischemia-induced reperfusion injury. A major part of the O_2 consumed by activated neutrophils is converted to O_2^- by membrane-associated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The O_2^- is further converted to H_2O_2 and OH^- as described above. Also, myeloperoxidase in neutrophils catalyzes the conversion of H_2O_2 to hypochlorous acid, which is also a

potent cytotoxic oxidizing agent. Cordeiro used L-arginine to reduce the neutrophil counts, which did reduce neutrophil-mediated tissue injury (Cordeiro et al. 1997a).

Vascular spasms during surgery are a well-known phenomenon. They may lead to loss of transplanted tissue. Vascular spasms are associated with ischemia, interstitial edema, tissue temperature, acidosis, metabolic derangement, and humoral or neural vasoactive substances (Seaber 1987). In addition, there are physiological and mechanical factors associated with vasospasm. For example, blood-induced segmental vasospasm has been identified (Hou et al. 1987), and an artery might constrict due to the myogenic response to stretching of the artery wall. A metabolically induced vasospasm may be induced by local vessel injury through damage to the endothelium of the artery. This would induce vasospasm in the damaged segment through depletion of prostacyclin and other nonprostaglandin vasodilators. Local vasoconstrictors, like endothelin-1, which is a potent vasoconstrictor released by vascular endothelium, and thromboxane A_2, a short-lived vasoconstrictor released from the circulating platelets, may also become activated during and after microsurgery (Seaber 1987, Samuelson et al. 1992), especially in the arterioles in the distal portion of the flap where the perfusion pressure is low and the concentrations of these damaging substances are high (Pang 1990).

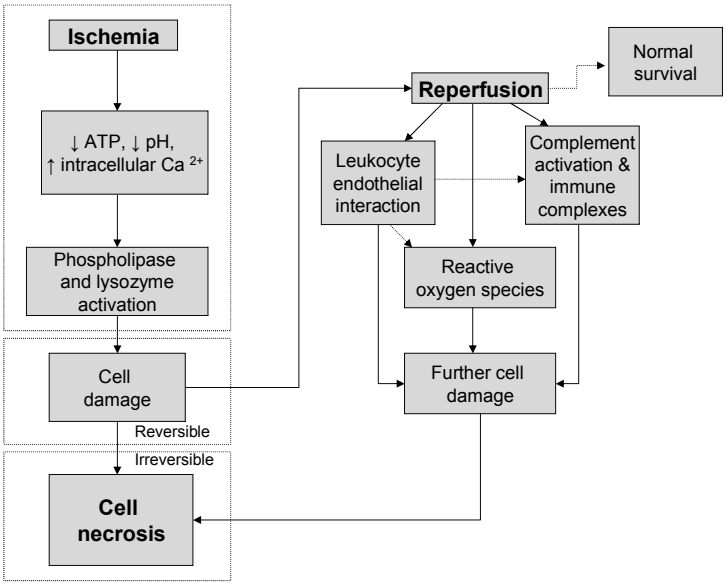

Figure 12. Sequence of events during ischemia and reperfusion.

2.4.2. Fat necrosis

Fat necrosis of the breast is a benign inflammatory process that may clinically, mammographically, and sonographically mimic malignancy. Fat necrosis appears most often after surgery (biopsy, lumpectomy, reduction mammoplasty, implant removal, breast reconstruction) and radiation therapy (Evers and Troupin 1991, Roisman et al. 1991, Hogge et al. 1995, Rosen 1997, Miller et al. 1998).

The mammographic spectrum of fat necrosis ranges from clearly benign to indeterminate to malignant-looking calcifications or masses (Bassett et al. 1978, Hogge et al. 1995). They may be radiolucent and have a thin, well-defined radiolucent and dense capsule, the "mycetoma" appearance (Evers and Troupin 1991, Van Gelderen 1994). They may be dense and circumscribed, ill-defined, or even spiculated (Bassett et al. 1978, Roisman et al. 1991, Miller et al. 1998).

At ultrasonography, these masses are usually solid, although they may be complex or cystic. Borders may be discrete or ill-defined. Posterior enhancement or shadowing may be present (Soo et al. 1998). The surrounding architecture may be distorted (Soo et al. 1998).

The pathophysiology of fat necrosis helps to explain the wide spectrum of mammographic findings. Microscopically, disruption of fat cells and hemorrhage with adjacent histiocytes is seen early in fat necrosis. Thereafter, multinucleated histiocytes, lymphocytes, and plasma cells appear, and hemosiderin is deposited. Necrotic fat and cellular debris are enclosed by fibrosis from the periphery. Usually years later, the fibrosis contracts into a scar but the collection of oil or fat may remain (Van Gelderen 1994, DiPiro et al. 1995). Older lesions tend to be more fibrotic and less cellular (Clarke et al. 1983). The degree of fibrosis affects mammographic findings. Early fat necrosis with, less extensive fibrosis is associated with a lipid cyst with a thin, fibrous capsule. As time passes, fibrosis becomes more extensive and a spiculated mass, indistinguishable from carcinoma, may develop (DiPiro et al. 1995). Calcifications represent a relatively late finding and occur often in association with fibrosis. However, early calcification of the fibrotic rim of a lipid cyst or collapse of a partially calcified lipid cyst may have an indeterminate mammographic appearance (Hogge et al. 1995).

In reducing the amount of disturbance to the abdominal wall donor site, use of the DIEP flap unavoidably reduces the number of perforators supplying blood to the flap. This could theoretically lead to a reduced supply of blood to the flap, thereby causing an increase in PFL and fat necrosis. On the other hand, PFL and fat necrosis may be prevented by removing the fat under Scarpa´s fascia in zone IV or by discarding zone IV. The latter is the protocol in most centers (Kroll et al. 1998, Blondeel et al. 2000).

3. Aims of the study

The main aim of the present study was to investigate the use of measurements of tissue oxygen tension and positron emission tomography (PET) measured blood perfusion in the evaluation of the hemodynamics and the blood flow of free flaps in cancer patients.

The specific aims were:

1. To assess the blood perfusion of microvascular free flaps quantitatively with PET in patients with head and neck squamous cell carcinoma (HNSCC) undergoing major radical surgery 3-4 weeks after high-dose radiotherapy.

2. To evaluate tissue oxygen measurement and PET measured blood perfusion as methods for predicting ischemia in microvascular free flaps of the head and neck.

3. To evaluate the feasibility of tissue oxygen measurement and PET in evaluating the perfusion of microvascular free breast reconstruction flaps.

4. To determine the perfusion heterogeneity in microvascular free breast reconstruction flap zones assessed with PET and to evaluate whether it is associated with vitality of the flap.

5. To evaluate the associations between free flap tissue oxygen tension and systemic blood pressure in patients operated on for HNSCC.

4. Patients, materials, and methods

All studies were prospective. These studies were carried out to investigate a reliable monitoring method for free microvascular flaps in head and neck cancer and in breast reconstruction patients. The studies took place during the years 2002-2008 at the Department of Otorhinolaryngology - Head and Neck Surgery, and the Department of Surgery, Turku University Hospital, Finland, and the PET Centre, University of Turku, Finland.

4.1. Patients

The vitality of 32 free flaps was studied in 30 patients operated on for head and neck cancer (I-II, V) or breast cancer (III-IV). There was some crossover of patients between studies which is presented in Tables 2 and 3. PET with [^{15}O] H_2O was used in 33 scannings of 29 patients to quantitate blood flow of the newly reconstructed area. Tissue oxygenation was evaluated with a polarographic catheter in 24 patients. Both methods were used in 24 patients (Table 1).

Table 1. Patient characteristics in studies I-V.

Study	N	Group	Age (years)
I	5	HNSCC	58.4
II	10	HNSCC	66.0
III	13	BC	49.9
IV	12	BC	49.8
V	10	HNSCC	62.7

N = number of patients; HNSCC = head and neck squamous cell carcinoma; BC = breast carcinoma. There was some crossover of patients in studies III and IV, and in studies II and V.

Patients who were in good or moderate clinical condition (Zubrod classification 0-2 (Oken et al. 1982)) were eligible for participation.

The study protocol was approved by the joint ethics Committee of the University of Turku and Turku University Hospital, and permission to use [^{15}O] H_2O in patient

studies was granted by the Finnish National Agency of Medicines. All patients were verbally informed of the study, its aims and procedures, after which each patient gave written informed consent before entering the study.

HNSCC patients underwent conventional diagnostic staging, including panendoscopy, contrast-enhanced CT (CT-imager: Siemens Somatom Plus 4, Erlangen, Germany) of the primary tumor site and the neck, and chest radiography. The location of the primary HN tumor, the histological diagnosis, and the clinical stages according to the criteria of the International Union Against Cancer (Sobin and Wittekind 1997) are given in Table 2.

Table 2. Characteristics of patients with head and neck squamous cell carcinoma in studies I, II, and V.

Flap #	Age years	Sex	TNM	Location	Grade	BMI	Flap (cm^2)	Study
1	57	F	T2N0M0	OC (Tongue)	I	27.6	48	I
2	72	M	T4N2M0	OP (BoT)	III	22.8	15	I
3	59	M	T3N0M0	OC (FoM)	II	30.0	32	I
4	52	M	T4N0M0	OC (Tongue)	II	21.1	23	I
5	52	M	T4N0M0 (r)	OC (Tongue)	II	20.5	45	I
6	64	M	T4NXM0	OC (RM)	II	18.3	40	I,V
7	53	F	T1N0M0 (r)	Hypopharynx	II	18.3	96	II
8	66	M	T2N1M0	OC (RM)	I	22.8	30	II,V
9	57	M	T4N0M0 †	OC (Tongue)	I	20.5	40	II,V
10	74	F	T3N1M0	OC (Tongue)	I	20.9	80	II,V
11	70	M	T3N0M0 (r)	OC (Buccal muc)	IV	25.5	154	II,V
12	62	M	T3N0M0	OC (RM)	II	23.5	35	II,V
13	57	F	T1N0M0 (r)	OC (Tongue)	I	19.6	260	II,V
14	72	M	T3N0M0	OC (Tongue)	I	23.1	56	II,V
15	65	M	T3N2M0	OC (Tongue)	II	21.1	28	II
16	81	F	T3N0M0	OC (Buccal muc)	I	21.3	41	II
17	52	M	T3N1M0	OC (Tongue)	II	23.2	30	V
18	53	F	T4N2M0 (r)	OC (RM)	I	14.9	120	V
Mean	61.9					22.5	63.9	
SD	10.3					3.2	62.5	

M = male; F = female; TNM = classification of malignant tumors UICC 2002; † = second primary tumor; (r) = recurrency; Grade = histological grade of the tumor; BMI = body mass index; Flap = cutaneous area of the free flap in square centimeters; OP = oropharynx; OC = oral cavity; BoT = base of tongue; RM = retromolar; muc = mucosa; FoM = floor of mouth

The characteristics of the BC patients enrolled in studies III and IV are presented in Table 3. In study III one patient (no.13) was studied only with $p_{ti}O_2$ monitoring,

otherwise the patients were the same in studies III and IV. The age range of the patients in these studies (III-IV) was from 26 to 67 years. One patient was a smoker (no. 3). One patient had arterial hypertension (no. 9) and took an ACE inhibitor. The BMI of the patient population ranged from 20.9 to 33.6.

After treatment, the patients were followed until December 31, 2008 or death. The median follow-up time after the PET study was 14 months (range 1.0-61.0). Further patient characteristics are presented in Tables 2 and 3.

Table 3. Characteristics of breast cancer patients (III – IV). In study III one patient (no.13) was studied only with $p_{ti}O_2$ monitoring, otherwise the patients were the same in studies III and IV.

Patient	Age	PAD	TNM	Flap	BMI	Vessels	No.perf.	Length of operation	Duration of flap ischemia	Hospital stay	Follow-up time (months)
1	53	DCIS	TisN0M0	MS2-TRAM	30.1	TD	2	300	94	7	29
2	41	DC	pT1mN1M0	MS3-DIEP	29.7	TD	1	480	117	6	20
3	58	DC	TisN0M0	MS2-TRAM	24.5	SC	3	325	55	7	23
4	53	LC	T1N0M0	MS3-DIEP	30.8	TD	2	310	67	6	36
5	43	DC	T3N1M0	MS3-DIEP	27.8	TD	3	518	142	9	31
6	39	DC	T2N1M0	MS2-TRAM	24.8	TD	4	425	138	6	22
7a	26	DCIS	TisN0M0	MS3-DIEP	33.6	TD	3	599	161	6	20
*7b				MS3-DIEP		TD	3		80		
8	67	DC	T2N0M0	MS3-DIEP	24.7	TD	2	365	130	7	29
9	61	DC	T2N0M0	MS3-DIEP	22.6	TD	3	325	63	7	35
10	57	DCIS	TisN0M0	MS2-TRAM	26.2	TD	4	320	84	10	14
11	54	DCIS	TisN0M0	MS3-DIEP	25.1	TD	2	375	59	6	18
12	46	DCIS	TisN0M0	MS3-DIEP	20.9	TD	2	315	61	7	27
13	51	DC	T1N1M0	MS2-TRAM	27.2	TD	4	565	na	8	30
Mean	49.9				26.8		2.7	402	96	7.1	25.7
SD	10.8				3.6		0.9	105	37	1.3	6.7

PAD = histological diagnosis of the breast cancer; TNM = classification of malignant tumors UICC 2002; BMI = body mass index; No. perf. = number of perforating arteries included in the flap; 7a = delayed DIEP flap of patient no.7; *7b = immediate DIEP flap of patient no.7; DCIS = ductal carcinoma in situ; DC = ductal carcinoma; LC = lobular carcinoma; MS2-TRAM = muscle-sparing transverse rectus abdominis myocutaneous flap; MS3-DIEP = deep inferior epigastric perforator flap; TD = thoracodorsal vein and artery; SC = subscapular vein; SD = standard deviation

4.2. PET

All PET studies were performed using a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, WI, USA) operated in 2-dimensional mode. The scanner consists of 18 rings of bismuth germanate detectors yielding 35 transverse planes each 4.25 mm in width giving a 15.3 cm axial length for the imaging field. The imaging field of view (FOV) was 55 cm in diameter. For the correction of photon attenuation a 10-minute static transmission scan was performed using 2 rotating rod sources containing $^{68}Ge/^{68}Ga$ (total 350 million counts).

