THE EFFECT OF GEL FORMULATED SILDENAFIL ON AXIALLY BASED RABBIT SKIN FLAP SURVIVAL – AN EXPERIMENTAL STUDY

Dr Koh Khai Luen

Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Surgery
(Plastic Surgery)

UNIVERSITI SAINS MALAYSIA

2018

ACKNOWLEDGEMENT

First of all, I would first to thank my supervisor, Assoc. Prof. Dr. Wan Azman Wan Sulaiman from Reconstructive Sciences Unit, Universiti Sains Malaysia for his relentless support and guidance during my research development that leads to successful completion of my thesis. He has encouraged me to become not only a plastic surgeon but a researcher and a well-spoken presenter at local and international conferences. This dissertation has won the Best Free Paper (Oral Presentation) in Malaysian Society of Plastic and Reconstructive Surgery, 3rd Annual Scientific Meeting at Putrajaya, Kuala Lumpur on November 2015 and International Travel Grant in the 9th Congress of World Society for Reconstructive Microsurgery at Seoul, Korea on June 2017.

Secondly, I would like to thank and congratulate my research partner, Mr Kho Siew Liang for his brilliant idea in the development of our thesis topic. At the same time, I would also like to thank the other investigators who were involved in this research project: Prof. Ahmad Sukari Halim, Dr. Yvonne Tee Get Bee and Assoc. Prof. Dr. Md Salzihan Md Salleh for their valuable opinion and guidance in various aspects of my study. Not to forget all the staffs in Animal House: Dr Noziah Binti Ghani, Dr Nur Izni Binti Mohd Zaharri, Mr. Koh Chun Haw, Mr. Zali and Mr Faizul in assisting us with the care of the animals during the study period. Without their passionate participation and input, the research project could not have been successfully conducted.

Finally, I must express my very profound gratitude and love to my wife, Ms. Tee Hui Peng, my parents and colleagues for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this dissertation. This accomplishment would not have been possible without them. Thank you.

TABLE OF CONTENTS **Page**

List of abbreviations

bFGF	Basic fibroblast growth factor
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
DCIA	Deep circumflex iliac artery
eNOS	Endothelial nitric oxide synthase
iNOS	Inducible nitric oxide synthase
LDF	Laser Doppler Flowmetry
LTA	Lateral thoracic artery
NO/cGMP	Nitric oxide/cyclic guanosine monophosphate
PDE	Phosphodiesterase
PU	Perfusion unit
SEPA	Superficial external pudendal artery
SIEA	Superficial inferior epigastric artery
SLS	Sodium lauryl sulfate
TA	Thoracodorsal artery
TRAM	Transverse rectus abdominis musculocutaneous
VEGF	Vascular Endothelial Growth Factor

List of tables **Page**

List of figures **Page**

Introduction

Study protocol

Manuscript

TITLE

THE EFFECT OF GEL FORMULATED SILDENAFIL ON AXIALLY BASED RABBIT SKIN FLAP SURVIVAL – AN EXPERIMENTAL STUDY

ABSTRACT

Introduction

Skin flap survival is one of the most important outcomes in reconstructive surgery. Various pharmacological agents have been studied previously with mixed results. Sildenafil has been shown to improved flap survival experimentally with variable dosing regimens and method of applications.

The objective of this study is to determine the effect of our formulation of sildenafil gel that will be applied topically on the epidermal surface of rabbit skin flap to augment flap survival.

Material and methods

Six New Zealand rabbits (weight range 2.5-3.2kgs, 3 Female and 3 Male) were divided into 2 groups; control group (n=3) and treatment group (n=3). A standard protocol of perioperative care, anaesthesia, antibiotics, dressings and feeding regimen were followed. A standard oversized rabbit skin flap (14X5cm) based on superficial inferior epigastric vessels was designed and raised. Subsequently, the flaps were rendered to two hours of ischemic time with vascular clamp at the pedicle, released and flaps were sutured back. An aliquot of 1ml gel consisted of sildenafil (3mg) was applied immediately after surgery to the distal half of the flap in treatment group while on 1 mg gel without sildenafil was applied to control group. Measurements with Laser Doppler Flowmetry (LDF) at both distal and central flap were performed in all rabbit pre-surgery, post-operative day 1, 3, 5, 7 and 10. Area of necrosis of the skin flaps were measured using 2-plane planimetry and photographed.

Result and discussion

Mean flap survival in the treatment group was 99.59%, nearing total flap survival as compared to the control group of 85.96% with significant improvement in flap survival of 13.35% as compared to control group. Early and late blood flow improvements were observed at both central and distal part of flap with significant increased seen at distal flap on postoperative day 1 (133.50 PU treatment group versus 30.63 PU control group, $p<0.05$) and on central flap at postoperative day 10 (152.17 PU treatment group versus 84.93 PU control group, $p<0.05$). We postulated that sildenafil improved skin flap perfusion via early vasodilatory effects (within 72 hours) and late angiogenic effects (after 72 hours) of skin flap surgery.

Conclusion

The present study has demonstrated that with single application of gel formulated sildenafil (3mg), there was a significant improvement in rabbit's skin flap survival via early and late increases of blood flow at the microcirculation.

Key words: 'topical sildenafil', 'skin flap survival', 'microcirculation blood flow'

TAJUK

KEBERKESANAN APLIKASI GEL FORMULASI TOPIKAL ‘SILDENAFIL’ UNTUK MENINGKATKAN KETAHANAN TRANSPLANTASI KULIT ARNAB SECARA EKSPERIMEN

ABSTRAK

Kejayaan prosedur transplantasi kulit merupakan ukuran penting di dalam bidang pembedahan plastik dan rekonstruktif. Terdapat pelbagai jenis ubat yang telah dikaji untuk meningkatkan tahap kejayaan prosedur tersebut tetapi menghasilkan keputusan yang tidak konsisten. *Sildenafil* telah terbukti secara eksperimen dapat meningkatkan kadar kejayaan prosedur transplantasi kulit menggunakan pelbagai dos ubat dan cara aplikasi yang berbeza-beza.

Objektif utama kajian ini adalah untuk menentukan keberkesaan formulasi *sildenafil* yang akan digunakan secara topikal ke atas transplantasi kulit arnab untuk meningkatkan ketahanan kulit transplantasi.

