

Review

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# Tissue conditioning - strategies to improve perfusion and reduce ischemia - reperfusion injury

Sabrina Krauss<sup>1</sup>, Jens Rothenberger<sup>1</sup>, Johannes Mayer<sup>1</sup>, Alexander Sogorski<sup>2</sup>, Manuel Held<sup>1</sup>, Theodora Wahler<sup>1</sup>, Adrien Daigeler<sup>1</sup>, Jonas Kolbensschlag<sup>1</sup>

<sup>1</sup>Department of Hand, Plastic, Reconstructive and Burn Surgery, BG Trauma Center Tuebingen, Eberhard Karl University Tuebingen, Tuebingen 72076, Germany.

<sup>2</sup>Department of Plastic Surgery, Burn Center, BG University Hospital Bergmannsheil, Ruhr-University Bochum, Bochum 44789, Germany.

**Correspondence to:** Dr. Sabrina Krauss, Department of Hand, Plastic, Reconstructive and Burn Surgery, BG Trauma Center Tuebingen, Eberhard Karl University Tuebingen, Tuebingen 72076, Germany. E-mail: SabrinaKrauss@gmx.net

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## Abstract

Ischemia as well as ischemia-reperfusion injury (IRI) can cause serious tissue damage and therefore is a feared complication in reconstructive surgery. This is the reason why researchers around the world invest their efforts to improve tissue viability after ischemic events. Tissue conditioning offers a broad scope of different techniques which can be applied pre-, peri- or postoperatively to adapt the affected tissue to the subsequent stress during and after ischemia to prevent or minimize IRI. The different ways of tissue conditioning in flap surgery include surgical delay, ischemic conditioning, remote ischemic conditioning as well as thermic preconditioning and other techniques, using growth factors, pharmaceutical agents, extracorporeal shock waves as well as stem cells. Therefore, we want to shed some light on the effects of ischemia and ischemia-reperfusion injury and further illustrate the different strategies of tissue conditioning with special concern to flap surgery but also regarding wound healing in general.

**Keywords:** Tissue conditioning, ischemia, ischemia-reperfusion injury, reconstructive surgery, flap surgery, wound healing

## INTRODUCTION

Cell damage caused by ischemia affects almost all clinical disciplines, seen in daily clinical routine for example as heart attacks, strokes, in organ transplantations as well as in reconstructive surgical procedures such as flap surgery<sup>[1]</sup>. Ischemia as well as ischemia-reperfusion injury (IRI) represent a formidable challenge



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in the transplantation of solid organs, in vascularized tissue allotransplantations (VCA) such as hand or face transplantations and in free flaps. In VCA for example, cold ischemia time is 6 h on average<sup>[2,3]</sup>. The importance of ischemia reperfusion-injury in tissue transplantation is clearly illustrated by its impact on early graft function as well as long-term outcomes<sup>[4]</sup>.

Insufficient tissue perfusion plays an important role in chronic wounds as well. Tissue hypoxia is responsible for a lack of energy metabolism, cell proliferation, angiogenesis, cytokine release as well as enzyme activity and therefore leading to an impairment of the tissue repair process<sup>[5-7]</sup>. Common causes for tissue hypoxia in chronic wounds are vascular alterations, oedema and fibrosis resulting in reduced oxygen partial pressure (pO<sub>2</sub>)<sup>[7]</sup>. An interesting discovery is that pO<sub>2</sub> in chronic wounds is 5-20 mmHg compared to 30-50 mmHg in control tissue<sup>[8]</sup>. In plastic surgery, tissue perfusion is of special concern due to the field of reconstructive surgery, where temporary tissue ischemia in free flaps as well as reduced perfusion in pedicled flaps has to be accepted to be able to treat tissue defects. In skilled and experienced hands, total flap loss is reported to occur in 1%-7% of cases, depending on recipient site and cause of the tissue defect<sup>[9-12]</sup>. But the rate of partial flap necrosis is much higher, with rates of 7%-20% in free flaps and even 20%-33% in pedicled flaps<sup>[13-16]</sup>. Partial flap necrosis often requires further operations, thus putting a significant burden on the affected patient as well as the healthcare system.

All those examples illustrate the significance of tissue blood supply and the need for methods to improve it. Or, as Gillies<sup>[17]</sup> once put it, “plastic surgery is the constant battle between beauty and blood supply”.

In this unsystematic review, we want to shed some light on the effects of ischemia and IRI on the affected tissue and illustrate different strategies of tissue conditioning focusing on its use in flap surgery.