All measurements were corrected for scatter, random counts, dead time, and physical radioactivity decay. The final in-plane resolution of the reconstructed image was approximately 6 mm full width of half maximum.

After transmission scanning, a 6-min dynamic emission scan (6 x 5 s, 6 x 15 s, 8 x 30 s frames) for BF_{PET} measurement was performed immediately after an intravenous injection of [^{15}O] H_2O (overall median dose in PET scans was 1,058 MBq (range 748-1,467 MBq)) dissolved in 2.5 mL saline which was given as a bolus (15 s; 10 mL/min), followed by a flush of saline over 45 s. Arterial blood was withdrawn with a peristaltic roller pump (Alitea, Stockholm, Sweden) at a speed of 6 mL/min to obtain the [^{15}O] H_2O input curve. Blood radioactivity concentration was measured using a 2-channel online coincidence detector (General Electric Medical Systems, Uppsala, Sweden). In case of malfunction of the arterial pump (n=2) the arterial input function (AIF) was determined noninvasively from the dynamic image data by region of interest (ROI) drawn over the left ventricle of the heart, as routinely performed in myocardial BF_{PET} analyses with PET and radiowater. To obtain a final in-plane resolution of approximately 5 mm full-width half maximum, the transmission corrected data were iteratively reconstructed in a 128 x 128 matrix.

4.3. Study protocol

In study I, five primary PET studies were performed on the second postoperative (PO) day, and one on the first PO day. Follow-up studies were performed on the 14th PO day for 3 patients, and on the 13th and on the 9th PO day for 2 patients, respectively. One follow-up study could not be performed for logistical reasons. In studies III-IV the PET scan was carried out on the first PO morning for 12 patients.

All PET imaging was performed while the patients were fasting. The patient was placed supine on the PET scanner couch with the head and neck area – or breasts – within the field of view (FOV). Laser-guided landmarks and felt pen markings were used, if feasible, so that the positioning of the patient for the second PET scan in study I could be performed in the same way as for the first examination. A catheter was

placed into the antecubital vein for injection of the [^{15}O] H_2O. Another catheter was placed in the contralateral radial artery, and in one case in the femoral artery for blood sampling. Normally the catheters were the same that were placed already for the anesthesia of the primary operation. The regional BF_{PET} of the entire flap was evaluated with PET using oxygen-15-labeled water to achieve data of the perfusion of the entire flap. Thereafter the patient was transferred to the regular ward.

4.4. Image analysis

4.4.1. Identification of free flaps from the PET image

Thirtytwo free flaps in 30 patients were studied. All flaps were easily detectable in the [^{15}O] H_2O study.

For evaluation of BF_{PET} in breast reconstruction free flaps, the skin surface was identified by slightly radioactive ^{22}Na-buttons (AEA Technology QSA GmbH, Germany) with an approximately 2 mm ^{22}Na-crystal framed by a plastic button with a diameter of 2.1 cm attached on the skin with an adhesive tape.

4.4.2. Definition of regions of interest (I-IV)

Regions of interest (ROIs) were drawn onto three (I-III) and four (IV) consecutive planes of perfusion images where the flap area was seen and summed to volumes of interest of 4.5-28 mL. All ROIs were drawn by the author of this thesis in each study to ensure similar analysis of the flap areas.

In HN patients (I-II) an elliptic ROI was placed over the contralateral muscle or corresponding area of tissue (healthy side) and another ROI in the microvascular flap. Anatomic landmarks seen on perfusion and MR (I) images, such as mandible, parotid glands, tongue, and the flap itself, were used to confirm the location of the area.

In BC patients (III-IV) a crescent-shaped ROI was placed over the contralateral breast tissue and another similar ROI in the breast reconstruction flap. Anatomic landmarks seen on perfusion and transmission images, such as the heart, the lungs, and the flap itself, were used to confirm the location of the area**.** In study IV additional ROIs were placed over zones I, II, and III of the breast flap, and over corresponding areas in the healthy breast.

In selecting the planes for ROIs and in drawing ROIs (ROI size ranged from 1.5 to 7.0 cm^2), large blood vessels and the heart were avoided to prevent contamination of radioactivity by spillover from tissues with high radioactivity. The ROIs were then used to obtain tissue curves for calculation of BF_{PET} (Figure 13).

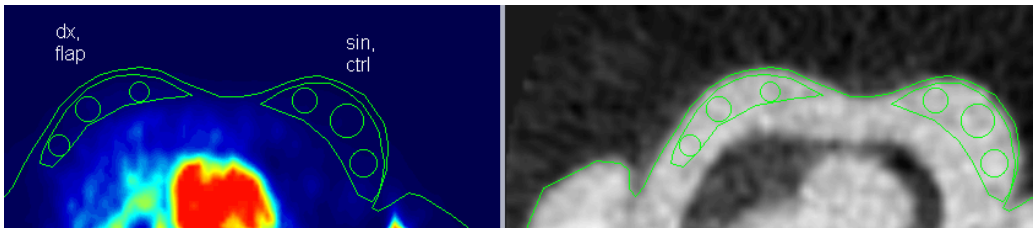

Figure 13. Regions of interest (ROIs, *green line*) drawn on the horizontal sectional plane of the parametric blood flow and transmission PET image (III-IV). dx, flap = right side of the thorax, microvascular reconstruction on this side; sin, ctrl = left side, normal breast tissue as control.

4.4.3. Image processing

Two-dimensional PET images were processed using the ordered subsets expectation maximization and median root prior reconstruction algorithm (2D OSEM-MRP) with 150 iteration and the bayesian coefficient 0.3 (Alenius and Ruotsalainen 1997).

The delay between the [^{15}O] H_2O input curve and the tissue curve was solved by fitting.

4.4.4. Calculation of perfusion and perfusion heterogeneity (I-IV)

Perfusion was calculated by the one-compartment model and the autoradiographic method (Nuutila et al. 1996, Ruotsalainen et al. 1997) voxel by voxel into flow images with a 90-s tissue integration time. The autoradiographic method is based on the principle of inert gas exchange between blood and tissues (Kety and Schmidt 1945).

Analysis of the perfusion heterogeneity was carried out with relative blood flow values in addition to the absolute blood flow values. A mean and standard deviation (SD) of perfusion values were calculated. The BF_{PET} values from each pixel were calculated and the BF_{PET} and BF_{PET} HG were analyzed by zones of the flap and the contralateral breast tissue. The partition value for adipose tissue was calculated as the ratio of water content in adipose tissue to water content in blood (Snyder et al. 1975). Relative dispersion (RD) of perfusion heterogeneity was calculated as SD divided by the mean (Duling and Damon 1987, Pitkanen et al. 1999). In studies III-IV, the voxel-based analysis was performed using proportional scaling with global normalization (relative scaling). In global normalization the values in each voxel are divided by a global mean value.

The BF_{PET} heterogeneity between different flaps and the heterogeneity of each flap was analyzed from selected ROIs from zones I to III in studies III and IV. The significance of different variables (zone, flap type, the number of perforators) in each flap was included in the heterogeneity analysis. The analysis was carried out, first, to analyze

whether the heterogeneity due to each variable is different depending on different zones of the flap. Then the analysis was carried out independent of zones.

4.4.5. Fractal analysis of a free breast flap perfusion heterogeneity (IV)

The spatial distribution of perfusion has been shown to increase scale-dependently in heterogeneity, as smaller regions of perfusion are evaluated (Iversen and Nicolaysen 1995, Glenny et al. 2000). Perfusion has also been shown to be heterogeneous in many tissues at the voxel by voxel level in PET studies using [^{15}O] H_2O. However, the limited spatial resolution of the PET scanner does not permit direct measurement of true vascular flow heterogeneity. Fractal dimension (D) obtained by fractal analysis describes the relationship between the relative dispersion and the size of the region studied, and has been used for the assessment of perfusion heterogeneity in microvascular units. In principle, it is possible to obtain data regarding physiological variables beyond the resolution of the imaging technique (e.g. PET) by using fractal analysis. Study IV was undertaken to evaluate fractal characteristics of PET perfusion data and to estimate perfusion heterogeneity in microvascular units in free flaps used for breast reconstruction.

Free breast reconstruction flap blood perfusion was measured using [^{15}O] H_2O PET and the fractal characteristics of blood flow in the flap were analyzed. The perfusion heterogeneity in microvascular units was estimated using the measured heterogeneity (relative dispersion, RD=SD/mean) and D values.

Fractal analysis was based on the equation by Bassingthwaighte (Bassingtwaighte et al. 1989), which describes the relationship of relative dispersion (RD) for a given mass m:

$$\mathbf{RD(m) = RD(m_{ref})\ (m/\ m_{ref})^{1-D}} \qquad \text{(equation 8)}$$

In equation 8, m_{ref} presents the mass of the reference sample (kg), $RD(m_{ref})$ is its concomitant relative dispersion and D is the fractal dimension, which describes the proportion of relative dispersion change with the changing sample mass (m). In this equation m and m_{ref} can also be replaced with volumes of pieces v and v_{ref} .

Square ROIs (4 x 4 voxels) were placed onto four cross-sectional planes of the flap zones of the reconstructed breast and onto the contralateral breast tissue. The ROI was placed over the flap tissue avoiding the pectoralis muscle. Thereafter, three-dimensional matrixes of voxel blood flow values (64 voxels) were regrouped to larger groups by combining adjacent voxels in a predetermined manner to the level where four combined pieces remained (Figure 14). The voxel perfusion values within each group were averaged and the mean, standard deviation (SD) and relative dispersion (RD = [SD/mean] 100 %) were calculated in all levels of groups (Kalliokoski et al. 2001).

The logarithm of RD of blood flow versus the logarithm of the number of the combined voxels was plotted, and the fractal dimension was calculated as 1 - k, where k is the slope of linear least-square regression line of the data (Kalliokoski et al. 2001).

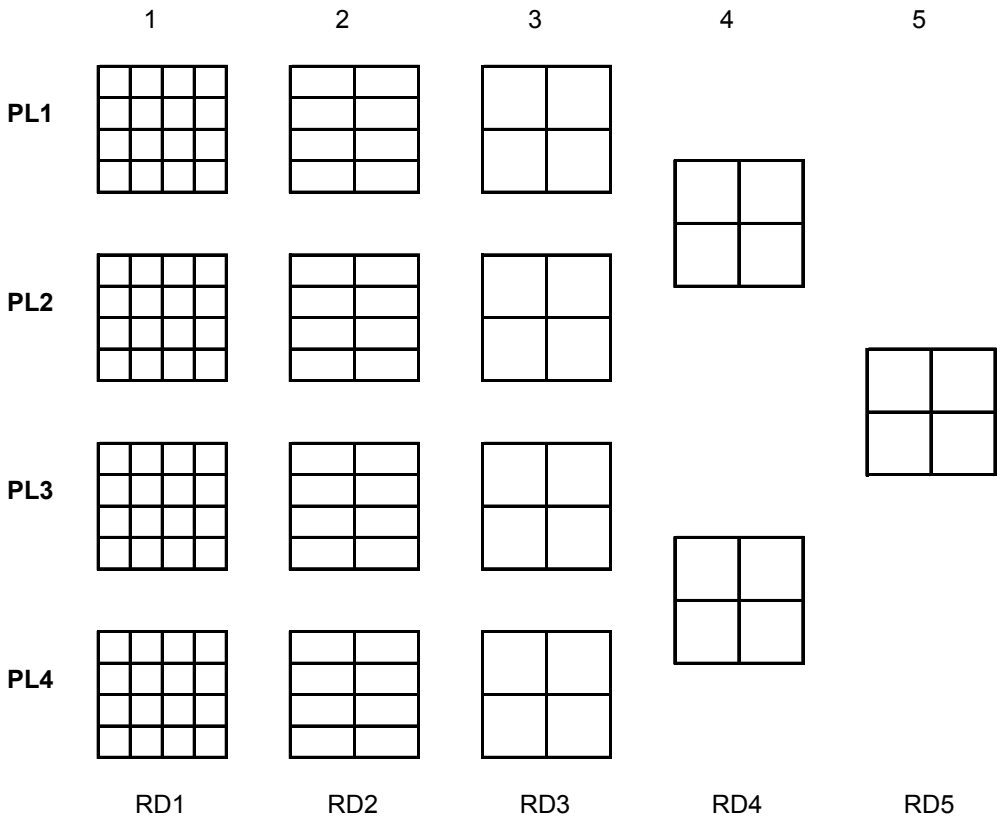

Figure 14. Square ROIs (4 x 4 voxels) were placed into four cross-sectional planes (PL) of the breast reconstruction flap zones I-III to determine the heterogeneity of the flap zones. Thereafter, 3D matrixes of voxel blood flow values (64 voxels) were regrouped to larger groups by combining adjacent voxels in to the level where four combined pieces remained.

4.5. Measurement of oxygen partial pressure ($p_{ti}O_2$) in tissue

The $p_{ti}O_2$ level of the newly reconstructed flap was continuously observed for a minimum of 48 hours with a Licox® (GMS, Kiel-Mielkendorf, Germany) polarographic probe, which was inserted into the subcutis of zone I of the breast reconstruction flap (Figure 3). The probe was inserted into the tissue by advancing it retrogradely along an insertion needle catheter lumen, which was then removed (Hirigoyen et al. 1995, Wechselberger et al. 1997, Kamolz et al. 2002) (Figure 15).