Metodologi

Enam ekor arnab *New Zealand* (berat di antara 2.5 kg dan 3.2 kg, 3 ekor jantan dan 3 ekor betina) dibahagikan secara rawak kepada dua kumpulan; kumpulan kawalan (n=3) dan kumpulan intervensi (n=3). Satu protokol standard meliputi pemakanan, bius, ubatan antibiotik, prosedur pembedahan dan penjagaan luka selepas pembedahan telah dipraktikan ke atas semua haiwan eksperimen. Satu rekabentuk *flap* kulit standard berdimensi 14 sm dan 5 sm yang bergantung kepada salur darah *superficial inferior epigastric vessels* telah dihasilkan pada semua haiwan eksperimen menggunakan kaedah pembedahan. Salur darah tersebut dikepilkan dengan *clamp* untuk menahan aliran darah masuk ke dalam flap kulit selama dua jam. Selepas itu, *clamp* dilepaskan untuk membenarkan aliran darah masuk ke *flap* kulit, diikuti dengan jahitan balik *flap* kulit kepada tempat asal. Satu milliliter (ml) formulasi sildenafil

(mengandungi dos 3 miligram (mg) *sildenafil)* telah diaplikasikan secara topikal pada sebahagian hujung flap kulit untuk kumpulan intervensi manakala gel dengan kuantiti sama (1 ml) tanpa *sildenafil* telah diaplikasikan pada kumpulan kawalan. Kertas grid standard (1 sm dan 1 sm) digunakan untuk membuat ukuran dimensi *flap* kulit dan *Laser Doppler Flowmetry (LD*F) telah digunakan untuk mengukur pergerakan aliran darah *flap* kulit pada hujung dan tengah-tengah flap. Semua ukuran dibuat sebelum pembedahan, sejurus selepas pembedahan, pada hari pertama, ketiga, kelima dan kesepuluh.

Keputusan dan diskusi

Purata kehidupan *flap* kulit di dalam kumpulan intervensi adalah 99.59 peratus berbanding 85.96 peratus di dalam kumpulan kawalan. Ini menunjukan terdapat peningkatan sebanyak 13.35 peratus dari segi kehidupan *flap* kulit. Di samping itu, terdapat penigkatan ketara aliran darah pada tengah-tengah dan hujung *flap* kulit pada hari pertama selepas pembedahan di dalam kumpulan intervensi berbanding kumpulan kawalan (133.50 PU kumpulan intervensi berbanding 30.63 PU kumpulan kawalan, $p<0.05$). Peningkatan aliran darah juga telah meningkat secara ketara pada hari kesepuluh di tengah-tengah *flap* kulit (152.17 PU kumpulan intervensi berbanding 84.93 PU kumpulan kawalan, $p<0.05$). Ini telah membuktikan bahawa formulasi sildenafil yang diaplikasikan secara topikal dapat meningkatakan tahap kejayaan *flap* kulit melalui dwi kesan iaitu; pengembangan salur darah pada peringkat awal dan kemungkinan penghasilan salur darah baru (*angiogenesis*) pada peringkat lewat (selepas 72 jam).

Kesimpulan

Kajian ini telah membuktikan bahawa aplikasi sekali formulasi 3 mg sildenafil secara topikal pada *flap* kulit arnab dalam eksperimen ini dapat meningkatkan kehidupan flap kulit melalui dwi kesan pada peringkat awal dan akhir pembedahan transplantasi.

INTRODUCTION

1. INTRODUCTION

1.1 Literature review

1.1.1 Background

Flap survival remains the most important outcome measure in any flap reconstructive surgery. Flap failure can result in total failure or partial failure secondary to inadequate flap perfusion. Failure of flap surgery will results in devastating complication to the patient with higher cost, prolong hospitalization and possibly more surgery with additional donor site morbidity. Recent refinements in microsurgery has ensure high success rate in free tissue transfer approaching 95 percent(1-3). However, most common clinical problem in flap surgery is partial necrosis rather than total flap failure. Satisfactory perfusion of bulky perforator flaps, random or pedicled flaps and functional muscle flaps remains a challenging problem for reconstructive surgeons(4).

Flap is defined as a unit of tissue comprises of its own intrinsic blood supply for survival(5). Flap can be transferred from donor to recipient site with its attached vascular supply (pedicled flap) or using microsurgical technique to anastomose its inherent blood supply to recipient bed (free flap). The intrinsic blood supply of a flap is the most critical determinant of successful transfer. Distal flap ischemia usually results from the inability to maintain critical capillary pressure to perfuse the most distal part of the flap where total flap failure is usually caused by global insufficiency of blood supply secondary to mechanical obstruction to the pedicle, or no reflow phenomenon(4). Partial or distal flap necrosis is unavoidable is certain clinical conditions despite refinements in microsurgery. It is attributed to the development of low flow state at the level of microcirculation caused by improper flap design, vasospasm, ischemic reperfusion injury, and systemic factors affecting microcirculation or physical compression of the flap.

Understanding the anatomic, hemodynamic and metabolic changes during flap transfer in a free or pedicled flap will determine the flap viability. Angiosome concept was first described by Taylor and Palmer in 1987 and has since become well accepted in the field of plastic and reconstructive surgery(5). It allows the conceptualization of the vascular supply to all tissues of the human body. An angiosome is a composite block of tissue supplied by a main source vessel. This concept indicates that the three-dimensional block of tissue is supplied by a major source of artery and acoompanying veins. The adjacent angiosomes are linked either by reduced calibre choke anastomotic vessels or vessels without reduction in calibre; the true (simple) anastomoses on the arterial side. The choke vessels can potentially dilate to the calibre of a true anastomosis after surgical delay or pharmacological manipulation. Improper flap design exceeding the intrinsic blood supply for the flap often leads to partial flap necrosis. Hence, this angiosome concept is applicable in any flap reconstructive surgery.

An acutely raised flap is both viable and ischemic. In normal hemodynamics, blood flow in the flap tip decreases 20% below normal levels within first 6 to 12 hours(1, 6). During the first 12- 18 hours, leukocyte-mediated endothelial damage, release of sympathethic vasoconstrictors and combination of decreased perfusion pressure flow dramatically decreases in the distal flap regions(7). Nutritive supply at the distal or marginal skin flap edge is compromise and this will lead to partial flap necrosis especially in the random part of the flap. When tip regions are exposed to severe ischemia for 6- 12 hours with no reflow-phenomenan sets in, tissue necrosis results in cellular changes in microcirculation level. By understanding the fundamental pathophysiology of flap microcirculation, various study has been conducted to further explained and proposed various pathogenic pathway leading to flap failure. As a result, various methods have been proposed to augment the flap survival based on these pathway.