### **Pathomechanisms of ischemia and IRI**

The persistence of insufficient tissue perfusion or total ischemia results in a loss of oxygen supply and therefore causes a change of cell metabolism as an adjustment to the lack of the aerobic pathway in the respiratory chain. This leads to an accumulation of metabolites as well as radicals and causes cell death<sup>[18,19]</sup>. Ironically, restauration of perfusion causes additional tissue damage due to inflammatory mechanisms caused by the release of oxygen compounds. Those lead to an activation of neutrophils and a consecutive adhesion between granulocytes and endothelial cells causing segmental vessel occlusion in postcapillary venules, transendothelial leukocyte migration and the release of tissue-damaging enzymes. This pathomechanism is referred to as the IRI<sup>[20-25]</sup>. With regard to flap surgery, flap survival is mainly depending on the integrity of the vascular pedicle and its “macrovascular” perfusion<sup>[26]</sup>. IRI on the other hand affects the microcirculation of the entire flap due to the inflammatory process and the increase in oxygen free radicals in the early stages of reperfusion<sup>[27]</sup>. Therefore, partial flap necrosis is often caused by an insufficient microcirculation mainly in the distal parts of the flap.

Total flap necrosis on the other hand is most often caused by thrombosis of the pedicle. Timely revision of the anastomosis is paramount to reestablish blood flow to the flap. However, such events can lead to an increase IRI which in term can lead to intravascular hemoconcentration, endothelial swelling, interstitial edema formation as well as inflammatory processes due to the reperfusion injury. In its highest degree, IRI can lead to a no-reflow phenomenon which also leads to complete flap loss<sup>[28-30]</sup>.

### **Tissue conditioning**

There are different means of pre- or peri- and postoperative techniques to adapt the tissue to the subsequent stress during and after ischemia to prevent or minimize IRI [Table 1].

**Table 1. Different ways of tissue conditioning**

Tissue conditioning
Surgical delay
Ischemic preconditioning
Remote ischemic preconditioning
Thermic preconditioning
Growth factors
Extracorporeal shock waves
Stem cells
Pharmaceutical preconditioning

### *Surgical delay*

Surgical delay is the predecessor of modern preconditioning techniques. By raising the flap itself without severing its pedicle, the vessels along the axis of the flap reorganize and increase in size, leading to a better perfusion of the distal flap due to a dilation of linking and choke vessels, causing a connection of adjacent vascular territories which could be demonstrated in animal models as well as in humans<sup>[31,32]</sup>. Direct linking vessels have a large caliber and connect adjacent vascular territories by connecting perforators themselves, while indirect linking vessels, also known as choke vessels, connect vascular territories via recurrent flow through the subdermal plexus<sup>[33,34]</sup>. The mechanisms behind this are still not fully understood but there are many animal studies that could show neovascularisation, vasodilation and reorganization of vessels due to surgical delay<sup>[35-37]</sup>. Although surgical delay proved to increase flap perfusion and therefore increased the survival of flaps in the clinical setting<sup>[38,39]</sup>, there are also major disadvantages to this strategy, especially the need for additional surgery and its risks for the affected patients as well as increased health care costs due to longer hospital stays. Especially in TRAM flaps surgical delay was used but due to the improvement of microsurgical methods free flaps as the DIEP flap have become a safer and more often applied alternative with less donor side morbidity<sup>[40-42]</sup>.

### *Ischemic preconditioning*

Ischemic preconditioning was introduced by Mounsey *et al.*<sup>[43]</sup> for conditioning of the myocardium, but has since been applied to different fields of surgery including flap surgery. Murry *et al.*<sup>[44]</sup> and Jennings *et al.*<sup>[45]</sup> found that brief, intermittent cycles of ischemia have a protective effect on the myocardium resulting in a delay or even protection of lethal injury to the myocardial cells due to metabolic changes in the affected cells in a dog model. Various animal studies showed, that ischemic preconditioning leads to an increase in capillary perfusion, the vascular response to changes in perfusion pressure, a decrease in leukocyte-mediated reperfusion injury, an increase of critical ischemia time tolerated by the affected tissue, a decrease of vasospasms as well as a decrease in the capillary no-reflow phenomenon<sup>[46-49]</sup>. All of these mechanisms lead to a significant decrease in flap necrosis in skin flaps as well as in muscle flaps<sup>[48]</sup>. Because of these positive effects, the ideal application of ischemic preconditioning was examined as well. It was found that three cycles of ischemic preconditioning of 10 min each are superior to the application of only one or two cycles. They also found that a cycle of 10 min of ischemia is superior to 5 min of ischemia<sup>[50]</sup>. It could also be shown that there is no difference in the positive effect of ischemic preconditioning on reducing muscle flap necrosis whether it is applied 24 h or immediately before flap elevation<sup>[51]</sup>. Interestingly, ischemic preconditioning has immediate as well as late protective effects: the immediate effect is an improvement of the blood flow hemodynamics and an attenuation of the leukocyte-mediated reperfusion injury whereas after 24 h of reperfusion the improvement of the hemodynamics has subsided while the protective effect against reperfusion injury was still present<sup>[46]</sup>. Although there are those numerous positive effects of ischemic preconditioning it hasn't found its way into clinical routine use. The main reason for this might be the additional time needed for preparing the pedicle and applying ischemia prior to flap elevation as well as the spreading of new techniques like indocyanine