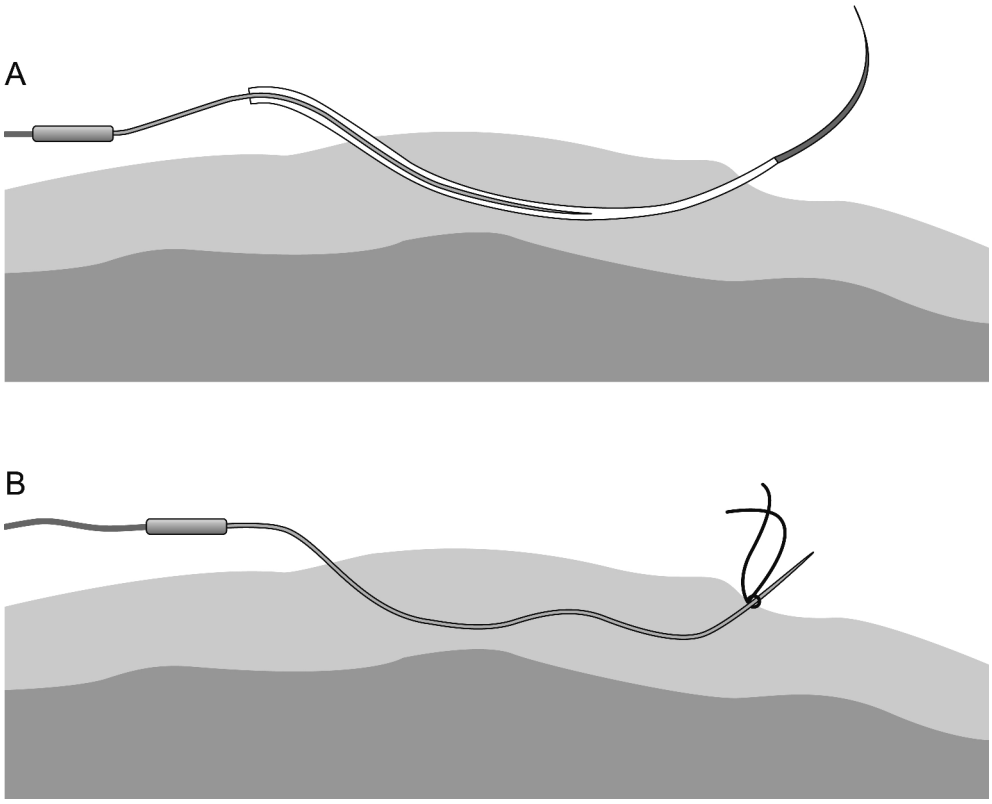

Figure 15. Schematic drawing of the Licox® polarographic $p_{ti}O_2$ probe catheter insertion. The probe was inserted into the tissue by advancing it retrogradely along an insertion needle catheter (VK2) lumen (*above*), which was then removed and the probe was secured (*below*).

The Licox® Revoxode CC1.2 polarographic probe (Figure 16) was used for all $p_{ti}O_2$ measurements; the probe has been designed for peri- and postoperative monitoring of the tissue oxygenation in a free flap. The probe was secured in place with interrupted nonabsorbable sutures placed through the skin around the external component of the catheter (Figure 15). In studies I, II and V, where intaoral or buried free flaps were studied, the core temperature was followed with the urinary catheter temperature probe, and the temperature was manually set in the Licox® measurement unit for temperature-adjusted $p_{ti}O_2$ (mmHg) results. In studies III-IV, the temperature of the flap was continuously measured with an electronic thermometer with surface probes (Duotemp TM101; Fisher & Paykel Health Care, Auckland, New Zealand) on the skin and the time course of a temperature-adjusted $p_{ti}O_2$ was graphically displayed and stored on a personal computer. A control temperature probe was set on the healthy skin of the trunk (studies III-IV).

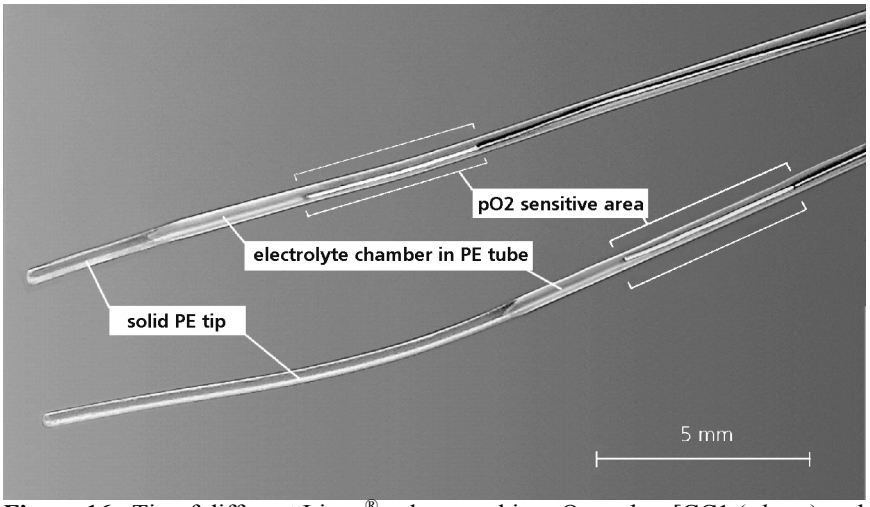

Figure 16. Tip of different Licox® polarographic $p_{ti}O_2$ probes [CC1 (*above*) and CC1.2 (*below*)]. Reprinted with permission from GMS.

During out-of-office hours the nurses taking care of the microsurgical patients were instructed to inform the surgeon in charge,

1) if there was a steady decline in the curve without recovery within 30 min,
2) if $p_{ti}O_2$ remained between 10 and 15 mmHg for over 30 min, or
3) if $p_{ti}O_2$ dropped below 10 mmHg, or
4) if a non-vital reaction of the flap occurred, such as a change in the flap color, or a decrease in the local temperature of the flap, suggesting circulatory deficiency of the flap.

The above $p_{ti}O_2$ limits used in this study were chosen based on previous literature (Hirigoyen et al. 1995, Wechselberger et al. 1997, Kamolz et al. 2002). The decision to reoperate was based on the $p_{ti}O_2$ values and a re-exploration was carried out if the $p_{ti}O_2$ value remained lower than 15 mmHg for over 30 min (III-IV). The decision was reconsidered if BF_{PET} values were readily available and considered sufficient.

4.6. Microsurgical procedures

4.6.1. HN reconstructions (I-II, V)

17 patients underwent 18 free flap reconstructions of the surgical defect (I, II, V); an experienced head and neck surgeon performed these operations. The same surgeon performed the tumor resection, the neck dissection and the free tissue transfer in the same session for 11 patients, and for 6 patients a plastic surgeon performed the free tissue transfer. The flaps were harvested as described (Soutar et al. 1983, Urken et al. 1995). Usually a free radial forearm flap (RFF, n = 12) was utilized. The

characteristics of the flaps used are presented in Table 4. The recipient vessels in the neck were most often the facial artery (n = 9) and the superior thyroid artery (n = 6). End-to-end arterial anastomoses were performed on the ipsilateral side for all patients. Ipsilateral veins branching from the internal jugular or external jugular veins served as recipient veins. Two veins were used in five patients. The anastomoses were performed with magnifying loops using 8-0 monofil nylon sutures.

Table 4. Reconstruction flaps in HN reconstructions. (I-II, V)

Type of flap	n
RFF	12
LD	3
Fibula	2
ALT	1

RFF = radial forearm flap; LD = latissimus dorsi flap; Fibula = osteocutaneous fibular flap; ALT = antero-lateral thigh flap

4.6.2. Breast reconstructions (III-IV)

Thirteen patients underwent free microvascular flap reconstruction of the surgical defect performed by three experienced plastic surgeons. Altogether fourteen free flaps were used for breast reconstructions. An immediate reconstruction after tumor resection was carried out in two patients and one immediate reconstruction was carried out after prophylactic mammary ablation in one patient who had a high hereditary risk of mammary carcinoma. Thus, 11 flaps were late reconstructions. (Table 5) In three patients, a sentinel node investigation was also carried out.

Table 5. Flaps in BC reconstructions.

Type of flap	n	Immediate	Delayed
TRAM MS2	5	0	5
TRAM MS3 (DIEP)	9	3	6
Mastopexia *)	4		
Reductioplasty *)	2		
None *)	8		

*) Contralateral operations.

A two-team approach was used, with simultaneous raising of the flap and preparation of the recipient vessels. The selection of abdominal flaps, including the free TRAM and DIEP flap, was based on preoperative and intraoperative assessment of the perforators (Nahabedian et al. 2002b). When a pulsating perforator was visualized, a DIEP (MS3) flap was used and included one to four perforators. In the absence of a dominant perforator, a muscle-sparing free TRAM (MS2) flap (Nahabedian et al. 2005, Bajaj et al. 2006) was preferred.

Thoracodorsal (TD) vessels were used for end-to-end anastomoses with the deep inferior epigastric vessels in 12 patients. The subscapular vein was used in one patient due to the absence of TD vessels which had been sacrificed in a prior axillary lymph node evacuation. In one patient two veins were utilized. The second anastomosis was performed between a superficial perforator and an intercostal branch of the axillary vein. During elevation of one flap, the main perforator vessel suffered an iatrogenic trauma from the electrocoagulator. A new anastomosis was carried out after resection of the injured part of the vessel.

All patients received low-molecular-weight heparin (enoxaparin) 40 mg daily as thrombosis prophylaxis. Despite this, one patient had a pulmonary embolism on the 3rd PO day, which was successfully treated with warfarin.

4.7. Patient monitoring

Patients were postoperatively monitored first either in the intensive care unit (ICU) (I-II, V) or in the surgical recovery room (III-IV), and thereafter in the regular ward. The systolic blood pressure level was set at above 100 mmHg. The core body temperature was followed during the first three PO days. HN patients breathed spontaneously after weaning from the respirator on the first postoperative day, while BC patients were normally extubated after the surgery. All HN patients in this series required a tracheostomy (I-II, V). The clinical assessment of the flap included capillary flap refill, and if required, bleeding time after needle puncture, initially at hourly intervals. HN patients received wide-spectrum intravenous antimicrobial agents for 7 days, dextran 40 (Rheo-Macrodex®, Pharmalink, Sweden) with papaverin (240 mg in 500 mL dextran 40) as a continuous infusion for 5 days, followed by aspirin 400 mg x1 usually through a percutaneous endoscopic gastrostomy tube for the following month.

The breast reconstruction patients were postoperatively monitored in the recovery room for the first PO night. The clinical assessment of the flap included capillary flap refill, temperature monitoring initially at hourly intervals, and, if required, the bleeding time after needle puncture of the flap was evaluated. The temperature of the flap was followed up with a surface probe and compared to a control skin probe. (III-IV)

4.8. Study endpoints

Overall survival of the patients and flap survival were selected as the endpoints of the study. The follow-up of head and neck cancer patients was at the responsibility of the treating physician in the otorhinolaryngology department and the multidisciplinary tumor board. The scheduled visits were: 1 month after surgery, then at 3-month intervals for the first and second years, then at 4-month intervals for the 3rd year, then at 6-month intervals for the 4th and 5th year after surgery. CT or MRI and endoscopy were not routinely carried out, although a postoperative CT-scan was taken during the

1st PO year in high-risk patients to obtain a reliable postoperative CT-status for later needs if recurrent tumor was suspected during follow-up.

Patients who had undergone breast reconstruction were followed up for a possible recurrence and for the vitality of the flap.

4.9. Statistical analysis

Data analysis was based either on analysis of variance for repeated measures or on one-way analysis of variance. Post-hoc analysis was done using the least significant difference procedure. The level of significance was set at .05. Computation was carried out using the SAS System for Windows, version 9.1.3 software. A more detailed description is presented in the methods section of each original publication.

5. Results

The PET perfusion studies were well tolerated by the patients, and usually the patients were able to complete the study as planned. In one patient, the PET study was cancelled because the patient needed urgent re-exploration of the free flap anastomoses only 90 minutes before the scheduled PET scan. One patient declined the second PET-scan (I) because of personal reasons. In two patients the arterial blood pump failed and for these patient studies a model-based noninvasive estimation of the input function was used to estimate absolute blood flow by drawing a ROI over the left ventricle assuming the input function to correspond with the results from arterial blood pump (Iida et al. 1992, Pitkanen et al. 1999). Altogether 29 patients were studied with PET in 33 scannings and 40 radiowater injections, and 24 patients were monitored continuously for an average of 3 postoperative days with $p_{ti}O_2$ measurement. (Table 6)

Table 6. Patient characteristics, mean flap blood flow and flap tissue oxygen measurement results included in each study.

Study	N	Group	Age (years)	BMI (kg/m^2)	BF_{PET} (mL/100g/min)	$p_{ti}O_2$ (mmHg)
I	5	HNSCC	58.4	24.4	4.8	na
II	10	HNSCC	66.0	22.2	8.5	46.8
III	13	BC	49.9	26.8	1.9	52.9
IV	12	BC	49.8	26.7	1.9	51.3
V	10	HNSCC	62.7	21.2	na	54.0

N = number of patients; BMI = body mass index; BF_{PET} = mean flap blood perfusion assessed with PET using [^{15}O] H_2O; $p_{ti}O_2$ = flap tissue oxygenation; na = not applicable; HNSCC = head and neck squamous cell carcinoma; BC = breast carcinoma

5.1. Functional evaluation of microvascular free flaps with PET

5.1.1. PET in HN patients

The quantitative BF_{PET} in HN free flap reconstructions could be assessed with PET using [^{15}O] H_2O in studies I and II. The BF_{PET} in HN free flaps was 17-74 % lower than in the contralateral healthy tissue. A low flap-to-muscle BF_{PET} ratio appeared to associate with inadequate flap perfusion, and thus with poorer flap success. The BF_{PET} results of the follow-up PET study on the second postoperative week were similar to the BF_{PET} values acquired from the primary PET scan according to study I.

