

## THE ULISSES™ DEVICE PRESERVING LIMBS IN THE 21<sup>ST</sup> CENTURY

**Background:** Combat and mass casualty situations, particularly where detonations have occurred, invariably produce a significant amount of soft tissue damage and can be associated with extremity avulsion. Industrial accidents and auto accidents can have the same tragic result. The perception of one's own body and its appearance can be significantly affected by the loss of a major appendage.<sup>1,2</sup> From a psychological standpoint, amputees can suffer from depression, anxiety, and in severe cases, be driven to suicide. Treatment options to counter traumatic limb amputation include: prosthetics, transplantation, or re-plantation.

Prosthetics, the most common solution, often manifest physical complications, such as sweating, heat rash, contact dermatitis, and ulceration of the residual limb at the attachment site. In addition, the prosthetic liner may serve to incubate bacteria, leading to serious infections.

While transplantation greatly reduces or eliminates the physical and psychological issues that occur with prosthetic use, transplant patients require daily doses of immunosuppressant, suffering the consequences of their side effects. This is why re-plantation represents the preferred treatment approach: it eliminates the complications of prosthetics and transplantation.

In order to facilitate replantation, the patient must be stabilized and transported to a facility capable of replantation. When that happens, the viability of the avulsed extremity may need to be preserved for several hours, or even days. Pathogenic contamination may also be an issue. Omoke et. al estimate that the infection rate in traumatically amputated limbs is on the order of 60%.<sup>3</sup> Warkentien et.al demonstrated a mold infection rate in excess of 68% in combat wounds.<sup>4</sup> The ability to perfuse avulsed tissue while simultaneously oxygenating and filtering the perfusate to remove pathogens would greatly enhance the potential of successful replantation.

**Technology Development:** For replantation to be a viable option, the patient must be stabilized and transported to an advanced care facility. This means the avulsed extremity needs to be preserved for several hours or even days. Unfortunately, skeletal muscle will not normally remain viable for more than 6 hours at ambient temperatures, due to the onset of ischemia-induced necrosis.<sup>5</sup> Tissue degradation ultimately leads to loss of cell homeostasis, releasing a multitude of factors that prime the tissue for free radical production - leading to irreversible ischemic and reperfusion injuries.<sup>5,6</sup> Thus, replantation of large limbs that have experienced prolonged ischemia may result in a number of serious complications.<sup>5-9</sup>

Current methods for preserving severed limbs fall into one of two categories: simple ischemic storage, and continuous perfusion storage. Both have been applied with and without the addition

of hypothermia. In clinical practice, cold ischemic storage is most often used because of its simplicity, low cost and relative effectiveness.<sup>10</sup>

Optimally, flushing the extremity with cold preservation solution and rapidly cooling to 4<sup>0</sup> C helps to minimize ischemia and metabolic derangement. In this case, clinicians combine hypothermia with a specially formulated preservation solution, such as the Euro-Collins, University of Wisconsin, HTK, or Viaspan solution.<sup>10,11</sup> This approach maintains viability for no more than 12 hours.<sup>10-13</sup>

In mass casualty situations and particularly in a combat setting, high-level trauma care is not readily available, and several limbs may simultaneously present for preservation. Twelve hours may not be sufficient for collecting the extremities, stabilizing the patients, and transporting them to a medical facility with the resources needed for limb replantation. A limb preservation technology capable of maintaining the limb for 24 hours or more would, therefore, facilitate prolonged forward care of detached limbs.

Two issues must be addressed for successful long-term storage and preservation: (1) the reduction of tissue metabolic demand, and (2) increasing the availability of critical substrates, such as oxygen and tissue nutrients. Extra-corporeal hypothermic perfusion with an oxygenated solution, a technique which combines these two principles, has been shown experimentally to preserve isolated canine cardiac muscle for as long as 24 hrs.