Various surgical and non-surgical methods have been proposed to augment the survival of flaps; surgical delayed remains the most well understood technique to improve flap survival. Early studies proposed that delayed mechanism is caused by alteration in sympathetic tone with loss of temporarily hyperadrenagic state in delayed flaps, dilatation of choke vessels and reorientation of vessels axially and early changes in tissue metabolism with increase tolerance to ischemia in delayed flaps(8, 9). The focus has shifted towards the late effect of vascular delayed especially with the recent advances in vascular stem cell biology. Various cytokines and angiogenic factors notably the VEGF (Vascular Endothelial Growth Factor) and bFGF (basic fibroblast growth factor) were demonstrated to increase following delay and subsequently improve neovascularization of flaps(10). However, this procedure has its disadvantage of only being available before the main surgery and requires a two-staged procedure with significant time delayed.

Many pharmacological agents have been introduced and studied experimentally to improve survival of flap, however clinical outcome is still controversial. These agents include sympatholytics, vasodilators, calcium channel blockers, anticoagulants, volume expander agents, prostaglandin inhibitors and antioxidants. It can be classify based on three pathogenetic entity namely on thrombosis (anti aggregants and anticoagulants), ischemia-reperfusion injury or the no-reflow phenomenan (drugs that modulate flap tolerance to ischemia) and vasospasms. Antithrombotic and antiplatelet agents were associated with risk of bleeding and outcome were often unpredictable(11, 12). On the other hand, various pharmacological agents targeting at ischemic reperfusion has shown promising results experimentally, however comparison among studies were difficult due to heterogenous experimental model with variable timing and dosing of agents used. Hence, these results were difficult to be translated into clinical practice. In addition, many of these agents studied may cause important systemic side effects and deemed

unsuitable for clinical use. At present, there are only several agents used clinically to prevent or relieve vasospasm intraoperatively during microsurgical free tissue transfers(13). Topical nitroglycerin has been used experimentally and in small clinical case series to improve flap survival, however with variable outcome(14-16). Hence, there is still a continue search for more effective pharmacological intervention with exact timing, method of delivery and dosing regimen without harmful side effects from the drug itself.

1.1.2 The medication– Sildenafil

Sildenafil citrate is an amphoteric drug with a ph-dependent solubility. Oral tablets is marketed as Viagra with doses of 25, 50 and 100 mg taken not more than once daily(17). Oral administration encounters various obstacles. The drug is vulnerable to considerable intestinal and hepatic first pass metabolism of approximately 71% of oral dose. The drug is metabolized in the liver by the enzyme cytochrome P3A4 with an oral bioavailability of 40%. Furthermore, numerous side effects such as blood pressure reduction, headaches, flushing and nasal congestion are concomitant with oral administration. With oral administration, the effect is relatively delayed with onset 30-45 minutes and short duration of action (half-life 4 hours). Repeated doses are required to sustain drug plasma levels(18).

The majority of sildenafil do not appear to penetrate the skin at a sufficiently high rate to show therapeutic effectiveness(19). Hence, the feasibility of transdermal route is limited to powerful actives presenting features such as appropriate low molecular weight and high lipophilicity. Additional challenges faced in formulating sildenafil given the fact that sildenafil citrate is amphoteric in nature and ph-dependent characteristics of the compound. The drug possesses poor solubility in both aqueous and oil phases. In different ph environment, the drug will have different levels of ionization following influence of the partition coefficient, as well as its permeation ability via the skin.

Therefore, modification of the permeation parameters of sildenafil citrate has been reported as a major task in development of an optimal drug for topical delivery formula. Yonessi and Saeedi investigated the effect of topical gel containing sildenafil (1% in different cosolvents) compared to oral sildenafil for erectile dysfunction(19). They reported significantly shorter onset of action and lower side effects for transdermal delivery. However, only 12.5% patients had complete erection with transdermal sildenafil as compared to 70% patients

receiving oral therapy. Other options to increase percutaneous absorption include addiditonal of several enchancers and to improve sildenafil delivery characteristics using nanocarriers.

In our laboratory, we have managed to formulate polymeric gel of Sildenafil with a mixture of 1g of sildenafil in 100 ml distilled water, carbomer (Carbopol 941) to give structural network and additional of sodium lauryl sulfate (SLS) as penetration enhancer(20). The psyhchochemical properties were determined and its stability with enhancer was ascertained. Ex vivo permeation study were performed with rabbit skin using a modified jacketed vertical Franz diffusion cell to ascertain the amount of sildenafil permeation of the formulated gel(21).

1.1.3 Animal model - Rabbit

Rabbit has served as an excellent model to study cutaneous vascular changes in a skin flap surgery. The rabbit abdominal flaps based on inferior epigastric vessels has been investigated in several experimental models, including epigastric fasciocutaneous free flap model for study of effect of venous hypertension(22), prefabricated abdominal skin flap(23), ischemia-reperfusion flap model(24), epigastric skin flap model for the study of delay phenomenon(9), SIEA fascia flap(25) and venous flap model based on thoracoepigastric vein(26).

The rabbit is chosen as the flap model in our study due to several advantages. It has been noted to be an excellent model for studies due to its ease of handling and husbandary, abundant blood supply, insignificant growth rate and more importantly large and consistent anatomy of the abdominal skin flap inferior epigastric vessels(8, 9). These advantages of larger and consistent vessels anatomy allow ease of surgical dissection and inferior epigastric vessels for clamping. The larger abdominal flaps as compare to rodents also provide better evaluation of flap perfusion and survival at different zones of the flap with various techniques including laser Doppler flowmetry and more accurate measurement of area of necrosis with paper template technique. In addition, rabbit has been used in various research for drug delivery system via transdermal route such as topical medication in view of the close homology to human skin especially the thickness of strateum corneum(27, 28). It also provide an excellent model to detect any significant skin reaction towards topical products, additives or enhancers used(29).