During the PO perfusion PET scans, all patients were in stable circulatory condition. PET was performed on the first, second, and third PO day in five, three, and two patients, respectively. The average BF_{PET} of the flap measured with [^{15}O] H_2O was 8.5 mL per 100 g min $^{-1}$ (SD 2.5, range 5.5-12.7) (Table 6), and 20.5 mL per 100 g min $^{-1}$ ($p < 0.001$, SD 7.3, range 10.6-31.0) in the contralateral reference muscle. There was a tendency for flap BF_{PET} to decrease over PO day 1 through day 3 according to study II (Figure 18).

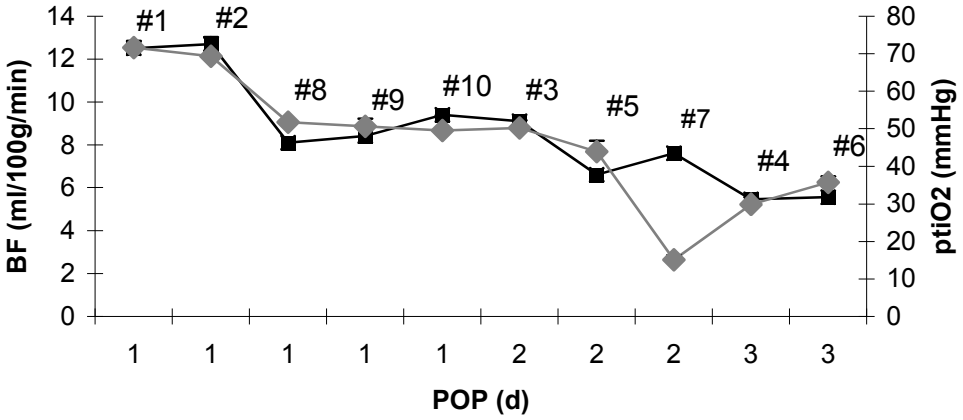

Figure 18. Correlation of flap blood flow (BF) assessed with PET versus tissue oxygenation (mean $p_{ti}O_2$ ±SD) of the flap during PET scan of each patient. The patients are in the order of the postoperative day when the PET scan was performed (in order as follows: patient nos. 1, 2, 8, 9, 10, 3, 5, 7, 4, 6). [*Filled square* is blood perfusion assessed with PET (mL/100g/min), *Filled diamond* is tissue oxygen partial pressure of the flap, ($p_{ti}O_2$, mmHg)]

5.1.2. PET in breast reconstructions (III-IV)

Twelve patients and 13 flaps underwent a dynamic BF_{PET} measurement using [^{15}O] H_2O, with a mean dose of 1,060±119 MBq (range: 860-1,247 MBq). Overall BF_{PET} was assessed quantitatively in all flaps with PET. The patients were hemodynamically stable during all PET scans. The patients were divided into subgroups in study III; group I consisted of patients without complications, group II consisted of patients with partial skin or fat necrosis, and group III consisted of patients whose anastomoses were re-explored. There were no statistically significant differences among groups I, II and III in BF_{PET} values. The BF_{PET} on the first postoperative day was 17 % lower in the newly reconstructed breast tissue in comparison to the contralateral normal breast tissue (2.8±1.3 vs. 3.2±1.0 mL per 100 g min^{-1}, respectively, $p < 0.001$) (III-IV). (Figure 19)

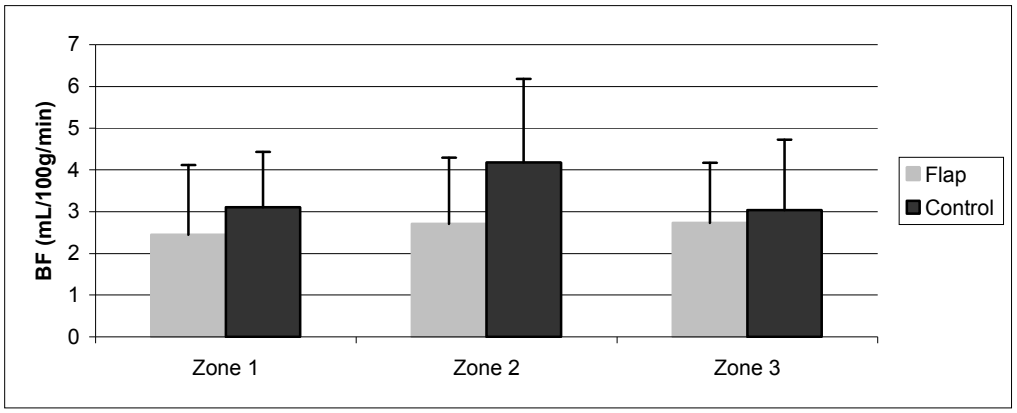

Figure 19. BF_{PET} measured from each zone and compared between the newly reconstructed breast flap (*Grey box*, perfusion in mL/100g/min) and contralateral normal breast tissue (*Black box*). Overall BF_{PET} on the first postoperative day appeared 17 % lower in the breast flap and in zone 2 the difference was significant ($p < 0.001$).

One re-operation suggested by the low $p_{ti}O_2$ results was avoided due to the normal BF_{PET} results. Few hours earlier, the same patient had undergone a re-exploration of the anastomoses due to the critically low $p_{ti}O_2$ values (< 10 mmHg), but the anastomoses were flawless. Unfortunately, the PET scan was not carried out until after this first unnecessary reoperation. The $p_{ti}O_2$ values dropped again three hours after the first re-exploration, but the BF_{PET} values acquired from the PET using [^{15}O] H_2O were considered sufficient and another reoperation was avoided and flawless flap survival occurred. (Figure 20)

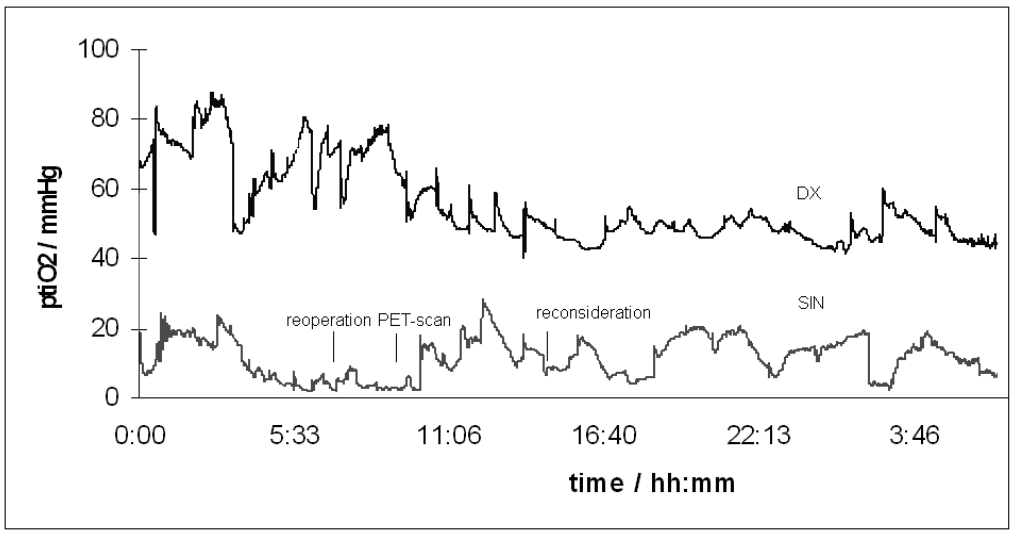

Figure 20. A $p_{ti}O_2$ measurement graph from a BC patient with a bilateral DIEP flap (No. 7); DX = DIEP on the right side; SIN = DIEP on the left side; reoperation = patient was operated on due to low (< 10 mmHg) $p_{ti}O_2$ values; PET-scan = PET perfusion scanning completed with sufficient results although $p_{ti}O_2$ values were still low; reconsideration = another reoperation was

considered due to the low $p_{ti}O_2$ values but cancelled on the basis of sufficient perfusion assessed with PET. The flap survived uneventfully.

5.1.2.1. Perfusion in breast reconstruction zones (IV)

Regional BF_{PET} of the adipose tissue in medial, central, and lateral parts of thirteen free flaps was assessed on the first postoperative morning with PET using oxygen-15 labeled water ($[^{15}O]$ H_2O) in 12 patients undergoing breast reconstruction with a DIEP or a TRAM flap.

The mean BF_{PET} values did not differ between DIEP and TRAM flaps ($p = 0.791$). The mean BF_{PET} values were higher in zone III compared to zone I ($p = 0.024$). (Table 7)

Table 7. Blood perfusion results (mL per 100g min^{-1}) from the evaluated zones of the breast flaps assessed with PET using radiowater.

Zone	Flap	Mean BF_{PET}	SD	Minimum	Maximum
I	TRAM	1.73	0.97	0.74	3.04
	DIEP	2.23	1.41	1.04	4.71
II	TRAM	2.05	0.87	1.43	3.34
	DIEP	2.43	1.34	0.87	4.54
III	TRAM	2.94	2.07	1.23	5.94
	DIEP	2.69	1.13	1.40	4.89

Mean BF_{PET} = the average blood perfusion assessed with PET using radiowater; SD = standard deviation; TRAM = transverse rectus abdominis myocutaneous flap, DIEP = deep inferior epigastric perforator (flap).

During follow-up fat necrosis was identified in three patients in the medial part (zone II) of the flap. However, the adipose tissue BF_{PET} assessed from all zones of the flap using PET with radiowater was normal. The BF_{PET} HG was higher in the control side (i.e. in the healthy breast tissue) compared to the flap ($p = 0.042$). The BF_{PET} HG was lower in zone III than in zone I ($p = 0.024$) and in zone II ($p < 0.001$). (Table 8)

There were no statistically significant differences in BF_{PET} of the different flaps according to the number of perforators ($p = 0.340$), the patients' age ($p = 0.624$), body mass index ($p = 0.681$), or the weight of the flap ($p = 1.000$) according to this study.

In three patients who later developed fat necrosis in the medial part of the flap, i.e. zone II, the early postoperative BF_{PET} and $p_{ti}O_2$ were normal.

Table 8. Perfusion heterogeneity (in percentage, %) from the evaluated zones of the breast flaps and from the corresponding areas of the contralateral healthy breast tissue assessed with PET

using radiowater (patient no. 7 excluded from this evaluation due to bilateral flap reconstruction).

Zone	Region	Mean (%)	SD	Minimum	Maximum
I	CTRL	29.4	8.0	18.2	42.2
	FLAP	24.9	9.8	9.1	41.4
II	CTRL	30.9	9.8	18.9	53.2
	FLAP	26.6	5.3	12.7	34.7
III	CTRL	24.9	4.4	15.8	34.3
	FLAP	20.0	5.1	11.3	29.3

Region = the area where the perfusion heterogeneity was assessed; CTRL = contralateral healthy breast tissue; FLAP = free breast reconstruction flap; Mean = the average blood perfusion heterogeneity percentage assessed with PET using radiowater; SD = standard deviation.

5.2. Monitoring microvascular free flaps with $p_{ti}O_2$ (II-V)

5.2.1. $P_{ti}O_2$ in head and neck cancer patients (II, V)

Eleven HN free flap patients were monitored with $p_{ti}O_2$ measurement (II, V). Continuous tissue oxygen measurement was carried out for 3.1 days (mean, SD 0.6, range 2-5). During the PET scanning the mean $p_{ti}O_2$ of the flaps was 46.8 mmHg (SD 17.0, range 15.1-71.6) (Table 6) and during the whole recording period of 3 days the mean $p_{ti}O_2$ was 42.1 mmHg (SD 10.6, range 21.4-56.2) (Figure 21). The difference was not statistically significant.

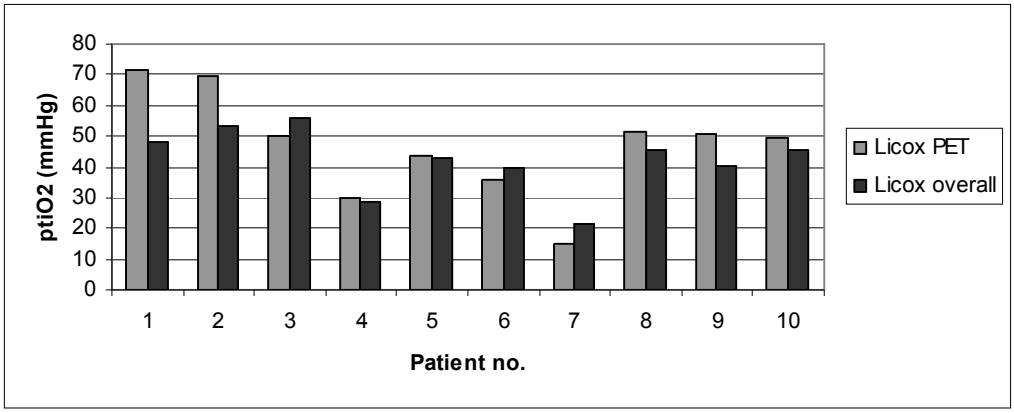

Figure 21. $P_{ti}O_2$ measurement results of HN patients during PET-scanning (*grey box*, Licox PET) and during the three-day monitoring (*black box*, Licox overall). (II)

In 3 free flaps out of 10 (II), a marked drop in $p_{ti}O_2$ levels took place and a perfusion

problem was suspected. Two of these occurred perioperatively during primary surgery and were due to anastomosis problems, which were not clinically visible without exploration. One patient had an arterial thrombosis twice due to kinking of the artery and one patient had a venous thrombosis. After thrombectomy and reanastomosis, the $p_{ti}O_2$ values rose from 0 to over 50 mmHg, and both flaps survived without further complications. In one patient a profound drop in $p_{ti}O_2$ (from 40 to 10 mmHg) was detected 4 h postoperatively. This was apparently due to postoperative turgor and too tight wound bandages. The core temperature of the same patient suggested concomitantly mild hypothermia (35.2°C), which partly affected the apparent low perfusion and thus the oxygenation of the large (260 cm^2) musculocutaneous flap. An increase in $p_{ti}O_2$ values to over 30 mmHg was achieved after loosening the bandages and by warming the patient to normal core temperature.

