Educational Administration: Theory and Practice
2024, 30(6), 1026-1033
ISSN: 2148-2403
https://kuey.net/

Research Article

Modelling Direct Ischemic Preconditioning in Arteriovenous Flap: Is It Safe?

Rani Septrina, MD [1*], Ronny Lesmana, MD, PhD [2], Reno Rudiman, MD, PhD [3], Prof. apt. Rizky Abdullah, PhD[4], Irra R. Widarda, MD[5], Ita N. Kusmayadi, MD[6], Stevy C. Suoth, MD[6], Caroline Dewi, MD[6]

[1*]Consultant of Microsurgery and Oncoplasty, Department of Surgery, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia
[2]Associate Professor in Biomolecular Physiology, Departement of Biomedical Sciences, Faculty of Medicine, Bandung, Indonesia
[3]Associate Professor in Surgery and Digestive Surgery, Department of Surgery, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia
[4]Professor in Clinical Pharmacy, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Bandung Indonesia
[5]Cosultant of Microsurgery and Oncoplasty, Departement of Surgery, Hasan Sadikin General Hospital, Bandung, Indonesia
[6]Trainee of Plastic Reconstructive Training Program, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia

***Corresponding author:** Rani Septrina
[*]Consultant of Microsurgery and Oncoplasty, Department of Surgery, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia, (Orcid ID: 0000-0001-5441-1380)

Citation: Rani Septrina et al. (2024) Modelling Direct Ischemic Preconditioning in Arteriovenous Flap: Is It Safe?, *Educational Administration: Theory And Practice, 30* (6), 1026-1033
Doi: 10.53555/kuey.v30i6.2945

ARTICLEINO

ABSTRACT

Background: Edema and congestion are drawbacks in Arterialized venous free flap (AVF) that leads to flap necrosis. Ischemic preconditioning (IPC) is procedure of alternate episodes of occlusion and non-occlusion in the feeding vasculature to promote protection against flap necrosis caused by ischemia-reperfusion.
Objective: To determine the efficacy of IPC in AVF.
Method: In this experimental study, 24 male Wistar rats were divided into 3 groups: standard AVF (PS), AVF with 5-minutes IPC (Ex-1), AVF with 10-minutes IPC (Ex-2). 3x3 cm skin flap created in left abdomen and arteriovenous anastomosis at epigastric vasculature. Clinical condition was examined to determine the extent of congestion and necrotic area. Mean comparison test was used to analyze the data. Using $p<0.05$, the results' significance was evaluated.
Results: 20 flaps were vital. As congestion, the Ex-1 group had a lower mean value (day 14^{th} = 4.51 ± 6.799; day 21^{st} = 0.00). In the study of the congested area from the immediate to the 21st day postoperatively, all groups had significant results with $p<0.05$. However, in the PS group, necrotic tissue was obtained with a mean end (day 14th = 4.34 ± 9.147; day 21st = 0.00), and no necrotic areas were found in the SHAM, Ex-1, and Ex-2 groups. In contrast, the area of necrotic tissue immediately up to the day 21st postoperatively did not show any significant results with $p>0.05$.
Conclusion: Direct IPC is safe in creating AVF. It has effect on reducing congestion and necrotic areas.

Keywords: Arterialized venous free flap; ischemic preconditioning; ischemia/reperfusion injury; congestion; necrotic

INTRODUCTION

Arterialized venous free flap (AVF) is one of method in composite tissue transfer that use subcutaneous network as blood inflow and outflow. One of vein will be anastomosed with recipient artery to perfuse the transplanted flap. Its advantages are vast area of donor, thin, pliable, easy to dissect and not sacrifice any source of artery(1,2). Nakayama introduced this non-conventional flap in 1980(3). The survival course of this flap characterized with its bluish, congestion, resolvable edema and changed of inflow vein to artery (arterialization). The arterialization in flow through venous flap was considered in increasing of edema and discoloration(2). Hyperemic venula and skin edema in AVF will reduce after 1 week post anastomosis, but in some cases will continue until 6 weeks. Baytinger et al also experienced edema in pedicled AVF in rats(3,4).

