Manuscript - The Impact of Ischemia on Limb Replantation

Authors
Francisco Serra Moura, Sarah Ellis, Priyatma Premchand Khincha

Affiliation
Department of Plastic Surgery, Royal Preston Hospital

Correspondence
Francisco Serra Moura
Email: francisco.serraemoura@nhs.net

Abbreviations
FDS – Flexor Digitorum Superficialis
IPC - Ischemic Preconditioning
IRI – Ischemia Reperfusion Injury

Abstract
Traumatic amputations of the extremity are relatively common catastrophic injuries that adversely affect a patient’s physical function and wellbeing. This article reviews the current management of amputated limbs and discusses the role of ischemia and the reperfusion processes in its future management. The decision to replant an extremity depends on numerous factors which include, but are not limited to, the time from injury, mechanism of injury, site of injury, patient factors and technical know-how of replantation surgery. Despite the significant progress in most of these aspects over the last decades, the management of the amputate has not significantly advanced. The outcomes of replantation are related to the amount of tissue injury incurred by the amputate and the time without blood supply. To further the optimisation of the amputated extremity and improve the success of replantation, the future of this field relies in the research into both pharmacological post-conditioning and ex vivo perfusion to overcome Ischemic Reperfusion Injury, particularly in proximal injuries.
1. Introduction

Traumatic amputations of the extremity are catastrophic injuries that adversely affect a patient’s physical function and wellbeing. In the USA, the risk of amputation injuries is 7.5/100,000 person years rendering it an infrequent but devastating injury. These cases are highly complex surgical emergencies that after careful consideration may undergo replantation - the reattachment of all soft tissues and bone. This is performed using microvascular techniques in a process referred to as revascularisation. Of note, surgical replantation involves prolonged rehabilitation that is also costly to the patient with subsequent time away from work.

The first reportedly successful hand replantation was reported by Malt & McKhann in 1962. This was shortly followed by the first successful thumb replantation carried out by Komatsu & Tamai in 1965. This was regarded as a significant surgical milestone providing evidence that microsurgical replantation is technically feasible which resulted in a temporary exponential growth in the volume of attempted replantations. Despite the stable incidence in amputation injuries over the last two decades, there has been a steady decline in the numbers of attempted replantations. This decreasing trend is attributed to rising health care costs, low reimbursement rates for digit replantation, evolving clinical decision making and the requirement for hand surgeons that possess microsurgical skills. Specialised regional microsurgical centers with rehabilitative facilities have been developed in response to managing these complex injuries. Hospitals that frequently manage such patients have better outcomes, with an overall replantation success rate of 70% for the thumb and digits. Nevertheless, the incidence of secondary procedures following replantation reported in the literature is approximately 50% but this can range between 15-80%.

1.1. The clinical challenge

The technical know-how of replantation surgery has become more refined since it was first performed over five decades ago. Furthermore, the clinical decision-making has improved in identifying which replantations will provide acceptable functional outcomes. However, one key aspect that has not changed and requires addressing is the management of the amputated part. Once amputated, tissues inherently lose their blood supply resulting in reduced oxygenation to tissues. This causes loss of ATP and dysfunction of ion-dependant membrane channels which result in cell swelling and death. Further ischemic compromise yields an acidic environment with a build-up of lactate and loss in cell calcium. Consequently, the outcomes of replantation are related to the amount of tissue injury incurred and the time without blood supply. For this reason, it is a time dependent emergency. Paradoxically, the restoration of blood flow (reperfusion) incites a further injury to tissues via free radicals. The magnitude of ischemia dictates this response. This article aims to review the current management of amputated limbs to combat these deleterious processes.
2. Pre-Hospital Management of the Amputated Limb

Patients with amputation injuries present directly to their local Emergency Department (Figure 1). An immediate traumatic life support assessment should be performed with referral (and prompt transfer) to a center with microsurgical expertise in extremity replantation.

![Figure 1. Illustration of the key steps in the journey of a patient with an amputated limb.](image)

The pre-hospital basic management of the amputated limb has not changed since the 1960’s. Current practice recommends wrapping the amputated extremity in sterile soaked gauze in a sealed plastic bag. This bag is then kept at 4°C after submerging it in iced water. The amputate should not be placed directly on ice as this may cause damage secondary to frostbite. The purpose of these steps is to extend the time before irreversible damage is sustained secondary to anoxia, and this is particularly relevant when the amputate comprises large volumes of muscle, which tolerate ischemia poorly. This is typically the case with proximal limb amputations. Muscle tissue has high metabolic demand and rapidly deteriorates in ischemic conditions, which leads to irreversible myonecrosis.

3. Surgical replantation

The decision to replant a limb depends on multiple factors (Table 1), expertise and surgical maturity.
Chung et al. advocate that, considering the improved patient-reported outcomes and long-term functional benefits, when technically feasible, replantation should be attempted in digital amputations involving 29:

- Three or more digits

Table 1. Relative indications and contra-indications for limb replantation. Abbreviations: Flexor Digitorum Superficialis (FDS).