Three patients had hematomas requiring revision, one patient on the first PO day and two patients on the second PO day. The hematomas were all clinically diagnosed and due to mild diffuse bleeding; no compression of the nutrient vessels or other hemodynamic problems occurred with the flaps.

Vascular disturbances were detected correctly in three of the ten (30 %) patients during the 3-day monitoring of the microvascular free flaps; there were no false positive or false negative results. The Licox® system detected the circulatory failures which necessitated revision before clinical signs emerged.

All 10 flaps were viable and in good condition at the time of the patients' discharge from the hospital. However, the epithelium of the ALT-flap of one patient was at follow-up partly necrotic, probably due to weak perforator vessels, and healed by secondary intent.

5.2.2. $P_{ti}O_2$ in breast reconstruction patients (III-IV)

The $p_{ti}O_2$ of each breast reconstruction flap was registered for a mean of 73.6 ± 8.6 hours postoperatively (range 53-90). The temperature of the breast reconstruction flap was followed and remained on average at 35.1 ± 1.2 and 35.7 ± 1.0 ºC with a range between 33.1-36.3 and 34.2-36.8 ºC during the follow-up. There were no statistically significant differences between the flap and the control skin temperature or between the flaps that developed complications or not.

The mean $p_{ti}O_2$ in free breast reconstruction flaps measured continuously from zone I of the flap (Figure 3A, p. 24) was 52.9 ± 3.3 mmHg during PET scanning (Table 6) and 48.1 ± 5.1 mmHg during the entire registration. There was a statistically significant difference between the groups in terms of $p_{ti}O_2$ values during the first 12 PO hours ($p < 0.05$). Two of the three patients in group III required re-exploration within 6 hours after the operation. At the time of PET scan, the clinical vitality of the flaps was flawless and the $p_{ti}O_2$ values were adequate in all but one patient. There was

no statistical significance between the primary ischemia time and $p_{ti}O_2$ measurement results. (Table 9)

There was no total flap loss in this series, and 7 patients had no complications. In total, 3 patients developed fat necrosis, i.e., hardening of the reconstructed tissue during follow-up. In all these patients, the areas of fat necrosis were noted in the periphery of the flap (zone II). In the donor area of one of these patients, who was a heavy smoker, there was skin necrosis requiring revision with a skin graft. Three patients (group III, Table 9) were re-explored 4 hrs and 6 hrs postoperatively and on the fourth PO day. One re-exploration was due to a clinical hematoma lateral to the flap which was due to diffuse bleeding from the fat tissue; there were no anastomosis problems, nor effects on the $p_{ti}O_2$ values. One patient had a late arterial thrombosis which was diagnosed clinically on the 4th PO day; this condition required re-exploration, thrombectomy and re-anastomosis to the artery. The Licox® catheter had been removed 1 day prior to this arterial thrombosis which occurred unusually late. This was a patient with two venous anastomoses in the primary operation due to rather small-calibre veins. The patient developed lateral fat necrosis, requiring an endoprosthesis 9 months postoperatively.

One false positive finding in the polarographic monitoring was found in one flap, suggested by $p_{ti}O_2$ values less than 10 mmHg within 4 PO hours. Clinically, re-capillarization was compromised supporting the tissue-oxygen measurement result. These findings were erroneus in terms of flap failure as shown by an immediate re-exploration, which did not reveal any anastomosis failures. There were no false negative results. Thus, the specificity of the $p_{ti}O_2$ measurement for predicting flap success was 92.3 % in this study.

Table 9. Characteristics of breast reconstruction patients and free flap operations. Group I consisted of patients with no complications, group II of patients with partial cutaneous or fat necrosis, and group III of patients who underwent a re-exploration of the flap.

	Age (yrs) (ns)	BMI (ns)	Duration (min)	Ischemia (min)
Group I (n=7)	47.1 ± 11.5 (ns)	26.7 ± 4.3 (ns)	381 ± 105 ($p < 0.05$)	84 ± 31 ($p = 0.013$)
Group II (n=2)	55.5 ± 3.5 (ns)	27.3 ± 4.0 (ns)	313 ± 18 ($p < 0.05$)	75 ± 28 ($p = 0.024$)
Group III (n=3)	46.8 ± 17.1	28.3 ± 3.8	512 ± 103	144 ± 16

yrs = years; ns = statistically non-significant difference between groups I and III, and II and III, respectively; BMI = body mass index; Duration (min) = duration of surgery in minutes; Ischemia (min) = ischemia of the flap from the time the artery is closed to the moment the perfusion is re-established. *Reproduced with permission of the copyright holder.*

5.3. Association between BF_{PET} and $p_{ti}O_2$ in free flaps (II-IV)

The blood perfusion values assessed with PET correlated statistically significantly with the $p_{ti}O_2$ values ($r = 0.97$; $p < 0.05$) measured during the PET scan in HN patients with uneventful recovery after flap reconstruction. However, in a patient with HNSCC whose tissue defect had been reconstructed with an anterolateral thigh (ALT) flap accompanied with a 5 by 8 cm rectus muscle with clinically rather small perforators, the $p_{ti}O_2$ values of the septocutaneous part of the flap were low (mean 15.1 mmHg during PET, SD 1.2 mmHg), whereas the blood flow assessed with PET from the muscular part was normal (7.6 mL per 100 g min^{-1}). (Figure 22)

The correlation of BF_{PET} and $p_{ti}O_2$ was not statistically significant in breast reconstruction free flaps (Figure 22).

The $p_{ti}O_2$ measurement of HN and BC flaps showed no statistically significant differences in uncompromised cases.

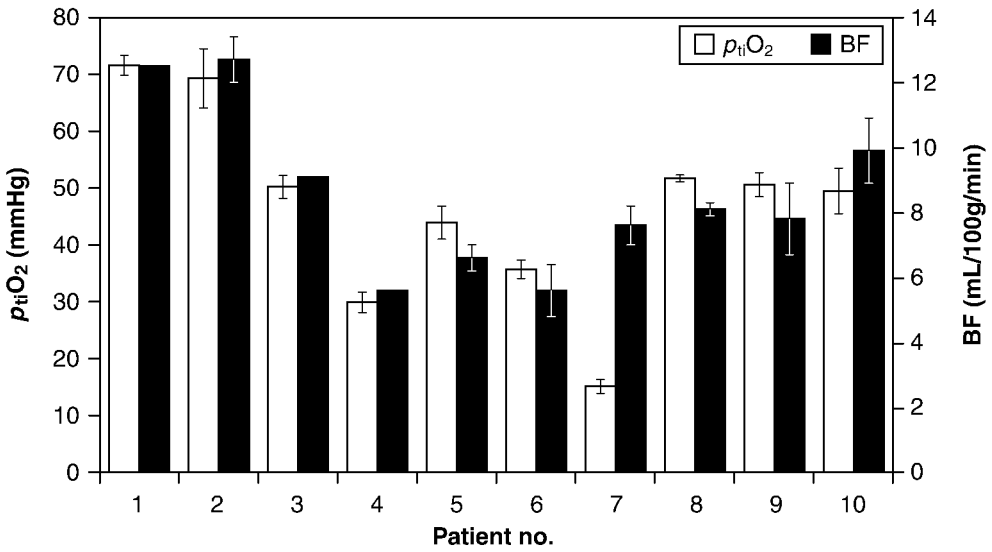

Figure 22. Licox® $p_{ti}O_2$ measurement (*empty box*) and absolute blood perfusion values (BF) assessed using PET (*filled box*), HN free flaps. Patient 7 had an ALT-flap that had rather weak perforators and muscular bulk. Although the early postoperative period was uneventful, the cutaneous part of the flap was lost while an ongoing secondary healing was detected 1 month postoperatively; the muscular bulk of the flap remained vital. The suboptimal $p_{ti}O_2$ measurement values represent the cutaneous part of the flap, while BF values are considered as normal and represent the muscular part of the flap. Data presented as mean±SD of $p_{ti}O_2$- and BF- values.

In one patient, re-exploration was considered on the first PO day due to critically low $p_{ti}O_2$ values in the flap; the flap skin was livid, and the re-capillarization and pin-prick test were compatible with compromised flap perfusion. However, BF_{PET} was considered sufficient, a second re-operation was avoided, and the flap survived uneventfully. (Figure 23)

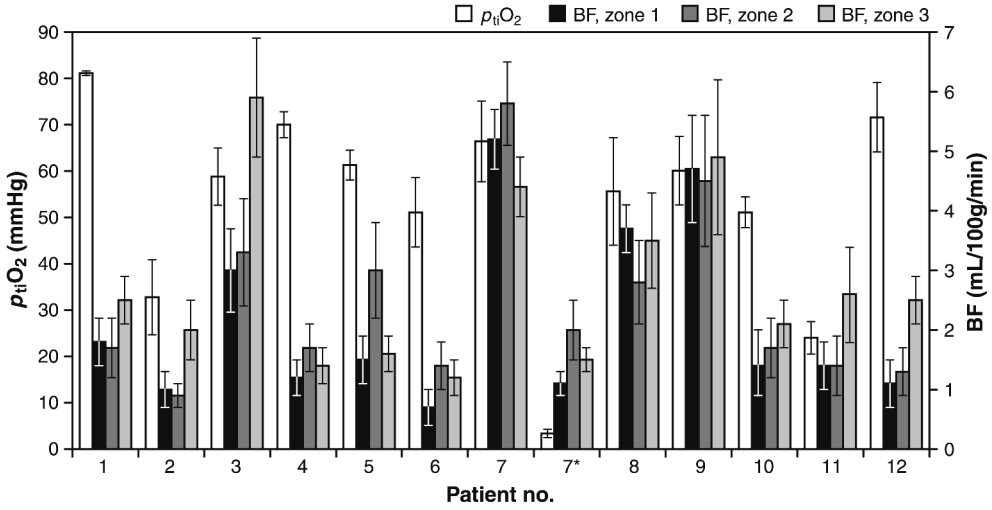

Figure 23. Absolute blood flow assessed with PET, zones I-III, and Licox® $p_{ti}O_2$ measurement, zone I, from each patient during PET scanning. Patient no. 7 had a bilateral DIEP operation and a re-exploration of the immediate DIEP flap (7*) anastomoses was carried out since the $p_{ti}O_2$ values were low before PET scanning. The BF_{PET} values were normal; a further re-operation in the same patient was avoided since the PET scan was considered more reliable, although $p_{ti}O_2$ values were critically low (< 10 mmHg).

5.4. Blood pressure and $p_{ti}O_2$ in HN patients (V)

Ten consecutive patients underwent resection of HNSCC followed by microvascular reconstruction with a free microvascular flap. The $p_{ti}O_2$ of each flap was continuously monitored for a minimum of 3 (range 3-4) PO days with the Licox® system. Systemic blood pressure (BP) was measured continuously from an arterial line during the operation and during the first PO day at the ICU. The correlation between $p_{ti}O_2$ and BP was analyzed. (Figure 24)

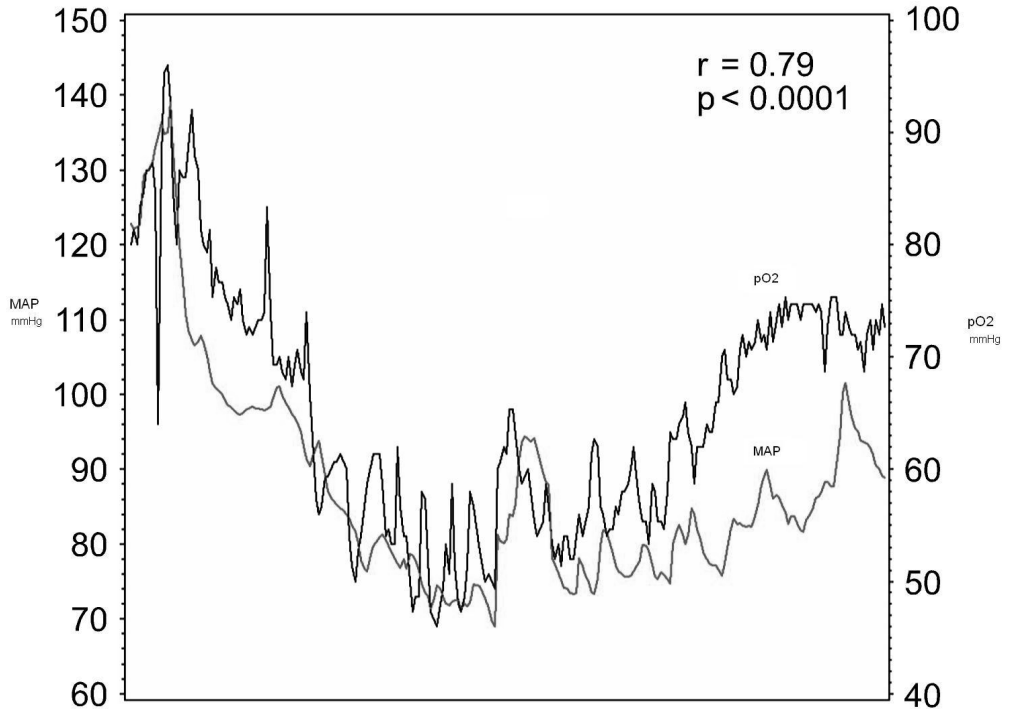

Figure 24. The correlation coefficient between BP and pO_2 in a free HN flap tissue; MAP = mean arterial blood pressure; pO_2 = flap tissue oxygenation in mmHg; r = Pearson product-moment correlation coefficient.

The correlation coefficient (r) between BP and $p_{ti}O_2$ of free flaps was varying from 0.08 to over 0.70.

In patients with suboptimal BP values i.e. MAP below 60 mmHg or with compromised flap BF, the correlation was low between MAP and $p_{ti}O_2$. $P_{ti}O_2$ was low in a flap with compromised cutaneous BF_{PET}, and the correlation with MAP was low ($r < 0.38$) during the early postoperative period. Another patient had a low correlation ($r = 0.08$) between BP and $p_{ti}O_2$ as the $p_{ti}O_2$ values declined in a free flap with a large cutaneous component exposed to room air temperature. The correlation between BP and $p_{ti}O_2$ of the flap improved significantly when the BF_{PET} of the flap recovered adequately.