After anastomosis, flap will look pale then congestion in early days post anastomosis(5,6). Sudden increase of arteriovenous shunt will cause active congestion(7,8) However, physiology of microcirculatory and mechanism of perfusion of this flap is still poorly understood.
Thrombosis and tissue necrosis are the risks of autologous tissue transplant. Tissue will experience ischemia condition when its supply was cut from its origin. After anastomosis, blood flow will restore and tissue come into reperfusion condition. This ischemia reperfusion injury (IRI) in free flap will trigger inflammatory response, reactive oxygen species and anaerobic metabolism thus flap will experience disruption of microcirculation (1). This event resulting in metabolic disfunction, endothelial disfunction and cellular apoptosis. Prolonged of IRI intraoperatively will result in "no-flow-phenomenon" and total flap necrosis(1,9). Kwok et al concluded that prolonged operation is the ultimate predictor in flap failure(10).
Flap conditioning is one of effort to increase flap survival by endothelial stimulation of hypoxia thus induce angiogenesis Ischemic Preconditioning is an alternate short cycle that consist of occlusion to create ischemia followed by releasing vasculature to reperfuse tissue(11). The other method is remote IPC (R-IPC) that works on the same cycle in the tissue that far from designated tissue(12) . Murray et al worked this IPC cycle in circumflex coronary artery with 4 cycle of 5 minutes occlusion/non-occlusion period. It decreased incidence of infarct myocardia for 75%(13). Numerous publications have been confirmed this successful method in flap surgery by in-vivo experiment. However, some controversy regarding direct IPC may produce stress to target organ and vessel trauma(14).
The objective of this study is to create a model of direct conditioning and elaborate different timing of Ischemic Preconditioning on survival of Arterialized Venous Flaps in the event of congestion and necrosis that become a drawback in this type of flaps.

MATERIALS AND METHODS

Animals

Male wistar rat (n=24) (300-350 g) obtained from *Biofarma Laboratory* were used in this experiment. All animal were acclimatized within 2 weeks in stress free laboratory.
All experiment was conducted in Postgraduate Small Animal Laboratory Universitas Padjadjaran.

Experimental Design

Rats were randomly divided into 3 groups after acclimatization. During flap raised, anastomosis and observation, all animal was anesthetized using 95mg/kg *ketamine hydrochloride* and 12mg/kg *xylazin* with intraperitoneal injection in right abdominal. Abdominal area was shaved before anastomosis and during each observation. Aseptic and antiseptic technique was performed prior flap elevation. Antibiotic ointment was applied on each observation to minimized the scratch of the rats.
Experimental treatments: Positive control group (PS) was created standard 3x3 cm of skin and subcutaneous flap, then performed end to end anastomosis of epigastric artery to epigastric vein by using 3-point stitch of 10-0 nylon (standard AVF). IPC is creating by choking epigastric artery and vein before anastomosis with vessel loop for certain minutes. Experiment group one (EX1) received 3-cycle of 5-minute IPC and experiment group two (EX2) received 3-cycle of 10 minute IPC. Flap then stitched back using nylon 5.0.

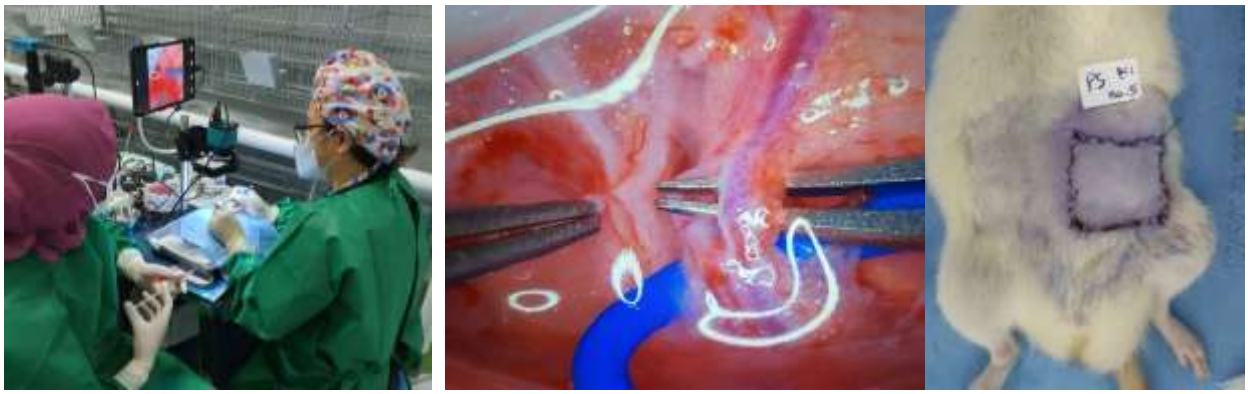


Figure 1. (Left) Dissection and anastomosis using industrial digital microscope, (Middle) dissection of epigastric vein and (Right) position of our AVF after elevation