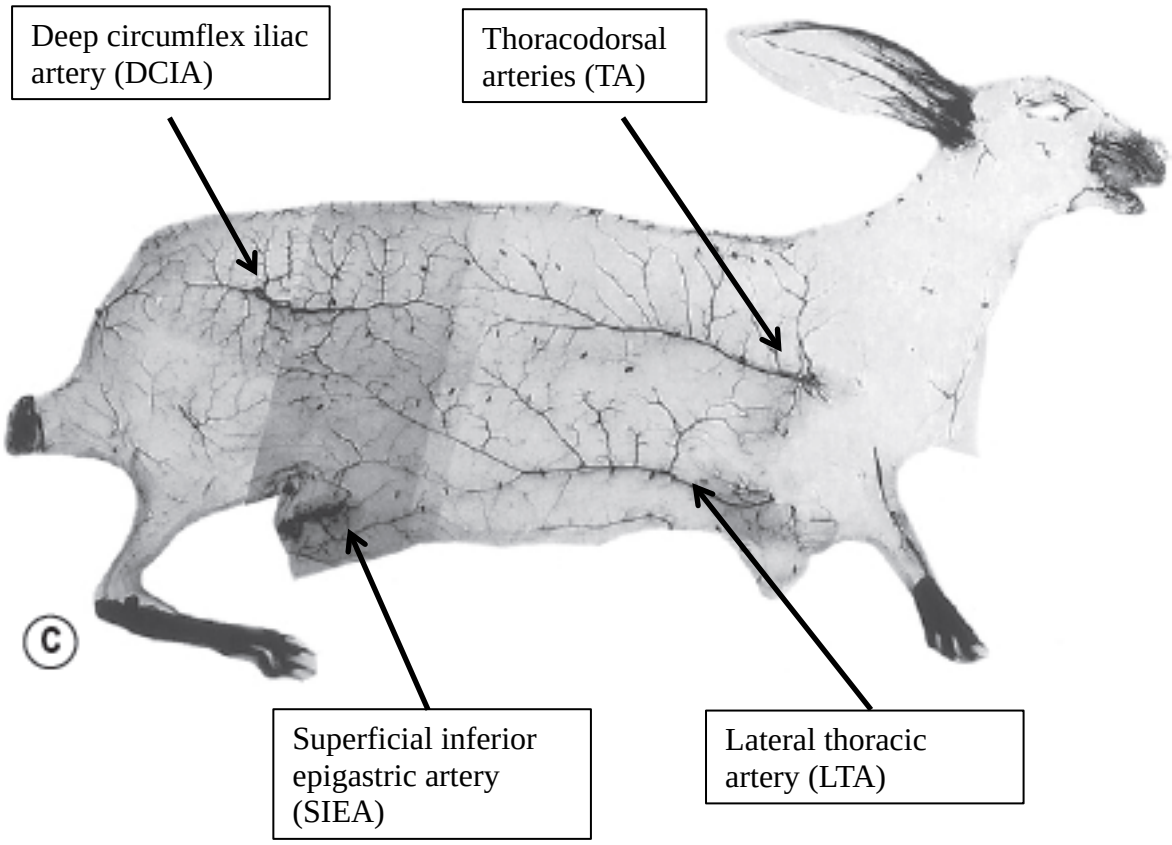

Figure 1. Blood supply of rabbit's abdominal and dorsal regions. Adopted from Taylor GI, Minabe T. The angiosomes of the mammals and other vertebrates. Plast Reconstr Surg. 1992;89:181(30)

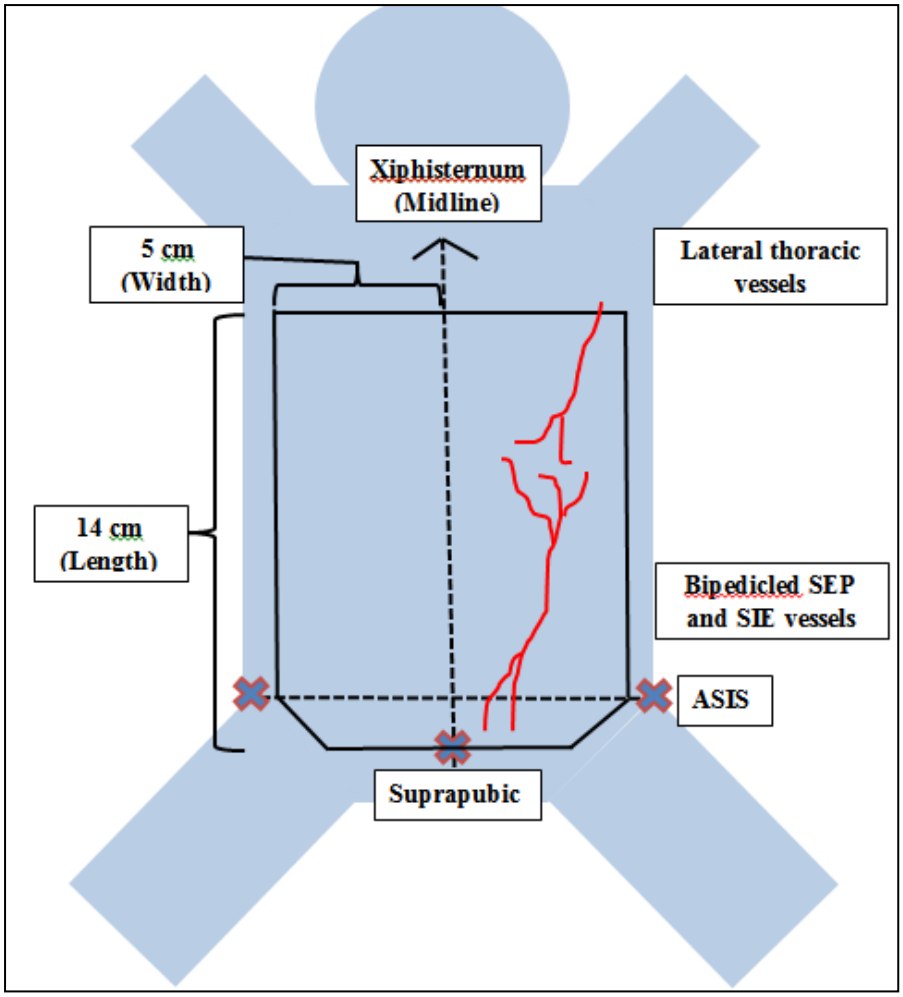

Figure 2. Modified abdominal flap design based on unilateral inferior epigastric vessels; translated from original design by Taylor and Minabe.