<table>
<thead>
<tr>
<th>Relative Indications</th>
<th>Relative contra-indications</th>
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<tbody>
<tr>
<td>Thumb amputation</td>
<td>Life threatening injury/ systemically unwell</td>
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<tr>
<td>Single digit amputation distal to FDS insertion</td>
<td>Location of finger injury proximal to FDS</td>
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<tr>
<td>Multiple Digit amputation</td>
<td>Mechanism of injury: Crush / Avulsion</td>
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<tr>
<td>Mid-palmar/hand/ proximal upper limb amputation</td>
<td>Multi-level injury</td>
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<tr>
<td>Pediatric amputation</td>
<td>Significantly prolonged ischemia time</td>
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<td>Patient factors: age, diabetes, smoking, motivation</td>
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When replanting an amputated part, the surgeon should ensure that function is maximised and that the predictive outcome is better than that of a prosthesis. The thumb provides 40-50% of hand function and has an essential role in opposition. One should recognise that a thumb with limited movement is more functional than other reconstructive procedures or prosthetics.30 Multi-digit amputations result in significant functional issues thus in these patients the thumb and middle fingers are prioritised to allow for a pincer grip. An important landmark regarding the level of amputation is the Flexor Digitorum Superficialis (FDS) insertion. There is ongoing debate in the literature regarding replantation of a single digit relative to this level. Evidence suggests that a replanted finger with amputation distal to FDS insertion is associated with limited function and has an overall negative effect on dexterity, especially with a poorly-functioning proximal interphalangeal joint.31 However, this is often attempted as the function of the replanted finger is enhanced by the additional length even if there is a stiff or fused DIPJ.32 Consequently, surgical practice varies internationally and by surgeon. In addition, some units across the globe perform microvascular replantations of distal fingertips.33
4. Ischemia & Reperfusion Injury

Ischemia is an important predictor of successful replantation.\textsuperscript{34} The ischemic process leads to elevated glycolysis, lactic acid production and subsequent intracellular pH reduction.\textsuperscript{35} Prolonged ischemia results in a variety of cellular metabolic changes (Figure 2). Ischemia leads to depletion of ATP in individual cells, altering the ionic pump functions and favouring entry of calcium, sodium, and water into it.\textsuperscript{21} This process also promotes the expression of pro-inflammatory gene products in the endothelium, whilst repressing protective gene products; thus inducing a cellular state that increases tissue vulnerability to further injury on reperfusion.\textsuperscript{36} If the duration of ischemia lengths beyond a critical point of tolerance, extensive liquefactive necrosis will result.\textsuperscript{37,38}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{flowchart.png}
\caption{Flowchart illustrating the cellular effects of ischemia. Abbreviations: ATP- Adenosine Triphosphate; IRI- Ischemic Reperfusion Injury; LAM- Lipoarabinomannans; NO- Nitric Oxide; ROS- Reactive Oxygen Species.}
\end{figure}

Irreversible ischemia reportedly occurs in muscle tissue after 4 hours of warm and 6-8 hours of cold ischemia time.\textsuperscript{39} In digital amputations, due to the lack of muscle tissue, replantation is not generally recommended when maximal warm and cold ischemic times exceed 12 hours and 24 hours, respectively.\textsuperscript{40} Alternatively, for proximal amputated limbs with high muscle mass, six hours of warm and twelve hours of cold ischemia is maximally advocated. However, the literature suggests that cold ischemia should
not influence the decision-making process because this does not compromise amputate survival and functional recovery. For instance, reports include successful replantation of digits at 94 hours of cold ischemia time and hands at 54 hours of cold ischemia time with satisfactory results. However, in proximal limb amputations, if this is not promptly replanted, there is an increased risk of life-threatening complications including rhabdomyolysis, myonecrosis and life-threatening IRI, on top of the already detrimental effect of fibrosis and scarring.

Many situations impede replantation within these recommended times but still warrant an attempt, particularly in paediatric patients or occupations that require high manual dexterity. Factors delaying time to replantation include multiple trauma, other life-saving surgery that takes priority, and multiple amputated part replantation.

IRI is a phenomenon that results from the abrupt reperfusion of the ischemic vascular bed that provokes a degree of tissue injury. Its pathogenesis has been studied and described as early as 1965 by Anon et al. who experimented with replantation of totally amputated canine extremities. Acute ischemia reperfusion of a severed limb leads to deleterious local as well as systemic effects by stimulating cytokine activation (specifically TNF-α and IL-6) and simultaneous multiple organ dysfunction. The abrupt reperfusion generates a burst of reactive oxygen species from the vascular endothelial cells in the post-ischemic amputate causing an inflammatory-like response to occur at the onset of reperfusion. Although literature is inconclusive regarding the source of the cytokine production, local production in the reperfused limb seems to be the most likely source, leading to a massive influx of polymorphonuclear leukocytes. The continuous rise in plasma concentration of IL-6 throughout the period of reperfusion, observed by Yassin and his colleagues suggests that reperfusion injury, rather than ischemia, is the stimulus for the cytokine production. This results in endothelial dysfunction, decreased endogenous nitric oxide generation, increased superoxide anion generation, and release of pro-inflammatory cytokines into the interstitial and vascular space. The vascular endothelium has been found to play an important regulatory role. Examples include the control of haemodynamic processes, vascular remodelling, immunity, metabolic functions, and anti- and pro-thrombotic processes. The term “arterial insufficiency” has been used to describe the inadequate and lack of uniform perfusion at microcirculatory level as a result of endothelial dysfunction.