Due to shortage of fund, we used single lens with 300x magnification industrial digital microscope that captured by 38 MP *KUAIQU YZ-3860* that we bought in online platform (*AliExpress*). The device then connected to laptop with application *S-eye* that enable us to differentiate artery, vein, pulsation and assessment of anastomosis patency. All anastomosis was done by one microsurgeon.

Data collection and observation

Data were collected immediate post operation, day 1 until 7, day 14 and day 21. Flap was clinically observe using loupe to assess area of bluish, capillary refill, edema and necrosis. Using plastic transparent scale, flap margin, area of necrosis and area of congestion was draw with fine tip permanent marker (*snowman OPF*). model was placed above white background and captured 15 cm perpendicular from the sheet using *Xiaomi* main camera 13MP, auto mode with blitz. Photo was analyzed for area of congestion and necrotic using *ImageJ* then compare to surface area of flap I percentage. All clinically vital flap will be dissected and observed under microscope for pulsation distal from site of anastomosis in day 21 after that rats were terminated. All sick or dead animal was dropped from research.

Statistical analysis

Numeric data were analyzed using SPSS ver. 24 for windows using mean comparison among multiple groups. Statistical significance was considered at $p<0.05$

RESULTS

From the total of 24 rats, 2 rats in EX1 and 2 rats in EX2 were died after anesthesia injection.
All 20 flaps were clinically vital (n=20, 100%) and all of them has visible pulse (n=20, 100%). Demographic data was shown in table 1. There were some flaps that experienced partial necrosis (3/20), but no total flap loss. All flaps experienced congestion, that will be discussed further.

Table 1. Summary of flaps in this experiment

Group	Vital	Pulse	Total Necrosis	Partial Necrosis	Edema	Congestion
PS (n=8)	8 (100%)	8 (100%)	0 (0%)	3 (37,5%)	8 (100%)	8 (100%)
EX1 (n=6)	6 (100%)	6 (100%)	0 (0%)	0 (0%)	6 (100%)	6 (100%)
EX2 (n=6)	6 (100%)	6 (100%)	0 (0%)	0 (0%)	6 (100%)	6 (100%)

Flap necrosis

Upon assessment of the necrotic area percentages, we found in standard AVF group, 3 flaps experienced partial necrosis. Necrosis was visible along the increasing of edema theory, which in day 4 to 6 then start declining in day 7 and resolved in day 21 ($p=0.010$). None of the IPC groups experienced any necrosis. However, table 2 shown that statistically in daily comparison there is no significance between standard treatment and experiment group. ($p>0.05$)

Table 2. Percentage of necrosis area

Variable	Standard treatment	IPC Experiment Group		P value
	PS	Ex-1	Ex-2	
	N=8	N=6	N=6	
Immediate				1.000*
Mean±Std	0.00	0.00	0.00	
Range (min-max)	0.00	0.00	0.00	
Day 1				0.522*
Mean±Std	0.99±2.804	0.00	0.00	
Range (min-max)	0.00-7.93	0.00	0.00	
Day 2				0.522*
Mean±Std	2.35±6.657	0.00	0.00	
Range (min-max)	0.00-18.83	0.00	0.00	
Day 3				0.197*
Mean±Std	3.39±8.949	0.00	0.00	
Range (min-max)	0.00-25.49	0.00	0.00	
Day 4				0.063*
Mean±Std	5.33±8.897	0.00	0.00	
Range (min-max)	0.00-24.61	0.00	0.00	
Day 5				0.063*
Mean±Std	5.56±9.548	0.00	0.00	
Range (min-max)	0.00-27.10	0.00	0.00	
Day 6				0.063*
Mean±Std	6.65±11.579	0.00	0.00	
Range (min-max)	0.00-32.93	0.00	0.00	
Day 7				0.063*