1.2 Rational of the study

The NO/cGMP pathway has gained interest of many recent studies due to the beneficial effect of nitric oxide on microcirculation. Recent studies has shown that sustained cGMP enhancing effect of NO have potential effect on wound healing, neuroprotection and neurogenesis, vascular relaxation on cardiac muscle and pulmonary arterial systems and angiogenesis through gene modulation(31-36). It acts directly on vascular endothelium by activating the second messenger signalling pathway for prostacyclin and nitric oxide, namely cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) to produce vascular smooth muscle relaxation through inhibition of myosin light chain kinase. Phosphodiesterase are a family of enzymes that inactivate cAMP and cGMP. There are eleven different types of phosphodiesterase (PDE) which are distributed throughout the body with only three selectively hydrolyse cGMP relative to cAMP(31). Both PDE-3 and PDE-5 have a high affinity for vascular smooth muscle and platelets, hence has been the most studied phosphodiesterase in skin flap surgery. By inhibiting phosphodiesterase, dephosphorylation of cGMP is prevented, thus allowing NO/GC/cGMP-mediated vasodilation to be sustained for a longer period.

One of the most widely used oral medication in the class of phosphodiesterase-5 (PDE-5) inhibitors is sildenafil. It is an agent that was initially developed as an antihypertensive agent. However, due to the unexpected side effect of promotion of penile erection in 1998, sildenafil citrate (Viagra, Pfizer) has been approved as the first oral drug by the United States Food and Drug Administration (FDA) for the treatment of erectile dysfunction. Subsequently, newer generation of PDE-5 inhibitors such as vardenafil, tadalafil and udenafil with different pharmacokinetics profiles were introduced(37). Sarifakioglu et al. is the first to conduct experimental study to evaluate the potential effects of sildenafil of skin flaps survival. He demonstrated that oral administration of sildenafil significantly improve skin flap survival in a

dose-dependent manner; 20 mg/kg/day for 7 days being the most significant(38). To achieve site specific delivery, prolonging exposure of action site and minimizing systemic side effects, Ulusoy et al showed that topically applied mixture of fibrin glue and 10 mg of sildenafil on the undersurface of flaps can improved skin flap viability(39). Other methods of administration of sildenafil via intraperitoneal or local injection with similar dosing regimens has produced similar results with improvement of skin flap viability(40-42). With the recent introduction of tadafil; PDE-5 inhibitor with longest half life of 17.5 hours, Oh et al has demonstrated that subdermal injection of tadafil 10mg/kg daily for 3 days showed significant improvement in flap survival(43). Barral et al. in his study using oral tadafil has produced similar trends towards improvement in skin flap survival(44), however less significant as compare to Oh et al.

Phosphodiesterase-3 (PDE3) predominantly dephosphorylate cAMP. Previous clinical study with amrinone; a selective PDE-3 inhibitor used clinically in US for the treatment of congestive cardiac failure, has shown that it positively influences the microcirculatory blood flow on transferred flaps and relieves intraoperative vasospasm(45) Cilostazol, a selective inhibitor of phosphodiesterase type III by which it increases cAMP, has recently been approved by the UK NICE (National Institute for Health and Care Excellence) and US FDA for use in patient with intermittent claudication secondary to peripheral vascular disease. Recent experimental trial using oral cilastazol on rats skin flap has shown to improve skin flap survival via induction of vasodilation through cAMP dependent pathway or nitric oxide production by increase iNOS(46).

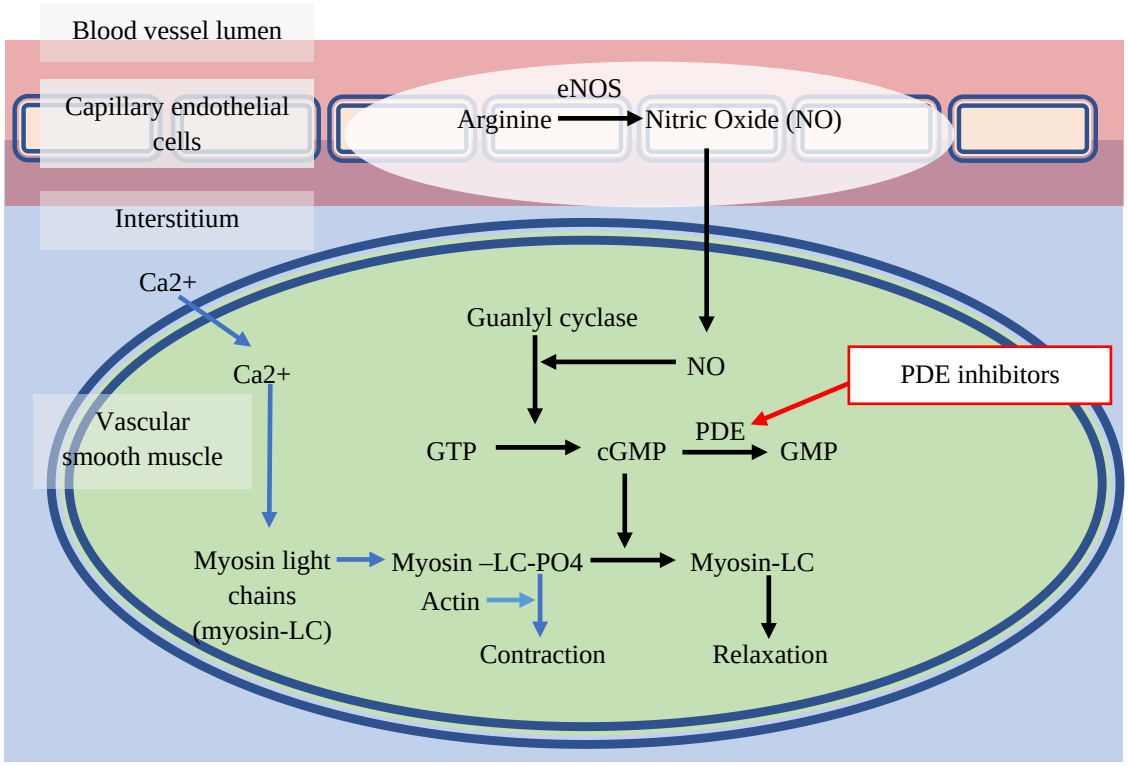

Figure 3. The NO/cGMP pathway on endothelial smooth muscle with mechanism of action of phosphodiesterase inhibitors. Redrawn from Katzung, B. G., Masters, S. B., & Trevor, A. J. (2012). *Basic and Clinical Pharmacology* (12th Edition ed.) SEC II: Cardiovacular and Renal pg 196-198l(18).