Numerous studies have since demonstrated the superiority of oxygenated perfusion storage over other methods.<sup>14-16</sup> For example Araki et al demonstrated that oxygenated perfusion preservation is effective in mitigating rat hind limb hypoxia for 24 hours.<sup>17</sup> Taeger et al showed that oxygenated, hypothermically-perfused muscles had a statistically significant greater ability to exert force compared to non-perfused muscles.<sup>18</sup> Domingo-Pech et al has also verified that preservation by extracorporeal circulation maintains limbs in good condition, with reversible ischemic lesions and less than 5% of the muscle fibers showing abnormalities.<sup>19</sup> Despite the effectiveness of this method of preservation, no commercially available devices that provide hypothermic, oxygenated, perfusion preservation for separated limbs have been developed. While laboratory systems have been assembled for experimentation, they are usually complex and impractical for clinical application, particularly under field conditions.

**ULiSSES™ – The Solution:** Our laboratory has been involved in cardiac preservation research for over 30 years. We have developed a lightweight, portable hypothermic, oxygenated perfusion preservation technology, under the ULiSSES™ brand (for Universal Limb Stasis System for Extended Storage). The ULiSSES™ technology is capable of maintaining interstitial oxygen and high energy phosphates in cardiac muscle at levels consistent with aerobic metabolism for at least 12 hours<sup>16</sup>, with recent prototypes and improved technology indicating a maintenance time of 24 hours, and longer.

Our studies of cardiac muscle performance also show that the contractility, as measured by stroke work, of adult, human-sized, canine hearts, preserved using this device for 14 to 24 hours is statistically identical to freshly recovered hearts.<sup>20</sup> In experiments where canine hearts were transplanted after 12 hours of oxygenated, hypothermic, perfusion storage, the transplanted hearts needed minimal pharmacologic support, with LVEDP (left ventricular end-diastolic pressure), cardiac output, and dP/dT (pressure-time derivative) showing no statistical difference from pre-transplant function. Conventionally-stored hearts required both pump and pharmacological support with significantly depressed LV (left-ventricular) function.<sup>21</sup>

We have also demonstrated that oxygen consumption during oxygenated, hypothermic perfusion storage is linearly, directly, and highly correlated with post-preservation tissue function.<sup>22</sup> In other findings, tissue recovered after 1.5 hours of ischemia initially showed low oxygen consumption followed by a gradual improvement, coming up to the expected level for the storage temperature.<sup>23</sup> This finding suggests a real potential for successful soft tissue resuscitation following prolonged ischemia.

Since cardiac muscle and skeletal muscle are similarly sensitive to ischemia, the concept of oxygenated hypothermic limb perfusion may have potential. Bretschneider et al showed that cardiac muscle stored at 4<sup>0</sup> C requires 0.125 ml-O<sub>2</sub>/min/100g.<sup>24</sup> Extrapolating the findings of Seiyama et al to 4<sup>0</sup> C, skeletal muscle would require 0.012 ml-O<sub>2</sub>/min/100g, which is roughly ten times less.<sup>25</sup> These findings strongly support the idea that hypothermic, oxygenated perfusion of separated limbs will have a high probability of maintaining skeletal muscle oxygen requirements and, ultimately, viability for extended time periods.

The ULiSSES<sup>TM</sup> device operates using a combination of fluidics, and mechano-elastic principles for the hypothermic, oxygenated, perfusion, preservation, of limbs and vascularized tissue. The device does not rely on electrical power to operate. Instead, it harvests energy from expanding compressed oxygen to simultaneously drive pulsatile perfusion of the limb and oxygenation of the preservation medium. Oxygen, fluid, and fluidics logic combine to perfuse, preserve, and resuscitate tissue at a rate that mimics a natural heartbeat. Capillary fibers with a large surface area, permeable to O<sub>2</sub>/CO<sub>2</sub>, provide gas exchange and efficient oxygenation of the perfusate. These innovations result in a dramatic reduction in size, weight and cost, leading to a portable, single use device that is expected to provide long term preservation.