Mean±Std	6.27±11.095	0.00	0.00	
Range (min-max)	0.00-31.67	0.00	0.00	
Day 14				0.063*
Mean±Std	4.34±9.147	0.00	0.00	
Range (min-max)	0.00-25.97	0.00	0.00	
Day 21				1.000*
Mean±Std	0.00	0.00	0.00	
Range (min-max)	0.00	0.00	0.00	
P value	0.010**	1.000**	1.000**	

*analyzed with one way ANOVA; **analyzed with repeated ANOVA

Flap Congestion

All measurement of the area using *ImageJ for windows* were compared to area of before anastomosis and presented in percentage. The observation showed that all flaps experienced episode of congestion immediate after anastomosis and declining in until day 21. As shown in table 3, Group of standard AVF (PS) experienced higher congestion rate compare to IPC group and persist in day 21. In both IPC groups, declining of congestion was drastically visible in day 1. We found edema is almost resolved in day 14 and complete resolved in day 21 in groups with 5-minute IPC cycle (EX1). The 10-minute IPC cycle group (EX2) experienced almost resolved in day 21. Analyzing inter groups using one way ANOVA, we found that it has significancy from immediate post anastomosis until day 21 ($p<0.05$). The same pattern was found intra groups statistic, all of them were significant (pPS=0.0001; pEx-1=0.0001; pEx-2=0.0001)

Table 3. Comparative measurement of flap congestion

Variable	Standard treatment	IPC experiment group		P value
	PS N=8	Ex-1 N=6	Ex-2 N=6	
Immediate				**0.002***
Mean±Std	79.92±17.267	66.54±15.647	73.42±17.275	
Range (min-max)	41.92-92.22	52.09-96.72	50.76-97.31	
POD 1				**0.0001***
Mean±Std	61.39±20.611	38.26±13.809	42.22±15.487	
Range (min-max)	34.40-99.93	20.56-59.52	27.28-70.38	
POD-2				**0.0001***
Mean±Std	46.99±13.312	33.63±12.105	35.91±10.724	
Range (min-max)	29.73-72.41	18.40-47.34	20.18-50.61	
POD-3				**0.0001***
Mean±Std	46.20±12.130	31.79±7.304	32.10±12.326	
Range (min-max)	25.16-61.70	24.36-40.83	11.82-48.36	
POD-4				**0.0001***
Mean±Std	44.92±13.439	20.74±4.234	28.52±12.580	
Range (min-max)	32.58-69.20	17.03-28.33	14.03-42.03	
POD-5				**0.0001***
Mean±Std	42.99±12.530	14.37±5.954	24.89±15.205	
Range (min-max)	27.84-67.20	3.31-19.98	10.66-49.88	
POD-6				**0.0001***
Mean±Std	45.08±12.477	14.29±3.909	32.00±18.757	
Range (min-max)	27.31-68.29	7.93-17.89	17.06-66.49	
POD-7				**0.0001***
Mean±Std	36.93±16.391	12.68±5.657	29.24±13.781	
Range (min-max)	19.37-73.39	4.80-19.07	15.53-54.03	
POD-14				**0.0001***
Mean±Std	35.94±17.168	4.51±6.799	27.77±11.120	
Range (min-max)	22.56-64.97	0.00-17.53	11.88-44.56	
POD-21				**0.003***
Mean±Std	22.18±16.405	0.00	3.82±5.948	
Range (min-max)	0.00-47.89	0.00	0.00-12.37	
P value	**0.0001****	**0.0001****	**0.0001****	