Table 1. List of experimental studies that uses phosphodiesterase class of drugs to improve skin flap survival

Author, Year,	Journal	Study design	Intervention	Major findings
Sarifakioglu et al. 2004(38)	BJPS	Experimental, rat model (n=32), Mcfarlane-type caudally based skin flap	Oral solution of sildenafil at 3,10 or 20 mg/kg/day for 7 days	Sildenafil administered orally have a dose-dependent effect to increase flap survival in random skin flaps. 10 and 20 mg/kg/day showed significant higher survival as compare to control (11.7% reduction) 83.3% (20 mg), 77.4% (10 mg), 71.6% (Control)
Ulusoy et al. 2005(39)	Annals of Plastic Surgery	Experimental, rat model (n=50), dorsal random-pattern skin flap	Topically applied mixture of fibrin glue (0.5 mL) and sildenafil at 2.5 mg or 10 mg placed under-flap once	Topically applied sildenafil combined with fibrin glue improved skin flap viability in a dose dependent manner (31% reduction) 10 mg sildenafil showed significant decrease in skin necrosis compare to 2.5 mg sildenafil 94.47% (10 mg), 87.9% (2.5mg), 67.3% (Control)
Ayyildiz et al. 2005(40)	Scand J Plast Reconstructive Surg and Hand Surg	Experimental, rat model (n=30), caudally based skin flaps	Orally administered sildenafil (1mg/kg/day) compare with local injection of sildenafil (0.5 mg/kg/day) for 7 days	Locally injected sildenafil is more effective than oral sildenafil to improve skin flap viability
Hart et al. 2006(41)	Laryngoscope	Experimental, rat model (n=109), McFarlane flap on dorsal skin	Intraperitoneal injection of sildenafil 9mg/kg/daily for 7 days	Intraperitoneally administered sildenafil decrease area of necrosis (24% reduction) Greater reduction observe at day 1 and day 3 (vasodiltory effect) compare to later day 5 and day 7 (angiogenic effect). 75.1% vs 64.7%(38, 39, 41, 47)

Tsai et al. 2008(42)	Aesth Plast Surg	Experimental, rat model (n=123), McFarlane flap on dorsal skin	Intraperitoneal injection of sildenafil solution (10mg/kg/day) compare with VEGF subdermal injection (4ug) and compare with combination	Combination of sildenafil and VEGF decreases the extent of avascular necrosis and stasis zone in skin flap but no difference statistically compare to sildenafil alone. Enhancement of flap survival mainly due to sildenafil alone.
Oh et al. 2008 (43)	American Dermatological Surgery	Experimental, rats (n=20), Mcfarlane caudally based axial flap	Subdermal injection of tadalafil 10mg/kg postoperatively, day 1 and day 2 at distal flap	Increase flap survival following subdermal injection of tadafil 10mg/kg (Reduction of necrosis 15.8%) Survival 78.1% vs 63.3%
Barral et al. 2011(44)	Acta Circugica Brasileira	Experimental, rat model (n=20), McFarlane skin flap on dorsal skin	Subdermal injection of sildenafil (0.5 mg/kg/day) immediately post op, and 48 hours later at nine points	Subdermal sildenafil is associated with lower skin flap viability (Increase necrosis 2.57%)
Brewer et al. 2012(47)	Eplasty	Experimental, rats (n=37), Mcfarlane random flap	Oral tadalafil 10mg, 20 mg/kg/day preoperatively and daily for 6 days	Increased flap survival with 10 mg or 20 mg/kg oral tadalafil but statistically not significant (Reduction of 4%) 81% vs 77%
Buruspat et al. 2015(46)	Annals of surgical innovation and research	Experimental, rat model (n=30) Mcfarlane random dorsal flap,	Oral cilostazol (40 mg/kg/day) preoperatively and daily for 7 days (Phosphodiesterase III inhibitor)	Significant improvement in flap survival at day 3, day 5 and day 7 compare to control group (Reduction of necrosis 9.68%)

In conclusion, phosphodiesterase inhibitors class of medication has been shown to improve skin flap survival in various experimental studies as shown. The experimental design, dosing and method of administration were consistent in most of the studies and consistent findings were produced. In addition, through this pathway, additional mechanism has been postulated to improve skin flap survival that has yet to be explained at this present moment. The shortfalls in majority of these studies were the use of oral, intraperitoneal of subdermal injection as administration method which complicate the ease of administration and potentially causes systemic side effects. Hence, topical application offers great potential to ease the administration on skin flap, to deliver site specific medication targeting at subdermal microcirculatory level and thus minimizes systemic side effect. Sildenafil, the oldest drug in this class of medication, are generally safe and has been used for years in human subjects for other treatment indications. Hence, sildenafil is chosen as active ingredient to be formulated as topical agents to improve flap survival. Rabbit is chosen as experimental model due to its close homology of rabbit's skin to human's integument as compared to rat model and axially based flap has more predictable vascularity and angiosome as compared to random flap. The aim of this study is to determine the effect of our gel formulated sildenafil application on inferior epigastric abdominal skin flap in rabbit model. We postulated that single application of sildenafil-formulated gel will improve blood perfusion of skin flap and reduce skin flap necrosis.

References:

1. Siemionow M, Arslan E. Ischemia/reperfusion injury: a review in relation to free tissue transfers. Microsurgery. 2004;24(6):468-75.
2. Blondeel PN, Morris SF, Hallock GG, Neligan PC. Perforator flaps: anatomy, technique, & clinical applications: CRC Press; 2013.
3. Gardiner M, Nanchahal J. Strategies to ensure success of microvascular free tissue transfer. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2010;63(9):e665-e73.
4. Pang CY aNP. Flap Physiology and Pharmacology. In: Plastic Surgery, Volume 1. In: Neligan PC GG, Warren R, Rodriguez ED, Losee JE, Song DH, Grotting JC, Chang J, and Van Beck AL, editor. Plastic Surgery 3rd Edition Volume 1: Principles. 1. Oxford: Elsevier; 2012. p. 573-86.
5. Gurtner GC, Neligan PC. Plastic Surgery: Principles: Elsevier Health Sciences; 2012.
6. Pang CY. Ischemia-induced reperfusion injury in muscle flaps: pathogenesis and major source of free radicals. Journal of reconstructive microsurgery. 1990;6(01):77-83.
7. Kerrigan CL, Daniel RK. Critical ischemia time and the failing skin flap. Plastic and reconstructive surgery. 1982;69(6):986-9.
8. Cederna PS, Chang P, Pittet-Cuenod BM, Razaboni RM, Cram AE. The effect of the delay phenomenon on the vascularity of rabbit abdominal cutaneous island flaps. Plastic and reconstructive surgery. 1997;99(1):183-93.
9. Dhar SC, Taylor GI. The delay phenomenon: the story unfolds. Plastic and reconstructive surgery. 1999;104(7):2079-91.
10. Holzbach T, Neshkova I, Vlaskou D, Konerding MA, Gansbacher B, Biemer E, et al. Searching for the right timing of surgical delay: angiogenesis, vascular endothelial

growth factor and perfusion changes in a skin-flap model. Journal of plastic, reconstructive & aesthetic surgery. 2009;62(11):1534-42.