5.5. Follow-up and survival

The median follow-up for breast reconstruction patients is currently 27.4 months (range from 13.9 to 35.7 mos) and 14.0 months (range from 1.0 to 65.3 mos) for HNSCC patients. A Kaplan-Meier curve of survival probability of the patients in the HN group is presented in Figure 24.

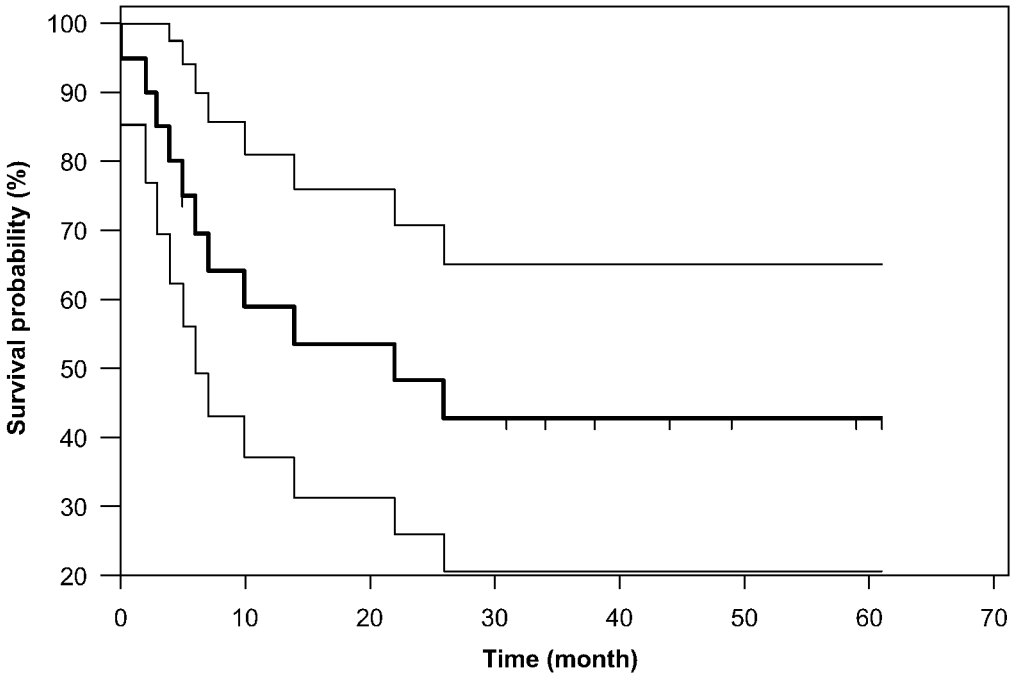

Figure 24. Survival probability of the HN cancer patients in studies I, II, and V. The 3 plots presenting mean ± 95% confidence limits.

All 14 flaps of 13 breast reconstruction patients survived, with three patients having either partial loss of the flap or fat necrosis in zone II of the flap (23 %). There were no total flap losses. The mean follow-up time of the patients is currently 14.4 months (range 6-21 months).

6. Discussion

The review of the literature on free flap monitoring reveals a plenitude of techniques available. The drawbacks and benefits of each method are known. Despite the numerous techniques that have been used, our knowledge of perioperative and postoperative changes in flap blood flow and perfusion is still inadequate. In this work, quantitative blood flow in free flaps was studied using PET and radioactive water and $p_{ti}O_2$ was followed continuously to examine tissue oxygenation by means of polarographic catheter probes inserted in the free flaps.

These two methods were chosen since they are feasible and were presumably suitable for the assessment of the present scientific questions. Moreover, there is wide experience in the Turku PET Centre of assessing BF_{PET} quantitatively in muscle and adipose tissue with PET using [^{15}O] H_2O. Of particular interest was to examine the correlation between absolute, quantitative, and momentary BF_{PET} with continuous, albeit superficial monitoring of $p_{ti}O_2$.

6.1. General discussion

Reconstructive surgery with free microvascular flaps requires close clinical PO follow-up if one is to recognize circulatory problems in time for re-exploration of the flap. Specially trained personnel are required to carry out these observations reliably. The principles of each different monitoring method have to be understood when comparing free flap monitoring results acquired with different methods.

Many technological developments of free flap monitoring have reached clinical applications, but no single method has gained wide acceptance. Various adjunctive techniques for postoperative monitoring are used. The wide variation in practice is probably due to several reasons, e.g., expensive equipment, different research interests, and the lack of a simple and reliable objective method of monitoring.

Various methods of monitoring have been developed and used in addition to conventional clinical follow-up. However, none of these methods has become widely used as a gold standard.

Although different methods to assess flap vitality are currently available, clinical observation with the assessment of skin color, capillary refill, and bleeding time characteristics is still important. However, if the free flap is buried during surgery, as in the reconstruction of upper esophagus with a circular radial forearm flap, direct observation is not possible and useful clinical monitoring methods are sparse.

Especially in head and neck tumor surgery the free microvascular flaps utilized are often buried under other tissue or are otherwise difficult to observe because of their

anatomical location. In buried flaps the loss rate was 6.5 % and for non-buried flaps 2.3 % in a series of 750 free microvascular flaps monitored with a handheld Doppler ultrasound probe (Disa et al. 1999). The hospital stay of patients undergoing reconstructive microsurgery nearly doubles if there are complications (Singh et al. 1999). These results and clinical experience suggest that there is a need for a more reliable monitoring method especially for flaps that are difficult to observe clinically.

The risk of thromboembolic events in cancer patients is increased during the first 3 postoperative days due to reconstructive surgery, the malignancy itself, previous surgical interventions, trauma, chemo- or radiotherapy (Siemionow et al. 1998, Olsson et al. 2001). Thus, monitoring of a free flap should continue for at least that length of time. Most problems of flap BF_{PET} occur and should be noted during surgery. Even the most experienced surgeons are faced with redoing anastomoses without any obvious cause for thrombosis of the flap vessels. Without monitoring, the possibility to save the flap is diminished if circulatory problems arise within the free flap.

Although the overall flap-failure rates of breast reconstructions are low, minor complications occur rather frequently. These complications require significant resources and flap loss could be prevented by early recognition. Despite wide attention in the literature, the hemodynamics of a free flap is still an issue that is not completely understood in the clinical setting (Snyder et al. 1975, Dinner et al. 1983, Lehtiö et al. 2001, Kalliokoski et al. 2003, Holm et al. 2006).

Although there were no true buried flaps in this study, the anatomical location of the flaps in this study was often difficult (e.g., retromolar and hypopharyngeal flaps in studies I, II, and V) which hampered direct and reliable clinical inspection.

6.1.1. Ethical considerations

The patients in this study were operated on for cancer or for the reconstruction after a previous cancer operation. 7 out of 13 breast cancer patients had received radiotherapy after primary surgery. 16 out of 17 HN cancer patients had received radiotherapy of which 3 patients received chemotherapy

Ionizing radiation may damage cellular DNA and lead to mutations, possibly resulting in carcinogenesis or disorders in germ cells. Because there is no safe amount of radiation that can be given to an individual, the risks and benefits must always be carefully considered in radiotherapy and diagnostic nuclear medicine. To ease the comprehension of the radioactive dose and to help the comparison between different diagnostic measurements, an equivalent dose to a biological tissue may be estimated in milliSieverts (mSv). The average dose received in one chest x-ray is approximately 0.03 mSv. This dose is quite small when compared to the large scaled computerized tomographic (CT) scans where the dose is 6-15 mSv (whole body CT). In the PET studies the dose was below 3 mSv, which provides a safe margin to the allowed maximum dose (10 mSv) in one PET study in Turku PET Centre. For additional

comparison, a radioactive dose is often related to the natural background radiation received each year in the Nordic countries (4-5 mSv). Thus, the radiation doses in our studies were equivalent to approximately less than one year's background radiation in Finland.

The International Commission on Radiation Protection has provided estimations about the impact of radiation on the incidence of cancer. If a person is exposed to 1,000 mSv of ionizing radiation during a long period of time, his individual risk of cancer increases approximately 5 %-units. Although in PET studies the dose is given during a relatively short period of time and the relation between the dose and the risk for cancer does probably not correlate linearly, it was estimated roughly for the subjects that a 10 mSv dose would increase the risk for cancer by approximately 0.05 %-units. Since cancer is quite common in the population, the effect of the radioactive doses received in nuclear medicine on the total appearance of cancer is probably low. All patients included in the study had the opportunity to review this information before consenting.

6.2. Methodological aspects

Blood perfusion of the flap assessed with PET and radioactive water yields a quantitative measure of blood flow from the entire region of interest and this usually covers the entire flap. When comparing different techniques of BF measurement, the results should be interpreted with caution. The depth of the tissue is one of the critical factors in the evaluation of reliable results. As an example, laser Doppler flowmetry is reported to be capable of collecting reliable data from as deep as several millimeters depending on the optical and anatomical properties of the site concerned. The actual depth measured is dependent on the probe and on the source wavelength. On the other hand, it is difficult to determine whether the measured perfusion relates to nutritional, thermoregulatory or both types of flow (Leahy et al. 1999). In NIRS the penetration depth depends on the size of the probe. The mean depth of measurement of the NIRS probe is approximately half of the distance between the tips of the emitting probes and the receiving optical fiber. With these superficial monitoring methods any dislocation of the probe position in relation to the underlying area influences the measurement outcome considerably (Stranc et al. 1998, Scheufler et al. 2004). Blood flow monitoring with LDF and NIRS at the current stage of development can only yield reliable data on the relative changes within an individual subject (Kuebler 2008). Although $p_{ti}O_2$ measurements provide absolute tissue oxygenation of the respective region, the region is limited to less than approximately 30 mm^3.

6.3. Tissue oxygen measurement ($p_{ti}O_2$) (II-V)

$P_{ti}O_2$ depends on the oxygen uptake of tissue cells and the transport rate in the microcirculation. The $p_{ti}O_2$ in muscle is 25-35 mmHg, in dermal connective tissue 40-

80 mmHg, and in brain tissue around 25 mmHg (Licox® Medical Systems and Neurosciences. 2002, Raittinen et al. 2005). High readings immediately after surgery are due to probe calibration and post-occlusive hyperemia by ischemia, and local microtrauma which damages the mitochondria of the cells and results in higher extracellular O_2 concentrations (Kamolz et al. 2002, Raittinen et al. 2005). Within a few PO hours, $p_{ti}O_2$ stabilizes and reaches a steady level. This phenomenon was also observed in this series in flaps with no circulatory problems (II-IV). Although the $p_{ti}O_2$ measurement results vary to some extent individually, the trend seems to be the same in all cases. Similar findings have been reported by Kamolz (Kamolz et al. 2002) and Raittinen (Raittinen et al. 2005). A sudden drop, or an obvious change in the level of the $p_{ti}O_2$ curve, reliably predicts circulation problems of the flap. PO anxiety and nursing procedures e.g. turning or suctioning the patient, can cause fluctuations in the $p_{ti}O_2$ curve (III). The personnel in charge of the monitoring have to be aware of the pitfalls and limitations of the method to permit reliable monitoring of the flap.

6.4. Evaluation of microvascular free flaps with [^{15}O]H_2O PET (I-II, IV)

Noninvasive manipulation of the transplanted tissue is essential in perfusion studies. The PET method does not involve the innervation of the tissue of interest. Manipulation and innervation both are known to affect perfusion HG.

Quantification of BF_{PET} presupposed arterial blood sampling in this study. In recent studies it appears that a model-based non-invasive estimation of AIF from the dynamic [^{15}O] H_2O PET images (Kudomi et al. 2008) is accurate and that an arterial line is no longer necessary. On the other hand, the patients in these studies typically had perioperatively pre-inserted arterial lines which were readily available for radioactive measurement using PET (II-IV) and no additional intervention was thus needed for arterial sampling.

In the pilot study (I) the results suggested that the ratio of BF_{PET} in the free flap to the BF_{PET} in the adjacent or contralateral e.g. muscle tissue might have predictive value with regard to flap vitality during the critical 3 days after surgery. It was evaluated that PET could be the method of choice to assess the perfusion of the free flap. Despite other monitoring methods (laser Doppler flowmetry, tissue oxygen measurement, microdialysis, clinical assessment) PET could provide additional information by quantifying the BF_{PET} of the entire flap before a decision to reoperate is made (II-IV).

There was a tendency for flap BF_{PET} to slightly decrease over the 3 first PO days. This could be explained by several reasons, e.g., the inflammatory reaction in the denervated flap, resulting in local vasodilatation in the early PO phase (lasting up to 24 h). Post-occlusive hyperemia could also explain to some extent the marked increase in the immediate PO BF_{PET} of the flap as we observed in studies I and II. After this period, a new balance between the flap and the recipient site begins with gradual reduction of all vascular and tissue modifications (within 4 weeks). The free flap

perfusion has been reported to increase between 2 weeks to three months as reported by Salmi, which could be due to the neoangiogenesis in the free flap (Salmi et al. 1995a). Daily measurements of blood perfusion with PET would probably have been informative in the same patient, but the increasing radiation dose, the cost, and the availability of the PET studies limit the feasibility of such repetition

PET assesses regional absolute blood flow in living tissue. Blood flow can be assessed quantitatively with radioactive water, which is an inert tracer. Clearly, the costs of PET technology are high, but still defendable, as is the case for the PET-FDG method which is increasingly and routinely used in clinical practice in major clinics, especially in oncology. There is an understandable demand for evidence of a positive effect on patient management and outcome once new diagnostic tests are introduced. The availability of PET facilities is growing and the results of this study might facilitate a cost-effective positioning of PET in routine patient care. In addition to PET-FDG there could be a place for [^{15}O] H_2O PET method for quantification of BF_{PET} when clinically needed.