*analyzed with one way ANOVA; **analyzed with repeated ANOVA

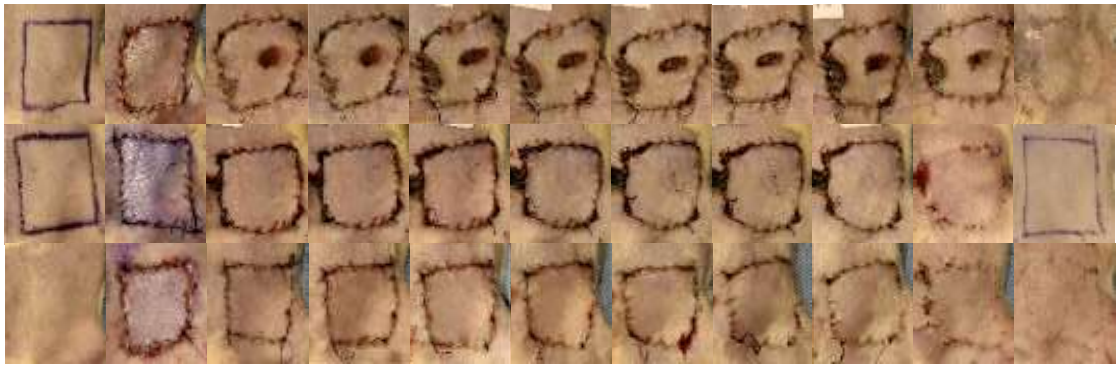
DISCUSSION

An arterialization in venous flap was created in order to enhance perfusion but still creates congestion and resolvable edema as its result of non-physiology flap. A classic circulation model from artery – capillary –

venule, theoretically, is not needed in this flap. Ensuring constant blood flow with arterialization was to open the shunt and perfuse the flap with arterial blood which was rich from oxygen (16). Pressure from artery that fills vein system was suspected to contribute to severity of congestion. If the pressure of tissue is exceeding the arterial pressure, the blood from vein will pump to artery in diastolic phase. This process creates congestion in the early arterialization and resolves after vascular adaptation occur(17). Therefore, an experiment using shunt restriction to create area of high and low pressure was done to make this flap more physiological with end result of reduced congestion(18). However, this intervention did not hinder congestion at all.

Another effort to increase flap survival was with pre-arterialization prior flap elevation and ligation of main arterial source. Side-to-side anastomosis of femoral artery and vein was conducted by Wungcharoen et al to ensure flap perfusion and much superior than original AVF(19). Flap delay was one of common procedure to increase flap survival. Yan et al showed that combination of pre-arterialization and delay procedure increase flap survival due to increasing of neovascularization and microvascular changes(20). Although these procedures increase angiogenesis, they require multiple stages.

All flaps in this experiment were vital and has visible pulse. This confirm that direct IPC procedure has no side effect for flap. Although some hesitation regarding this procedure arose due to vascular trauma and preferred remote IPC(21). Modification of digital industrial microscope was visible to be used in training super microsurgery in animal model. The three-point suture in small caliber vessel is also proven to be efficient way of anastomosis.



Picture 2. Flap before procedure and follow up immediate after anastomosis, day 1 until 7, day 14 and day 21. Above: standard AVF. Middle: AVF + 5-minute IPC. Bottom: AVF + 10-minute IPC.

Partial flap necrosis in this experiment were an uneventful effect of AVF creation and visible from day 2 with its visible peak in day 6. This confirms publication from Bajawa et al that stated after 48 hours post operative, flap look cyanotic and continuous to do so until day 7 when demarcation with healthy skin was clearly visible(22,23).

In comparison with standard delayed procedure, Ceylan et al were concluded that venous flap with 3 cycle of 10 minutes IPC has smaller area of necrotic(24). This is in line with our experiment that all IPC flaps experience no necrotic. Repetition of three occlusion/non occlusion cycle was proven to be effective to increase flap survival. Wang et al was applied 2 cycle of 5 minutes and 10 minutes IPC to TRAM flaps in rat and proved that their survival rate is higher than control group(25) Three cycle of IPC apparently sufficient to produce peak blood flow to the flap and increase blood saturation by 29% from the baseline(21). Our protocol had inline result with Coskunfirat et al, that IPC prior to ischema showed a significant flap survival portion compare to its standard procedure(26).

The drawbacks of AVF are flap edema and congestion. Our experiment was also showed that all of our flaps experienced congestion started from immediate after anastomosis and slowly resolved. Bajawa et al stated that flap will have congestion in 24 hours after procedure(22). Baytinger et al stated that congestion and edema were mostly prominent in day 7. Neo angiogenesis and cell proliferation were responsible to that event(27). Other publication also stated that 40% of AVF were still experiencing congestion up to 6 weeks post operative. In our standard AVF, congestion and edema Partial flap necrosis in this experiment were an uneventful effect of AVF creation and visible from day 2 with its visible peak in day 6. This confirms publication from Bajawa et al that stated after 48 hours post operative, flap look cyanotic and continuous to do so until day 7 when demarcation with healthy skin was clearly visible(22,23).