11. Kuo Y-R, Jeng S-F, Wang F-S, Huang H-C, Wei F-C, Yang KD. Platelet glycoprotein IIb/IIIa receptor antagonist (abciximab) inhibited platelet activation and promoted skin flap survival after ischemia/reperfusion injury. Journal of Surgical Research. 2002;107(1):50-5.
12. Abla LEF, Gomes HC, Percario S, Ferreira LM. Acetylcysteine in random skin flap in rats. Acta Cirurgica Brasileira. 2005;20(2):121-3.
13. Vargas CR, Iorio ML, Lee BT. A systematic review of topical vasodilators for the treatment of intraoperative vasospasm in reconstructive microsurgery. Plastic and reconstructive surgery. 2015;136(2):411-22.
14. Dunn CL, Brodland DG, Griego RD, Huether MJ, Fazio MJ, Zitelli JA. A single postoperative application of nitroglycerin ointment does not increase survival of cutaneous flaps and grafts. Dermatologic surgery. 2000;26(5):425-7.
15. Coruh A, Abaci K, Gunay GK. Effect of topical nitroglycerine on the survival of ischemic flow-through venous flaps in rabbits. Journal of reconstructive microsurgery. 2004;20(3):261-6.
16. Gdalevitch P, Van Laeken N, Bahng S, Ho A, Bovill E, Lennox P, et al. Effects of nitroglycerin ointment on mastectomy flap necrosis in immediate breast reconstruction: a randomized controlled trial. Plastic and reconstructive surgery. 2015;135(6):1530-9.
17. Wright P. Comparison of phosphodiesterase type 5 (PDE5) inhibitors. International journal of clinical practice. 2006;60(8):967-75.
18. Deck D, Winston L, Katzung B, Masters S, Trevor A. Basic & clinical pharmacology. 2012.

19. Elnaggar YS, El-Massik MA, Abdallah OY. Fabrication, appraisal, and transdermal permeation of sildenafil citrate-loaded nanostructured lipid carriers versus solid lipid nanoparticles. International journal of nanomedicine. 2011;6:3195.
20. Kho SL. Formulation of Sildenafil Gel. Unpublished manuscript. 2015;Universiti Sains Malaysia, Malaysia.
21. Kho SL, Koh KL. Transdermal permeation profile and drug release mechanism of polymeric gel of sildenafil using ex vivo skin permeation method. Unpublished manuscript. 2016(Univerisit Sains Malaysia, Malaysia).
22. Rooks MD, Gould JS, Masear V, Powell JH, Gould S. Effects of venous hypertension on rabbit free flap survival. Microsurgery. 1991;12(4):253-61.
23. Takato T, Komuro Y, Yonehara H, Zuker RM. Prefabricated venous flaps: an experimental study in rabbits. British journal of plastic surgery. 1993;46(2):122-6.
24. Gürlek A, Schusterman MA, Evans GR, Gherardini G. Blood flow and microcirculatory changes in an ischemia-reperfusion injury model: experimental study in the rabbit. Journal of reconstructive microsurgery. 1997;13(05):345-9.
25. Giessler GA, Friedrich PF, Shin RH, Bishop AT. The superficial inferior epigastric artery fascia flap in the rabbit. Microsurgery. 2007;27(6):560-4.
26. Tan MP, Lim AY, Zhu Q. A novel rabbit model for the evaluation of retrograde flow venous flaps. Microsurgery. 2009;29(3):226-31.
27. Kantarcı G, Özgüney I, Karasulu HY, Güneri T, Başdemir G. In vitro permeation of diclofenac sodium from novel microemulsion formulations through rabbit skin. Drug development research. 2005;65(1):17-25.
28. Pople PV, Singh KK. Development and evaluation of topical formulation containing solid lipid nanoparticles of vitamin A. Aaps Pharmscitech. 2006;7(4):E63-E9.

29. Saulis AS, Mogford JH, Mustoe TA, Tredget EE, Anzarut A. Effect of Mederma on hypertrophic scarring in the rabbit ear model. Plastic and reconstructive surgery. 2002;110(1):177-83.

30. Taylor GI, Minabe T. The angiosomes of the mammals and other vertebrates. Plastic and reconstructive surgery. 1992;89(2):181-215.

31. Jackson G, Gillies H, Osterloh I. Past, present, and future: a 7-year update of Viagra®(sildenafil citrate). International journal of clinical practice. 2005;59(6):680-91.

32. Jamshidzadeh A, Azarpira N. The effects of topical sildenafil on wound healing in rat. Iranian Journal of Pharmaceutical Sciences. 2011;7(1):43-8.

33. Dussault S, Maingrette F, Ménard C, Michaud S-É, Haddad P, Groleau J, et al. Sildenafil increases endothelial progenitor cell function and improves ischemia-induced neovascularization in hypercholesterolemic apolipoprotein E–deficient mice. Hypertension. 2009;54(5):1043-9.

34. Bandera BC, Pham T, Hill-Pryor C, Bah-Sow M, Franco N, Prasad BM, et al. Role of growth factors in improved skin flap viability caused by phosphodiesterase-5 inhibitor. The American Surgeon. 2010;76(6):614-7.

35. Pyriochou A, Zhou Z, Koika V, Petrou C, Cordopatis P, Sessa WC, et al. The phosphodiesterase 5 inhibitor sildenafil stimulates angiogenesis through a protein kinase G/MAPK pathway. Journal of cellular physiology. 2007;211(1):197-204.

36. Farsaie S, Khalili H, Karimzadeh I, Dashti-Khavidaki S. An old drug for a new application: potential benefits of sildenafil in wound healing. Journal of Pharmacy & Pharmaceutical Sciences. 2012;15(4):483-98.

37. Toque HA, Priviero FB, Teixeira CE, Claudino MA, Baracat JS, Fregonesi A, et al. Comparative relaxing effects of sildenafil, vardenafil, and tadalafil in human corpus

cavernosum: contribution of endogenous nitric oxide release. Urology. 2009;74(1):216-21.

38. Sarifakioglu N, Gokrem S, Ates L, Akbuga UB, Aslan G. The influence of sildenafil on random skin flap survival in rats: an experimental study. British journal of plastic surgery. 2004;57(8):769-72.