Early experiments using a first order prototype demonstrated that an oxygen partial pressure of 420 mmHg in the perfusing solution at 4<sup>0</sup> C can be attained after only 40 min of operation (Figure 1). Based on flow at 40 ml/min and a pulse rate of 70/min, the prototype delivered 1.0-ml O<sub>2</sub>/min to the stored tissue at 4<sup>0</sup> C. The delivered oxygen thus satisfied the oxygen requirements of a skeletal muscle mass of approximately 7.5 kilograms. At this rate of perfusate oxygenation, saturation is extrapolated to occur after 70 min, resulting in the ability to support the metabolic oxygen needs of about 12 kg of skeletal muscle. More recent prototypes have greatly improved upon these early results.

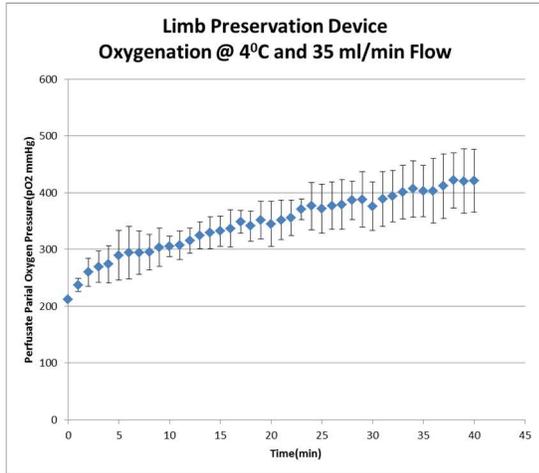


Figure 1



Figure 2

In order to demonstrate feasibility, a scaled-down version of the early prototype was used to perfuse a rat hind limb recovered 90 minutes after euthanasia. (Figure 2) The limb was perfused with oxygenated Krebs Henseleit solution at approximately 18<sup>0</sup> C for two hours. Oxygen consumption by the limb was 0.11 ml-O<sub>2</sub>/min/100g. This finding was consistent with the findings of Seiyama et al, which showed rat skeletal muscle oxygen consumption at 15<sup>0</sup> C is 0.10 mlO<sub>2</sub>/min/100g.<sup>25</sup>

But it is critical that oxygenated perfusion begin as soon as possible after the limb is separated. For example, in a separate experiment, rodent hind limbs were perfused with oxygenated Krebs Henseleit solution at 19<sup>0</sup> C for seven hours, starting over three hours after recovery. Initial oxygen consumption was very low, rising to a plateau of only 0.023ml-O<sub>2</sub>/min/100g after three hours of perfusion. (Figure 3) It is clear from this observation that more than three hours of warm ischemia time resulted in significant, irreversible muscle injury.

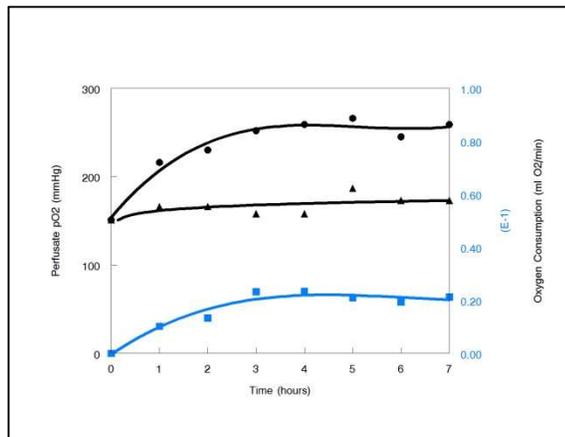


Figure 3

In previous studies, our findings suggested that oxygen consumption during preservation and organ function following re-warming were directly correlated.<sup>22</sup> Extrapolating from those observations suggest that a little more than 23% of the rodent skeletal muscle survived. This finding is consistent with the observations of Belkin et al, who demonstrated a 25% survival of rodent skeletal muscle after 3 hours of warm ischemia.<sup>26</sup> Again, early perfusion of separated limbs is essential.

In short, these observations, as well as others in the literature, strongly support the notion that the ULiSSES™ technology will have a significant impact on limb preservation and limb resuscitation. We expect this new technology to truly make a difference.

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