In comparison with standard delayed procedure, Ceylan et al were concluded that venous flap with 3 cycle of 10 minutes IPC has smaller area of necrotic(24). This is in line with our experiment that all IPC flaps experience no necrotic. Repetition of three occlusion/non occlusion cycle was proven to be effective to increase flap survival. Wang et al was applied 2 cycle of 5 minutes and 10 minutes IPC to TRAM flaps in rat and proved that their survival rate is higher than control group(25) Three cycle of IPC apparently sufficient to produce peak blood flow to the flap and increase blood saturation by 29% from the baseline(21). Our protocol had inline result with Coskunfirat et al, that IPC prior to ischema showed a significant flap survival portion compare to its standard procedure(26).

The drawbacks of AVF are flap edema and congestion. Our experiment was also showed that all of our flaps experienced congestion started from immediate after anastomosis and slowly resolved. Bajawa et al stated that flap will have congestion in 24 hours after procedure(22). Baytinger et al stated that congestion and edema were mostly prominent in day 7. Neo angiogenesis and cell proliferation were responsible to that event(27). Other publication also stated that 40% of AVF were still experiencing congestion up to 6 weeks post operative. In our standard AVF, congestion and edema were most prominent in day 5-6 post operative and although declining, this group was having minimal congestion in day 21. This might be due to denervated vein that created dilated vein, unlike arteriole that able to contract(24,25,28,29). All of our IPC flaps were experienced congestion than reduced significantly in day 2. In 5-minutes IPC group, the congestion was almost resolved in day 14 and complete resolved in day 21. In 10-minute IPC group, congestion almost resolved in day 21 (4%), compared to standard AVF. This confirm that in flap transfer, IPC procedure creates protective and adaptative mechanism for Ischemic/Reperfusion Injury. Our experiment proved that IPC create the same mechanism in AVF.

CONCLUSION

Direct Ischemic Preconditioning procedure, either 5-minute or 10-minute cycle, was proven safe for creation AVF. This conditioning procedure to AVF does not create any flap necrotic and tends to reduce congestion that might improve survival of the flap. This adjunct procedure may take more time intraoperatively but has significant mechanism in protecting flaps for adverse event of IRI in AVF.