The more deep-seated buried flaps are used in reconstructive surgery, the more the need for reliable monitoring techniques exists. In some cases, conventional flap monitoring methods give controversial results. When tissue oxygenation measurements suggest circulatory impairment, and the clinical evaluation is difficult due to the location of the flap, a non-invasive evaluation of the flap is needed. A PET scan is a feasible method of assessing the quantitative blood flow of the microvascular flap. A PET scan with a simultaneous CT scan (PET-CT) is an even more accurate way of obtaining reliable objective data on the perfusion of the newly reconstructed free flap with more precise anatomical landmarks in addition to functional status.

The findings in these studies indicate that flap blood perfusion measured with PET correlates well with the oxygenation status of the flap. When circulation in the flap becomes impaired and other monitoring methods are unavailable or the results are contradictory, a [^{15}O] H_2O PET method will give further valuable and reliable information on the amount of BF_{PET} to the free flap.

6.5. Monitoring of microvascular free flaps with $p_{ti}O_2$ (II-V)

Although tissue oxygen tension measurement appears to be a sensitive and reliable method for free microvascular flap monitoring (Wechselberger et al. 1997, Kamolz et al. 2002, Raittinen et al. 2005), there are methodological limitations that need to be considered. Wechselberger reported in a series of 17 different free microvascular flaps one “false” alarm in a buried flap, as the probe was misplaced. A larger series of 60 free flap patients monitored with the Licox® system was published by Kamolz (Kamolz et al. 2002). They detected all patients who had circulatory failure with no false positives or negatives. Of these 60 patients, 6 were head and neck cancer patients operated on and reconstructed with a free microvascular flap. Raittinen (Raittinen et

al. 2005) identified all hemodynamic failures necessitating revision (n=6) with the Licox® system in a series of 37 head and neck free microvascular flap reconstructions. The overall flap survival rate was 97.2 %. Three false positive alarms were given by the Licox® system and no false negative results. According to the studies in this thesis, all PO flap perfusion problems in HN patients were detected using the Licox® monitoring system with no false negative results. In breast reconstructions the heterogeneity of the BF_{PET} in the flap from the lower abdomen (MS2- or MS3-TRAM) flap and the vulnerability of the flap to cooling limit the reliability of the $p_{ti}O_2$ monitoring.

High readings immediately after probe insertion are due to probe calibration and post-occlusive hyperemia (Kamolz et al. 2002). According to the literature the $p_{ti}O_2$ values stabilize and reach a steady level within a few hours. This phenomenon was also observed in this study but already within a few minutes. Thereafter the readings tended to slightly decrease during the three-day monitoring period. These findings are similar to those reported by Kamolz (Kamolz et al. 2002) and Raittinen (Raittinen et al. 2005). An obvious change in the level of or a sudden drop in the $p_{ti}O_2$ curve predicts reliably circulation problems.

On the other hand, in breast reconstruction flaps, the $p_{ti}O_2$ results are more susceptible to even misleading results due to the greater temperature variation of the flap exposed to room air temperature. Thus, frequent flap temperature measurement, or combined tissue oxygen and tissue temperature probe insertion is recommended for acquisition of more accurate data. Also the measuring depth of the $p_{ti}O_2$ probe is limited due to the need of fixation of the probe near the surface of the flap to achieve reliable results (Figure 15).

Although the $p_{ti}O_2$ curve acquired by the Licox® system is easy to read, there are series of events that can cause false alarms. Postoperative nursing procedures, e.g. turning or suctioning the patient, cause fluctuations in the $p_{ti}O_2$ curve, as does patient anxiety. Mild sedation, correction of the patient's position and administration of additional oxygen improve tissue oxygenation. In case of a real circulatory problem, the $p_{ti}O_2$ readings decline, and these procedures are of no avail.

According to study V it is of utmost importance to keep the patient's BP within certain limits (e.g. in our hospital MAP should stay above 60 mmHg) to optimize the perfusion in a re-anastomosed tissue transfer to assess reliable results from the tissue oxygen measurement and thus to increase the flap success rate.

The correlation between BP and $p_{ti}O_2$ was high ($r > 0.70$, $p < 0.001$) in patients with uneventful flap survival. However, the correlation was poor ($r < 0.10$) in patients with suboptimal BP values (i.e. MAP < 60 mmHg) or with compromised flap BF. $P_{ti}O_2$ was low in a flap with compromised cutaneous BF, and the correlation with systemic BP was low ($r < 0.38$) during the early postoperative period. Another patient had also a poor correlation ($r = 0.08$) between BP and $p_{ti}O_2$ as the $p_{ti}O_2$ values declined in a free flap with a large cutaneous component exposed to room air temperature. The

correlation was low in flaps with compromised cutaneous BF. The correlation between BP and $p_{ti}O_2$ of the flap improved significantly when the BF of the flap recovered adequately. (V)

6.6. Postoperative evaluation of flap blood flow with [^{15}O] H_2O PET and $p_{ti}O_2$ (II-IV)

The different nature and limitations of the methods that measure BF to the flap have to be considered when analyzing the results of continuous tissue oxygenation measurement and the perfusion of the free flaps assessed with PET.

There were no statistically significant differences in BF_{PET} and $p_{ti}O_2$ measurement between the breast recostruction flaps DIEP and TRAM. Due to the study protocol (explorations were carried out according to the $p_{ti}O_2$ values) there was a statistically significant difference between low tissue-oxygen values and re-explorations. The specificity of the $p_{ti}O_2$ method in this study was 92.3 %; one false positive result caused one unnecessary exploration but one unnecessary re-exploration was avoided on the basis of the BF_{PET} results. Whether the unnecessary re-operation could have been avoided by the information acquired from a PET scan remains unclear, because the patient was operated on based only on the $p_{ti}O_2$ measurement results and no [^{15}O] H_2O PET was available. One has to remember that although an 'extra-eye for the night' is available by the novel monitoring methods, the $p_{ti}O_2$ measurement provides data from only a limited area of of approximately 7 mm^2 around the 5 mm $p_{ti}O_2$ sensitive area. The false positive result in the $p_{ti}O_2$ measurement of patient 7 could have been the consequence of the operative trauma to the main perforator causing a hypoxic zone at the $p_{ti}O_2$ measuring area (Table 3).

The $p_{ti}O_2$ alarm levels in different types of flaps should probably be re-evaluated. The $p_{ti}O_2$ studies with the greatest number of patients have usually been performed in the head and neck region where the local temperature of the free flap is maintained closer to the body temperature without further maneuvres, particularly when the flap is intraoral. The different locations and types of flaps should be considered when the alarm levels of $p_{ti}O_2$ measurements are evaluated. Automatically temperature-adjusted (e.g. Licox® Revoxode CC1.1P) probes should be preferred in flaps with large skin areas to avoid false alarms when using the $p_{ti}O_2$ system.

Monitoring of the flaps exposed to the room air temperature, e.g., breast reconstructions and flaps of the extremities, is probably susceptible to bias towards false positive results with the superficial methods (Bassingtwaighte et al. 1989, Ohjimi et al. 2005). The monitoring carried out in these areas with the superficial methods could lead to unreliable results in terms of the vitality of the whole flap.

When monitoring flaps exposed to room air temperature using $p_{ti}O_2$ measurement, an additional method for monitoring the deeper parts of the flap should be available. The

use of a secondary method to assess the vitality of the newly reconstructed flap should be considered. The expenses of such a method should also be evaluated, as should the radiation dose to the patient. If repeat studies are carried out, more data are accessed, although at the cost of an increasing dosage to the patient (Takeishi et al. 2008). Clinical prudence requires, on the other hand, that patients at higher risk of PO complications should be more closely monitored.

The clinical status of the flap (color, turgor, pin prick and capillary refill) made by an experienced microsurgeon is the basis of a decision to re-operate or not. However, when conventional monitoring methods, for example, $p_{ti}O_2$ measurements give controversial results or are unavailable, a secondary method for a more reliable assessment of the perfusion and thus the vitality of the free flap is needed.

According to the studies in this thesis, the measurement of BF_{PET} with [^{15}O] H_2O PET method is of great help in the evaluation of the perfusion of microvascular transplants, in cases when the clinical examination is not reliable due to the location of the flap, or the O_2 probes have been removed or are not working properly. The use of a temperature-adjusting polarographic probe (Licox® Revoxode CC1.1P) has been taken into practice by the plastic surgeons in our hospital and results have become more reliable. The alarm levels have also been re-evaluated resulting in fewer false alarms.

The functional imaging capability of PET and, in particular, the BF_{PET} studies using radiolabeled water appear promising and could be considered a feasible secondary method to ensure the vitality of the flap, especially for carefully selected patients with increased PO risk of flap complications.

In three BC patients who developed fat necrosis (nos 1, 3, and 5, Table 3) during follow-up, the $p_{ti}O_2$ values were normal during the early postoperative phase as well as the BF_{PET} values assessed with the [^{15}O] H_2O PET technique were normal in all zones on the morning after the operation. The average ATBF was slightly lower in zone I than in zones II and III, which somewhat contrasts with previous results (Ohjimi et al. 2005, Holm et al. 2006, Holm et al. 2007). The explanation for this controversy could be different monitoring methods used in these previous publications measuring cutaneous perfusion and not ATBF (Hallock and Rice 2004, Holm et al. 2006). Furthermore, the heterogeneity was greater in zones I and II in comparison to zone III, which supports the assumption that monitoring blood flow from the more superficial parts of the flap might not present the perfusion of the whole flap. The perfusion of the cutaneous part of the free flap indirectly evaluated by the $p_{ti}O_2$ measurement from zone I is supplied mainly by the subdermal vascular plexus, while the perfusion of the deeper adipose tissue evaluated by PET scanning is supplied by the fascial vascular plexus immediately superficial to Scarpa´s fascia. On the other hand, parts of the deep layer of adipose tissue are often included in the free flap, but no differences in the formation of fat necrosis has been detected between the incidence of fat necrosis beneath the Scarpa´s fascia. Excessive thinning of the flap may disrupt the blood supply, leading to ischemia, necrosis, and flap loss (Boyd et al. 1984, Moon and Taylor 1988). The lower mean ATBF in zone I could be explained by the exceptionally high HG in that same area.

The area to be monitored should probably be the area in the medial part of the flap, which is most susceptible area to flap ischemia. On the other hand, in zone I the cutaneous blood flow is probably the strongest, while the blood flow in the adipose tissue does not necessarily follow the same pattern. The blood flow results in our study assessed with PET using [^{15}O] H_2O suggest that zone I has lower overall mean perfusion in the adipose tissue in comparison to zones II and III. This stands in contradiction to some previous literature reports (Holm et al. 2006). All in vivo studies concerning blood flow have studied relative blood flow of the cutaneous or subcutaneous parts of the flap supplied by the subdermal plexus and only a portion of the fatty layer of the flap is fed in a vertical direction from vessels derived from the subdermal and fascial vascular networks. For this reason, the adipose layer is probably the most peripheral site of blood circulation in the TRAM flap (Takeishi et al. 2008).

6.7. Perfusion heterogeneity in breast reconstruction free flaps (IV)

The blood flow heterogeneity (BF_{PET} HG) was evaluated in study IV with breast reconstructions. The BF_{PET} HG in the free flap elevated from the lower abdomen was determined in zones I-III of the breast reconstruction flaps (Figure 3). According to previous studies the adipose tissue BF_{PET} in breast reconstructions is reported heterogeneous (Virtanen et al. 2002, Levitt 2007).

There are not many publications on postoperative perfusion HG of microvascular breast flaps in vivo, but a few studies on perfusion HG of pedicled breast reconstructions available (Yamaguchi et al. 2004, Holm et al. 2006). The different characteristic patterns of regulation of blood flow hemodynamics in pedicled flaps and in free flaps have to be distinguished from each other. Blood flow studies between different zones of the breast flap have been made with pedicled flaps. In vivo and ex vivo studies have to be considered in the evaluation of the results.

The viability of unipedicled flaps could be predicted by evaluation of blood supply with indocyanine green dye fluorescence videoangiography (Yamaguchi et al. 2004, Holm et al. 2006). Yamaguchi confirmed an individual “perfusion map”, where zone II of the breast flap was sometimes not as well perfused as zone III. The results achieved in study IV were similar to the study by Yamaguchi, although not directly comparable because Yamaguchi used pedicled TRAM flaps and in study IV free flaps were used (Yamaguchi et al. 2004). Similarly, Kim studied the incidence of fat necrosis in zone II of pedicled TRAM flaps and found that the incidence is significantly higher in zone II than in zone III. The incidence of fat necrosis was positively associated with the relative amount of zone II tissue included in the flap and the total weight of the mastectomy specimen (Kim et al. 2007). These results suggest that perfusion is weaker in zone II than zone III. There was no significant positive correlation between the weight of the flap used for reconstruction and the incidence of fat necrosis in study IV, although the number of patients was small.

[^{15}O] H_2O PET permits noninvasive, direct and regional in vivo assessment of perfusion without interference from other tissues. The method has been validated and it is widely used for assessment of BF_{PET} in skeleletal muscle (Ruotsalainen et al. 1997), and adipose tissue (Virtanen et al. 2001). The lower water content of adipose tissue (15 %) compared with muscle (79 %) has to be taken into account to avoid underestimating the blood perfusion in the adipose tissue when the method is applied to adipose tissue (Snyder et al. 1975).

The mean BF_{PET} in zone III appeared higher compared to zone II, which finding is in line with previous studies by Yamaguchi, Holm, and Kim (Yamaguchi et al. 2004)(Holm et al. 2006)(Kim et al. 2007), who reported similar results concerning zones II and III. In a study by Hallock (Hallock 2001) of 13 free TRAM flaps the mean BF assessed with laser Doppler flowmetry was significantly reduced in an orderly fashion: the flow in zone I was highest, then zone II, zone III, and finally zone IV. The relative values acquired with a laser Doppler flowmeter vary unless data is recorded exactly at the same measuring point; the comparison of different areas is not reliable. Ardilouze did not detect any difference in the subcutaneous ATBF between the right and the left sides at either level using the ^{133}Xe washout method. They found that subcutaneous ATBF of the anterior abdominal wall was greater at the upper level than the lower level. (Ardilouze et al. 2004)

The BF_{PET} measurements in study IV concerning the adipose tissue of the TRAM flap were not different from DIEP flaps depending on zones. The mean BF_{PET} values were not statistically different between DIEP and TRAM flaps. The BF_{PET} values in TRAM flaps appeared somewhat lower than those with DIEP flaps and comparable with those previously measured in human subjects (Summers et al. 1996, Virtanen et al. 2001).