**Figure 1.** Remote ischemic conditioning: Examining the changes in tissue perfusion on the right hand of the test person after inducing ischemia on the left arm with a tourniquet

green (ICG) angiography, dynamic infrared thermography (DIRT), and photospectrometry which facilitate flap design<sup>[28,52,53]</sup>. One exception where ischemic preconditioning is routinely applied is the pedicled groin flap. It still poses as a viable option when free or local flaps cannot be used to reconstruct tissue defects<sup>[54]</sup>.

#### *Remote ischemic conditioning*

The idea of remote ischemic preconditioning was introduced by Przyklenk *et al.*<sup>[55]</sup> in 1993 with their findings in a dog model that brief cycles of ischemia in a remote vascular bed are also capable of protecting myocardial cells from damage caused by the occlusion of coronary arteries. Plastic surgeons applied this idea to their line of work. Today three forms of remote ischemic conditioning exist: pre-, per-, and post-conditioning. In animal models it could be demonstrated that the induction of ischemia and reperfusion in a body part distant from the flap prior to its elevation could reduce the occurrence of flap necrosis in adipocutaneous flaps as well as in muscle flaps. They also found that ischemia and reperfusion could be induced non-invasively by the application of a tourniquet on a body area distant from the flap prior to flap elevation<sup>[56,57]</sup>. After those first promising results many experimental studies focused on the ideal type of application regarding remote ischemic conditioning. They found that inflating the tourniquet on the upper extremity had better effects on cutaneous microcirculation than on the lower extremity and that three circles of 10 min ischemia each were superior to shorter cycles or more frequent applications<sup>[58,59]</sup> [Figure 1]. Those findings indicate that remote ischemic preconditioning has to be a systemic phenomenon but the mechanisms behind “classic” and remote ischemic conditioning are not yet fully understood. Various factors such as the release of nitric oxide, heat-shock proteins, adenosine, ATP-sensitive K<sup>+</sup> channels, cyclooxygenase as well as bradykinin through sensory nerve stimulation seem to be involved<sup>[60-66]</sup>. The protective effect of remote ischemic preconditioning against ischemia reperfusion-injury can be divided into two different timeframes. The acute effects last about four hours after the initial application of the preconditioning stimulus whereas the late effects occur after 24 h and last for at least another 24 h<sup>[67]</sup>. Furthermore the clinical application of remote ischemic conditioning is rather convenient as it is non-invasive and time-effective. The first study to examine remote ischemic conditioning in the clinical setting regarding flap surgery has shown promising results including an improvement of microcirculation in pedicled and free flaps<sup>[68]</sup>. Although there are many promising results regarding remote ischemic preconditioning there are also certain drawbacks. Some recent studies in the field of cardiac surgery revealed that the positive effects of remote ischemic preconditioning in laboratory studies may not be applied successfully in the clinical setting meaning there are no significant effects on the clinical outcome<sup>[69]</sup>.

#### *Thermic preconditioning*

Other research groups have focused on the protective effects of hypo- and hyperthermia concerning

ischemic events and reperfusion-injury. By heating tissue to a temperature of 42 °C heat shock proteins (HSPs) are upregulated. HSP-70, HSP-72 and HSP-32 are reported to induce mechanisms which protect musculocutaneous, fasciocutaneous and skin flaps from ischemic injury if the hyperthermic preconditioning was conducted 6 to 24 h before flap elevation in animal studies<sup>[70-72]</sup>.

Hypothermia has protective effects caused by the downregulation of cellular metabolism as well as the induction of HSPs. Cooling the donor area 24 h before raising of the flap has shown to increase the expression of HSP-32 as well as significantly increase capillary perfusion and reduce skin flap necrosis in an experimental setting<sup>[73]</sup>.