REFERENCES

1. Naalla R, Chauhan S, Dave A, Singhal M. Reconstruction of post-traumatic upper extremity soft tissue defects with pedicled flaps: An algorithmic approach to clinical decision making. Chinese J Traumatol - English Ed [Internet]. 2018;21(6):338–51. Available from: https://doi.org/10.1016/j.cjtee.2018.04.005
2. Garlick JW, Goodwin IA, Wolter K, Agarwal JP. Arterialized venous flow-through flaps in the reconstruction of digital defects: case series and review of the literature. Hand. 2015;10(2):184–90.
3. Pittet B, Quinodoz P, Alizadeh N, Schlaudraff KU, Mahajan AL. Optimizing the arterialized venous flap. Plast Reconstr Surg. 2008;122(6):1681–9.
4. Rozen WM, Leong J. Arterialized venous flow-through flaps with dual discontiguous venous drainage: A new modification to improve flap survival. Plast Reconstr Surg. 2012;130(1):229–31.
5. Kayalar M, Kucuk L, Sugun TS, Gurbuz Y, Savran A, Kaplan I. Clinical applications of free arterialized venous flaps. J Plast Reconstr Aesthetic Surg. 2014;67(11):1548–56.
6. Woo SH, Kim KC, Lee GJ, Ha SH, Kim KH, Dhawan V, et al. A retrospective analysis of 154 arterialized venous flaps for hand reconstruction: An 11-year experience. Plast Reconstr Surg. 2007;119(6):1823–38.
7. Yan H, Fan C, Zhang F, Gaoo W. Arterialized Venous Flaps in Reconstructive and Plastic Surgery Hede. In: Intech [Internet]. InTechOpen; 2013. p. 179–201. Available from: http://www.intechopen.com/books/trends-in-telecommunications-technologies/gps-total-electron-content-tec- prediction-at-ionosphere-layer-over-the-equatorial-region%0AInTec
8. Woo SH, Kim SE, Lee TH, Jeong JH, Seul JH. Effects of blood flow and venous network on the survival of the arterialized venous flap. Vol. 101, Plastic and Reconstructive Surgery. 1998. p. 1280–9.
9. Ballestin A, Cascado JG, Abellan E, Vela FJ, Blasquez R. Ischemia-reperfusion injury in a rat microvascular skin free flap model : A histological , genetic , and blood flow study. PLoS One. 2018;13(12):1–16.
10. Kwok AC, Agarwal JP. An analysis of free flap failure using the ACS NSQIP database. Does flap site and flap type matter? Microsurgery. 2017;37(6):531–8.
11. Wang WZ. Investigation of reperfusion injury and ischemic preconditioning in microsurgery. Microsurgery. 2009;29(1):72–9.
12. Huang L. What Happened if Various Kinds of Postconditioning Working on the Preconditioned Ischemic Skin Flaps. PLoS One. 2013;8(9):1–6.
13. Bousselmi R, Lebbi MA, Ferjani M. Myocardial ischemic conditioning: Physiological aspects and clinical applications in cardiac surgery. J Saudi Hear Assoc [Internet]. 2014;26(2):93–100. Available from: http://dx.doi.org/10.1016/j.jsha.2013.11.001
14. Kraemer R, Lorenzen J, Kabbani M, Herold C, Busche M, Vogt PM, et al. Acute effects of remote ischemic preconditioning on cutaneous microcirculation - A controlled prospective cohort study. BMC Surg [Internet]. 2011;11(1):32. Available from: http://www.biomedcentral.com/1471-2482/11/32
15. Zhang Y, Xu H, Wang T, He J, Wei J, Wang T, et al. Remote limb ischemic post-conditioning attenuates ischemia-reperfusion injury in rat skin flapby limiting oxidative stress. Acta Cir Bras. 2016;31(1):15–21.
16. Lam WL, Lin WN, Bell D, Higgins JP, Lin YT, Wei FC. The physiology, microcirculation and clinical application of the shunt-restricted arterialized venous flaps for the reconstruction of digital defects. J Hand Surg Eur Vol. 2013;38(4):352–65.
17. Lin Y Te, Loh CYY. Application of concatenated arterialized venous flaps in finger reconstruction. Ann Plast Surg. 2019;82(3):292–5.

18. Lin Y Te, Hsu CC, Lin CH, Loh CYY, Lin CH. The position of 'shunt restriction' along an arterialized vein affects venous congestion and flap perfusion of an arterialized venous flap. J Plast Reconstr Aesthetic Surg [Internet]. 2016;69(10):1389–96. Available from: http://dx.doi.org/10.1016/j.bjps.2016.05.013
19. Wungcharoen B, Santidhananon Y, Chongchet V, Pradidarcheep W. Pre-arterialisation of the arterialised venous flap: An experimental study in the rat. Br J Plast Surg. 2001;54(7):621–30.
20. Yan H, Brooks D, Jackson WD, Angel MF, Akdemir O, Zhang F. Improvement of Prearterialized Venous Flap Survival with Delay Procedure in Rats. J Reconstr Microsurg. 2010;26(3):193–200.
21. Kraemer R, Lorenzen J, Kabbani M, Herold C, Busche M, Vogt PM, et al. Acute effects of remote ischemic preconditioning on cutaneous microcirculation - a controlled prospective cohort study. BMC Surg [Internet]. 2011;11(32):1–7. Available from: http://dx.doi.org/10.1016/j.ajem.2016.04.021
22. Bajwa N, Au J, Jarrahy R, Sung S, Fishbein MC, Riopelle D, et al. Non-invasive terahertz imaging of tissue water content for flap viability assessment. Biomed Opt Express. 2017;8(1):460.
23. Casal D, Mota-Silva E, Pais D, Iria I, Videira PA, Tanganho D, et al. Optimization of an Arterialized Venous Fasciocutaneous Flap in the Abdomen of the Rat. Plast Reconstr Surg - Glob Open. 2017;5(8):1–8.
24. Ceylan R, Kaya B, Çaydere M, Terzio›lu A, Aslan G. Comparison of ischaemic preconditioning with surgical delay technique to increase the viability of single pedicle island venous flaps: An experimental study. J Plast Surg Hand Surg. 2014;48(6):368–74.
25. Wang H, Li Z, Liu X. Effects of various protocols of ischemic preconditioning on rat tram flaps. Microsurgery. 2008;28:37–43.
26. Coskunfirat OK, Ozkan O, Dikici MB. The effect of ischemic preconditioning on secondary ischemia in skin flaps. Ann Plast Surg. 2006;57(4):431–4.
27. Baytinger VF, Kurochkina OS, Selianinov K V., Baytinger A V., Dzyuman AN. The possibility for use of venous flaps in plastic surgery. AIP Conf Proc. 2015;1688:1–5.
28. Matsumura H, Yoshizawa N, Watanabe K, Vedder NB. Preconditioning of the distal portion of a rat random-pattern skin flap. Br J Plast Surg. 2001;54(1):58–61.
29. Keskin D, Unlu RE, Orhan E, Erkilinç G, Bogdaycioglu N, Yilmaz FM. Effects of remote ischemic conditioning methods on ischemia-reperfusion injury in muscle flaps: An experimental study in rats. Arch Plast Surg. 2017;44(5):384–9.