IRI may involve not only the ischemic limb being replanted but also induce systemic damage to distant organs, potentially leading to multi-system organ failure. Although a similar risk in cytokine increase is observed postoperatively following major surgery, endotoxemia in the absence of bacterial translocation remains a feature uniquely attributed to the cellular mediators produced locally in the reperfused tissue. Animal experimental studies have shown that the lung is particularly vulnerable to remote ischemia compared to the hepatic and renal
systems. Several theories have been postulated, concluding that this may be due to the sequestered pulmonary leukocytes which are activated by the humoral mediators generated in the reperfused tissue. This causes damage by plugging pulmonary capillaries and disrupting their vascular endothelium.\textsuperscript{53-55} It is also possible that resident mast cells and macrophages amplify this injury in the lung by producing potent neutrophil chemo-attractants like leukotriene B4, in addition to histamine, serotonin, and platelet activating factor.\textsuperscript{55} Other organs frequently involved include the cardiac, renal and gastrointestinal systems.

Clinically, IRI can manifest diversely, ranging from benign transient arrhythmias to fatal multiorgan dysfunction syndrome.\textsuperscript{56} The response to IRI varies greatly among individuals, with increased vulnerability in the high risk group.\textsuperscript{35} Risk factors for IRI are categorised into fixed and modifiable risk factors. Fixed risk factors include advanced age, male gender, hereditary factors and significant muscle mass. Modifiable risk factors include tobacco smoking, hyperlipidaemia, hypertension, obesity and diabetes mellitus.\textsuperscript{21}

There have been many controlled, experimental models which have demonstrated therapeutic strategies – alone or in combination – in the prevention of IRI. This includes ischemic preconditioning, antioxidant therapy, calcium antagonists and leukotriene B4 antagonists to name a few.\textsuperscript{57–59} However, these have tended to yield equivocal results in the clinical setting, or have not been approved for human trials. Therefore, as it stands, timely reperfusion of the ischemic limb remains the cornerstone of clinical practice.

Ischemic preconditioning (IPC) refers to adaptive mechanisms that are triggered by brief episodes of ischemia and reperfusion. This protects tissues from the sustained post reperfusion-ischemic injuries that may occur.\textsuperscript{60} IPC, however, is not pertinent to unexpected amputated extremities. In such situations, manoeuvres such as intermittent reperfusion or post-conditioning are more relevant.\textsuperscript{61,62} Both intermittent reperfusion and ischemic post-conditioning refer to the same concept whereby alternating episodes of reperfusion are employed after a prolonged episode of ischemia, as seen in amputated extremities. Intermittent reperfusion has been shown to attenuate the IRI as seen in IPC although not to the same extent.\textsuperscript{61} Subsequently, the term post-conditioning, first reported by Zhao et al.,\textsuperscript{62} has been documented as an effective protective strategy in different disciplines and animal models.\textsuperscript{65-67}

The future of this field relies upon optimisation of the amputated extremity by addressing the ischemic and reperfusion process particularly in proximal limb amputations. In order to improve ischemia time, temporary catheter shunting can be used in major limb replantation.\textsuperscript{66} Studies evaluating ex vivo perfusion with anticoagulated blood and medications including allopurinol and streptokinase have demonstrated prolonged warm ischemia time although these are not common in pre- or intra-operative surgical practice.\textsuperscript{67,68} Additionally, the perfusion of the amputate using a modified solution before allowing
normal blood to flow has also been explored with promising results.69 Yet, a randomised multi-center trial has shown no difference among those patients with acute ischemic limbs treated with routine thrombo-embolectomy and normal blood reperfusion and those treated with thrombo-embolectomy and controlled reperfusion (autologous blood mixed with crystalloid solution, with supplemented allopurinol).70

5. Conclusion

There is no doubt that for successful replantation surgery to take place an organised network able to promptly manage, refer and transfer patients with these devastating injuries to a high volume center with available microsurgical expertise is essential. Nonetheless, minimal scientific progress in this field has been witnessed to meaningfully improve the care provided to the patients falling victim to such injuries. The principal limiting step is to improve the handling of the amputated part. Research into pharmacological post-conditioning is a possible strategy when combined with ex vivo perfusion to overcome IRI preventing oxygen free radical injury to skeletal muscle, particularly in proximal injuries. Regardless of its promising research, its therapeutic benefits await to be seen.

6. Acknowledgements

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