39. Ulusoy MG, Koçer U, Karaaslan Ö, Cuzdan SS, Ayyldz A, Üstün H. Improved flap viability with site-specific delivery of sildenafil citrate using fibrin glue. Annals of plastic surgery. 2005;55(3):292-6.

40. Ayyildiz A, Uysal A, Koçer U, Karaaslan Ö, Huri E, Germiyanoglu C, et al. Effect of sildenafil citrate on viability of flaps: an experimental study in rats. Scandinavian journal of plastic and reconstructive surgery and hand surgery. 2005;39(4):204-8.

41. Hart K, Baur D, Hodam J, Lesoon-Wood L, Parham M, Keith K, et al. Short-and Long-Term Effects of Sildenafil on Skin Flap Survival in Rats. The Laryngoscope. 2006;116(4):522-8.

42. Tsai JW, Ayubi FS, Hart KL, Baur DA, Parham MA, Moon JK, et al. Evaluation of the effect of sildenafil and vascular endothelium growth factor combination treatment on skin flap survival in rats. Aesthetic plastic surgery. 2008;32(4):624-31.

43. Oh M, Chang H, Minn KW. The Effects of Tadalafil on Axial-Pattern Skin Flap Survival in Rats. Dermatologic Surgery. 2008;34(5):626-30.

44. Barral SM, Araujo ID, Vidigal PVT, Mayrink CAC, Araujo AD, Costa PRd. Effects of sildenafil on the viability of random skin flaps. Acta Cirurgica Brasileira. 2011;26(4):314-9.

45. Ichioka S, Nakatsuka T, Ohura N, Sato Y, Harii K. Clinical use of amrinone (a selective phosphodiesterase III inhibitor) in reconstructive surgery. Plastic and Reconstructive surgery. 2001;108(7):1931-7.

46. Burusapat C, Paengnoi J, Satayasoontorn K. An experimental study of the effect of pre-operative administration of cilostazol on random skin flap survival in rats: double blinded randomized controlled trial. Annals of surgical innovation and research. 2015;9(1):2.

47. Brewer MB, Stump AL, Holton LH, Janes LE, Silverman RP, Singh DP. Effects of systemic tadalafil on skin flap survival in rats. Eplasty. 2012;12.

STUDY PROTOCOL

2. STUDY PROTOCOL

THE EFFECT OF TRANSDERMAL SILDENAFIL ON AXIALLY BASED RABBIT SKIN FLAP SURVIVAL – AN EXPERIMENTAL STUDY

Student: Dr Koh Khai Luen

Matric No: PUM 0095/14

Programme: Master of Surgery (Plastic Surgery)

Co-investigators:
1. Assoc. Prof. Wan Azman Wan Sulaiman
2. Dr Yvonne-Tee Get Bee
3. Mr. Kho Swee Liang
4. Prof. Ahmad Sukari Halim

2.1.1 Introduction

Flap survival remains the most important outcome measure in any flap reconstructive surgery. Flap failure can result in total failure or partial failure secondary to inadequate flap perfusion. Failure of flap surgery will result in devastating complication to the patient with higher cost, prolong hospitalization and possibly more surgery with additional donor site morbidity. Recent refinements in microsurgery has ensured high success rate in free tissue transfer approaching 95 percent(1-3). However, most common clinical problem in flap surgery is partial necrosis rather than total flap failure. Satisfactory perfusion of bulky perforator flaps, random or pedicled flaps and functional muscle flaps remains a challenging problem for reconstructive surgeons(4).

Understanding the anatomic, hemodynamic and metabolic changes during flap transfer in a free or pedicled flap will determine the flap viability. A flap is a unit of tissue that maintains its own blood supply while being transferred from their original bed of body (donor site) to an adjacent or distant area (recipient site)(5). The intrinsic blood supply of a flap is the most critical determinant of successful transfer. Angiosome concept was first described by Taylor and Palmer in 1987 and has since become well accepted in the field of plastic and reconstructive surgery(6). It allows the conceptualization of the vascular supply to all tissues of the human body. An angiosome is a composite block of tissue supplied by a main source vessel. This concept indicates that the three-dimensional block of tissue is supplied by a major source of artery and acoompanying veins. The adjacent angiosomes are linked either by reduced calibre choke anastomotic vessels or vessels without reduction in calibre; the true (simple) anastomoses on the arterial side. The choke vessels can potentially dilate to the calibre of a true anastomosis after surgical delay or pharmacological manipulation. Improper flap design exceeding the intrinsic blood supply for the flap often leads to partial flap necrosis. Hence, this angiosome concept is applicable in any flap reconstructive surgery.

Distal flap ischemia usually results from the inability to maintain critical capillary pressure to perfuse the most distal part of the flap where total flap failure is usually caused by global insufficiency of blood supply secondary to mechanical obstruction to the pedicle, or no reflow phenomenon(4). Distal necrosis is attributed to the development of low flow state at the level of microcirculation caused by improper flap design, vasospasm, ischemic reperfusion injury, and systemic factors affecting microcirculation or physical compression of the flap. An acutely raised flap is both viable and ischemic. In normal hemodynamics, blood flow in the flap tip decreases 20% below normal levels within first 6 to 12 hours(1, 7). During the first 12- 18 hours, leukocyte-mediated endothelial damage, release of sympathetic vasoconstrictors and combination of decreased perfusion pressure flow dramatically decreases in the distal flap regions(8). Nutritive supply at the distal or marginal skin flap edge is compromise and this will lead to partial flap necrosis especially in the random part of the flap. When tip regions are exposed to severe ischemia for 6- 12 hours with no reflow-phenomenon sets in, tissue necrosis results in cellular changes in microcirculation level. By understanding the fundamental pathophysiology of flap microcirculation, various study has been conducted to further explained and proposed various pathogenic pathway leading to flap failure. As a result, various methods have been proposed to augment the flap survival based on these pathways.

Various surgical and non-surgical methods have been proposed to augment the survival of flaps; surgical delayed remains the most well understood technique to improve flap survival. However, this procedure has its disadvantage of only being available before the main surgery and requires a two-staged procedure with significant time delayed. The use of pharmacological agents to improve flap survival was and is still largely studied experimentally(9-11). Several agents used has shown good and promising results experimentally, however these drugs required high dosage, impractical method of administration and may cause significant adverse effects; hence it was difficult to be translated into clinical use. Hence, there is still a continue search for an effective