The significance of the muscle included in the TRAM flap with regard to the hemodynamic characteristics has to be considered. The microsurgeon elevating the flap chooses usually perioperatively whether to use, e.g., a DIEP or a TRAM flap according to the clinical appearance of the pedicle. Therefore, in non-blinded non-randomized studies the results could be biased in favor of the DIEP flaps when comparing different types of flaps chosen by the status of the vasculature of the flap during elevation, due to the fact that the dominant perforator is available and probably strong in comparison to the TRAM flap which has the pedicle elevated with muscle.

The wider use of the perforator flaps in breast reconstruction has brought into focus the significance of preoperative planning and identification of the most suitable perforators. In study IV there were no significant differences regarding BF_{PET} between the different flaps by number of perforators. The number of perforators in the free perforator flap is probably not as important as the quality, the size and pulsation of the perforating artery or the size of the perforator vein. In addition to the Doppler ultrasound scanning of the perforators, modern multidetector CT scanners are used for preoperative planning; they provide valuable and more detailed information of the location, anatomic relationships, course, and caliber of the perforators (Masia et al. 2008).

This study gives additional information on the varying regional blood flow of different zones of free breast reconstruction flaps. The results aid in the selection of a proper monitoring method and for applying probes of various monitoring methods. Further study is needed to evaluate a possible predictive value of the measurement of BF_{PET} acquired from each zone in the detection of ischemia or fat necrosis development. Study IV supports the revisited division of zones II and III of the lower abdominal flap as suggested by Dinner (Dinner et al. 1983) and again by Holm (Holm et al. 2006).

6.8. Study limitations and a cost-benefit evaluation

It has been argued that current methods for the in vivo assessment of tissue blood flow in humans, such as perfusion magnetic resonance imaging or PET, are usually not easily available for physiological or diagnostic purposes, which require more flexible, accessible, and rapid monitoring techniques (Kuebler 2008).

Currently, the spatial resolution of PET scanners in clinical use is no more than 5 mm. High resolution has been obtained at the expense of high patient radiation doses and high cost. Still, this level of resolution may not suffice and it could be a limiting factor for assessing the BF_{PET} of thin fasciocutaneous free flaps. Therefore, the utility of the [^{15}O] H_2O PET method in evaluating the success rate of, e.g., fasciocutaneous free flaps needs to be investigated in a larger study and preferably with a PET-CT-scanner to acquire concomitantly more detailed anatomical structures. Slightly radioactive ^{22}Na-buttons on the flap skin surface allow more reliable identification of the flap surface.

If patient moves during PET scanning, this may be a potential confounding factor especially in the imaging of head and neck, but also of breast area. Although the PET scan using radiowater is quite fast, positioning of the head and movement of the patient have to be frequently controlled during scanning. The patient has to be informed about the progress and the duration of the study. Patient motion during dynamic PET studies is a well-documented source of errors. Motion correction methods are available and especially required in dynamic cardiac studies during physical exercise. At rest, patient motion has been found in 18 % of the frames, but during pharmacological stress the fraction increased to 45 % and during physical exercise to 80 % (Naum et al. 2005). Motion correction methods were not used in the present PET studies because all patients were scanned at rest and stayed still during the PET procedure. Patient-movement was continuously followed through a radiation protective glass, and furthermore, patients were frequently informed about the proceeding of the PET scan, and informed to stay stationary during the scan.

Monitoring the $p_{ti}O_2$ of the free flap with a polarographic catheter limits the monitored area to the surface tissues of the flap. The breast reconstruction free flap from the lower abdomen is usually at least couple of centimeters thick, and therefore the deeper adipose

parts of the free flap extend beyond the monitoring range of the probe's oxygen sensitive area of 5 mm in length and of approximately 7 mm^2 area, resulting in approximately 10 to 30 mm^3 $p_{ti}O_2$ measurement volume depending on the movement of the probe. The Licox measurement system limits somewhat the free movement of the patient, although the recovery of the patient from the treatment in the early postoperative days, confine the patient's mobility and exercising capability even more. However, there is no limit of normal movement of the patient due to the probe as long as the probe is not pulled out. Moreover, the probe can be unplugged from the central unit and fixed with, e.g., an adhesive tape to the patient if desired. Furthermore, the smartcard calibrates the probe immediately when docked back to the central unit.

The overall cost of surgical treatment of a patient with e.g. advanced HNSCC consists of the surgical operation, the ICU- and the general ward days (€15,500 in our unit). Revision surgery including an extra ICU-day increases the total costs by approximately 27 % (€4,200). Often the need for revision surgery arises during out-of-office hours, which increases costs even more. The direct and indirect costs of late revision surgery due to a partial or total flap loss are substantial. A [^{15}O] H_2O PET scan increases the total expenses by 6.4 % (€991). However, if revision surgery could be avoided with one [^{15}O] H_2O PET study, the cost-benefit of the PET scan becomes much more favorable. In a study by Singh, the median hospital stay for microvascular reconstructions of head and neck defects was 16 days, but the development of complications increased the median hospital stay by 7.5 days (Singh et al. 1999).

In addition to the investment costs of a monitoring device, the disposable single-use materials required in each monitoring method, e.g., single-use probes, attachment pads, and insertion needles, have to be considered when evaluating and comparing the costs of the different monitoring methods. For instance, the Licox polarographic probe with a probe-specific smart card is disposable with the cost of approximately €354 per unit. Also the inserting needle (VK2) is disposable with the cost of €18 per unit at present. The need for specialized personnel needs also to be considered. For example, Doppler ultrasound is widely used and reportedly relatively inexpensive, but the cost of an experienced radiologist is not usually included in cost calculations.

The number of patients is small in this study, but the number of observations is large in, e.g., each $p_{ti}O_2$ monitoring period as well as the results of the PET perfusion scans were controlled in various parts of the free flaps to ensure reliable results. Although the [^{15}O] H_2O PET method has not been used in free flap monitoring previously, the wide experience in muscle and adipose tissue perfusion studies have shown the reliability of PET perfusion scan results. The minimum number of patients required is also ethically defendable due to the radiation dose administered to the patients. With modern PET/CT scanners further research of this method is recommended.

6.9. Future applications

The $p_{ti}O_2$ method makes it possible to study the buried flaps, but reliable insertion and fixation of the probe and especially removal of the probe in the early postoperative period are challenging precedures with buried flaps. Furthermore, the critical alarm levels concerning different flaps have to be more thoroughly investigated in future studies.

The PET method described in this thesis may be further simplified since an arterial line is no longer necessary to obtain absolute quantitative BF_{PET} data. The development of PET scanners has given the possibility to obtain reliable quantitative BF_{PET} data without blood sampling during the study (Kudomi et al. 2008). Thus, the reality of getting a quantitative blood flow study of a patient with a newly reconstructed free flap with a PET scanner during out-of-office hours is closer to reality in centers with modern technology, especially if an on-line batch production of radiowater is available (Ruotsalainen et al. 1997, Sipilä et al. 2001). In the future, this method may allow studies of even buried flaps together with CT, which would provide more detailed data of the postoperative anatomy.

PET-CT scanners are becoming more easily available for diagnostic purposes. In the near future it could be possible to do a brief PET scan with radiowater to assess reliable, quantitative BF_{PET} data of the entire flap, if there is a suspicion of compromised blood flow in the flap. This could be reality in PET centers with an on-line batch production of radiowater (Sipilä et al. 2001). Commercial cyclotrons with radiowater production capability are entering the market. Thus, in the near future, the [^{15}O] H_2O PET method could be available for most patients undergoing microsurgery. The [^{15}O] H_2O PET method could be considered as a feasible secondary method for verification of the vitality of the flap in selected high-risk patients, before a decision to reoperate is taken.

7. Conclusions

In the present work, blood perfusion (BF_{PET}) in free flaps was monitored for the first time in the literature with the [^{15}O] H_2O PET method. Moreover, the flaps were continuously monitored with tissue oxygen partial pressure measurements ($p_{ti}O_2$).
The following conclusions can be drawn from studies I-V:

I The purpose of this pilot study was to investigate the feasibility of PET using [^{15}O] H_2O in the evaluation of BF_{PET} in microvascular reconstructions following operation for head and neck cancer. All free flaps could be clearly identified in the primary perfusion images the first postoperative day as well as in the secondary PET images during follow-up. A higher flap-to-adjacent muscle tissue BF_{PET} ratio appeared to correlate with flap survival. The [^{15}O] H_2O PET method is feasible for quantitative assessment of BF_{PET} of whole free flaps in patients operated on for oral HNSCC.

II The aim of this study was to evaluate two different methods for predicting ischemia in microvascular head and neck (HN) free flaps. The perfusion problems were detected with $p_{ti}O_2$ measurement and re-explorations succeeded without any flap losses. Blood flow of the HN flaps assessed with [^{15}O] H_2O PET appeared to correlate well with the $p_{ti}O_2$ results. This study suggests that $p_{ti}O_2$ measurement is a feasible method for continuous postoperative (PO) monitoring of HN free flaps. The perfusion of the whole flap can be assessed with PET, which may help assess flap vitality when clinical examination and evaluation by other means are unreliable or give controversial results.

III This study was undertaken to determine whether $p_{ti}O_2$ measurement with a Licox® catheter probe system is a feasible method for continuous postoperative monitoring of free flaps in breast reconstructed patients. The $p_{ti}O_2$ measurement results were compared with BF_{PET} assessed with [^{15}O] H_2O PET and the correlation was not as evident with breast reconstruction flaps as with HN free flaps. Nevertheless, the study suggests that when clinical examination and evaluation by other means is unreliable or gives controversial results, the perfusion of the whole flap may be assessed reliably with PET.

IV The purpose of this study was to measure the BF_{PET} in free flap breast reconstructions. The BF_{PET} and BF_{PET} heterogeneity from the adipose tissue of the flaps were measured separately in each zone on the first PO morning. The mean BF_{PET} of the adipose tissue (ATBF) values did not differ between DIEP and TRAM flaps. The overall ATBF values assessed with PET were the highest in the lateral part (zone III) of the DIEP flap, and were slightly higher in comparison to zone II and zone I. Fat necrosis developed in the medial part of the flap (zone II) in three patients, while early postoperative BF_{PET} was normal. There was no statistically significant correlation between the BF_{PET} assessed from the adipose tissue using [^{15}O] H_2O PET and the subsequent frequency of

fat necrosis. The division of the cutaneous perfusion of the lower abdomen flap zones previously described in the literature did not correlate with the adipose tissue blood perfusion results.

V This study investigated possible associations between the $p_{ti}O_2$ in flaps and systemic blood pressure (BP). A significant correlation with systemic BP and free flap $p_{ti}O_2$ was documented in non-compromised flaps, but the correlation was low in flaps with compromised cutaneous perfusion. The correlation between BP and $p_{ti}O_2$ of the flap improved significantly when the perfusion of the flap recovered adequately. This study suggests that although the Licox® catheter probe oxygenation measurement system is reported as a feasible method for continuous postoperative monitoring of free flaps in patients operated on for oral HNSCC, it is of utmost importance to keep the BP within certain limits to optimize the perfusion in a re-anastomosed tissue transfer. Postoperative pain and suboptimal BP need to be attended to for adequate perfusion to the flap.

8. Summary

The esthetic and functional reconstruction of a tissue defect after ablative surgery with free flaps is a challenge for the surgeon. Although the use of free flaps has become clinical routine, flap complications are often unpredictable. Thus, a continuous and reliable monitoring method of the function of the free flap is of utmost importance. Timely interventions for flap salvage are needed, if flap failure caused by vascular problems is to be minimized.

The aim of the present work was to investigate different postoperative methods to monitor the condition of free flaps. Based on previous experience and literature data the tissue oxygen tension ($p_{ti}O_2$) measurement system was scrutinized as a potential method for clinical monitoring. Thirty patients with 32 microvascular free flaps were evaluated by measurements of oxygen tension and blood perfusion using dynamic [^{15}O] H_2O PET.

According to the results of the studies of this thesis $p_{ti}O_2$ offers a reproducible and reliable way of measuring flap vitality in most cases. It is possible to measure reliably local tissue oxygenation of the free flaps located, e.g., in the oral cavity with the present method. However, oxygen tension can only be measured reliably if the probe stays exactly at the measuring point, thus fixed to the flap surface and accordingly, in free flaps exposed to room air temperature vulnerable to cooling, such as breast flaps, the method seems to be less reliable.

Blood flow evaluation with PET using radioactive water (the [^{15}O] H_2O PET method) is an elegant non-invasive method to measure absolute quantitative blood flow in regions of the whole free flap and surrounding tissue. The limitations of this method, e.g., thus far poor availability, costs, and radiation burden to the patient, have to be considered, and therefore this method is thus far an adjunctive method taken to when flap blood flow compromise is suspected if other monitoring methods, such as $p_{ti}O_2$ measurement, give controversial results or are not available.

The studies provide further data on the complex mechanisms of hemodynamic responses in free flaps, on the means of how to improve flap survival, and on the causes of free flap failures. In addition to presenting further information on $p_{ti}O_2$ measurement, a method for evaluating quantitative blood flow in microvascular free flaps with PET using [^{15}O] H_2O (the [^{15}O] H_2O PET method) is presented for the first time in the literature. The present method of flap BF_{PET} evaluation with [^{15}O] H_2O could be utilized in further studies and possibly in clinical studies, as well.

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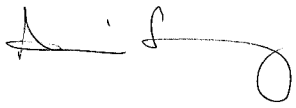

Aleksi Schrey

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