KEMENTERIAN PENDIDIKAN, KEBUDAYAAN,
RISET DAN TEKNOLOGI
UNIVERSITAS PADJADJARAN
KOMISI ETIK PENELITIAN
RESEARCH ETHICS COMMITTEE
Jl. Prof. Eyckman No. 38 Bandung 40161
Telp. & Fax. 022-2038697 email: kep@unpad.ac.id, website: kep.unpad.ac.id

No. Reg.: 2204050448

PERSETUJUAN ETIK
ETHICAL APPROVAL

Nomor: 572/UN6.KEP/EC/2022

Komisi Etik Penelitian Universitas Padjadjaran Bandung, telah mengkaji dengan teliti proposal penelitian yang menggunakan subjek Hewan Coba dalam penelitian yang berjudul:

The Research Ethics Committee Universitas Padjadjaran Bandung, has been throughly reviewed proposal for reaserch with animal subjects in reaserach entitled:

"PENGARUH *ISCHEMIC PRECONDITIONING* PADA VASKULARISASI *ARTERIALIZED VENOUS FREE FLAP* YANG MELIBATKAN PENSINYALAN AUTOFAGI MELALUI PROTEIN LC3, P62, BECLIN-1"

Nama Peneliti Utama *Principal Researcher*	:	Rani Septrina
Pembimbing/Peneliti Lain *Supervisor/Other Researcher*	:	Ronny, dr.,M.Kes.,AIFO.,PhD Dr. Reno Rudiman, dr., M.Sc., SpB-KBD Prof. Rizky Abdullah, Apt Phd,
Nama Institusi *Institution*	:	Program Pascasarjana Program Studi Doktor Fakultas Kedokteran Universitas Padjadjaran

proposal tersebut dapat disetujui pelaksanaannya.
hereby declare that the proposal is approved.

Ditetapkan di : Bandung
Issued in
Tanggal : 20-06-2022
Date

Ketua,
Chairman,

Nur Atik, dr, M.Kes., PhD
NIP. 19811010 200801 1 019

Keterangan/*notes*:
Persetujuan etik ini berlaku selama satu tahun sejak tanggal ditetapkan.
This ethical clearance is effective for one year from the due date.
Pada akhir penelitian, laporan pelaksanaan penelitian harus diserahkan ke Komisi Etik Penelitian.
In the end of the research, progress and final summary report should be submitted to the Research Ethics Committee.
Jika ada perubahan atau penyimpangan protokol dan/atau perpanjangan penelitian, harus mengajukan kembali permohonan kajian etik penelitian.
If there be any protocol modification or deviation and/or extension of the study, the Principal Investigator is required to resubmit the protocol for approval.
Jika ada kejadian serius yang tidak diinginkan (KTD) harus segera dilaporkan ke Komisi Etik Penelitian.
If there are Serious Adverse Events (SAE) should be immediately reported to the Research Ethics Committee