#### *Other ways of tissue conditioning*

Pretreatment with growth factors which induce neovascularization has been investigated by some research groups. Until now, the application of vascular endothelial growth factor (VEGF) is the most widely investigated growth factor. The intravascular and subcutaneous application of VEGF proved to induce angiogenesis and increase flap survival in a rat model<sup>[74,75]</sup>. There has also been a gene therapeutic approach which showed a reduce of ischemic tissue in random skin flap models in rats by transfection with VEGF plasmids<sup>[76]</sup>. Other studies investigated the effects of human basic fibroblast growth factor (bFGF). Pretreatment with subcutaneous application of bFGF at the future donor site as well as the pretreatment of the recipient site of the flap via gene transfection showed an increase in vascularity and tissue perfusion in skin flap models in rats<sup>[77,78]</sup>.

Other research groups have focused on the effects of extracorporeal shock wave application (ESWA) on angiogenesis and tissue perfusion. Through ESWA, an increase in blood flow and angiogenesis as well as an improvement in tissue metabolism could be achieved in a mouse model<sup>[79-81]</sup>. In a rat model it could even be found that preoperative ESWA has a similar effect as surgical delay regarding microvessel density and perfusion, being non-invasive and easily applicable<sup>[82]</sup>. ESWA even decreases inflammatory reactions and therefore has a protective effect against reperfusion-injury<sup>[83]</sup>.

A further approach is the use of stem cells in tissue conditioning. In an experimental setting with Lewis rats it could be demonstrated that the venous application of adipose derived stem cells during reperfusion after an ischemic event could reduce necrosis in skin flaps and local application of adipose derived stem cells to the wound bed before suturing the flap could increase tissue perfusion and skin flap survival<sup>[84,85]</sup>.

Another means for tissue preconditioning is a pharmaceutical approach. Possible drugs should counteract the pathophysiological processes responsible for ischemia or ischemia reperfusion-injury. Of interest are drugs which improve microcirculation as heparin, drugs which cause vasodilation like beta-mimetics, selective calcium channel blockers and nitric oxide donors or drugs with anti-inflammatory effects like prostaglandin-analogues or cyclooxygenase inhibitors as could be shown in different animal models<sup>[86-91]</sup>.

#### *Tissue conditioning and wound healing*

Many of the above mentioned approaches in tissue conditioning are not only promising in plastic surgery but also seem to be an option for the improvement of wound healing, especially in wounds which are difficult to treat due to their size or the patients comorbidities and the resulting physiological changes in the tissue. For example there are many clinical studies investigating the positive effects of platelet rich plasma (PRP) on wounds, as it contains a large amount of cytokines, growth factors and chemokines<sup>[92,93]</sup>. Another, yet mainly experimental approach is the use of hypoxia to induce angiogenesis and improve wound healing. Therapeutic aspects using hypoxia include preconditioning cells in vitro or inducing hypoxia-mediated pathways in vivo by gene therapy or pharmaceutical agents, which eventually leads to an increase in pro-angiogenic growth

factors and other angiogenic mediators in the wound bed<sup>[94]</sup>. Remote ischemic conditioning for example has also proven to be of use in diabetic patients. There are clinical studies that could show a significantly improved healing of diabetic foot ulcers due to remote ischemic conditioning<sup>[95,96]</sup>. And the effects of extracorporeal shock waves have been investigated in the clinical setting in burn patients as well. Researchers could show better results in terms of wound healing because of its positive effects on angiogenesis<sup>[79,80,97]</sup>.

## CONCLUSION

Despite all the progress made in plastic and especially flap surgery, total or partial flap loss caused by ischemia or ischemia reperfusion-injury is still feared and has common complications. As temporary ischemia is inevitable in free flaps and the tissue of pedicled flaps is initially exposed to changes in perfusion as well, strategies to improve tissue viability are of vital importance. Many of the above mentioned, promising ways of tissue conditioning have yet only been tested under laboratory conditions or in animal models so further studies including clinical trials are needed. Those findings could not only offer great benefits in plastic surgery but in other fields of medicine as well, including the treatment of extensive or chronic wounds as well as transplant surgery. To lean on the words of Gillies<sup>[17]</sup>, tissue conditioning harbors the possibility to be an important weapon in the battle between beauty and blood supply.

## DECLARATIONS

### Authors' contributions

Designed and wrote the article, performed literature research and produced the figures: Krauss S  
Assisted in designing the article, offered ideas concerning content of the article, corrected the article and proofread the final version: Kolbensschlag J  
Read, corrected the article and discussed the content: Rothenberger J, Mayer J, Sogorski A, Held M, Wahler T, Daigeler A

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None.

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All authors declared that there are no conflicts of interest.

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Not applicable.

### Consent for publication

Consent for publication was obtained.

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