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INNOVATION

Therapeutic limb hypothermia for the treatment of traumatic acute limb ischemia

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ABSTRACT

Acute limb ischaemia (ALI) is an emergent clinical condition that strains pre-hospital resources and impacts healthcare costs and patient quality of life. Hypothermia has long been used in clinical and research settings to mitigate ischaemic damage in tissues, but prompt reperfusion is needed to prevent loss of limb or function from ALI. To address the unmet need for pre-hospital intervention of threatened limbs awaiting definitive specialty care, we have focused on controlled application of hypothermia. Over years of animal experiments, phantom limb creation, and materials selection, we conceptualised and created a portable limb-cooling device that can be used alone or combined with a traditional tourniquet or resuscitative endovascular balloon occlusion of the aorta. Here, we describe our process of building and testing the device, from computer simulation through animal-limb metabolic studies, to prototype testing.

Abbreviations: ALI: acute limb ischaemia; pDCR: prolonged damage control resuscitation; REBOA: resuscitative endovascular balloon occlusion of the aorta; TEC: thermoelectric cooler

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1. Introduction

Acute limb ischaemia (ALI) is defined as a sudden interruption of limb perfusion that threatens limb viability. It may be caused by trauma, embolic or thrombotic arterial occlusion, or life-saving haemostatic therapies such as tourniquet use or resuscitative endovascular balloon occlusion of the aorta (REBOA) [1–4]. ALI presents a significant burden to patients and to the healthcare system - not only does it substantially impact patient morbidity and mental health, it also generates high healthcare cost and resource utilisation. Annually, ALI leads to roughly 40,000 amputated limbs at a monetary cost of $7 billion, which is projected to increase as the general population ages [5]. The incidence and cost of ALI is even greater in times of combat. In 4 years of war in Iraq and Afghanistan, limb-threatening extremity injuries resulted in over $42 million in immediate costs and are estimated to cost an additional $1.2 billion in lifetime disability [6]. But more importantly, studies of U.S. service members have shown that lower-extremity vascular injury impacts quality of life and may result in worse physical and mental outcomes despite a greater than 80% limb salvage rate [7,8]. This is consistent with studies indicating that limb salvage in traumatic ALI may not predict functional outcome [9], which further suggests that current treatment of traumatic limb injury is inadequate and demands improvement.

Definitive management of traumatic ALI requires intervention by a surgeon, which often necessitates rapid transfer to a tertiary care facility. Tourniquets are being more commonly used for prehospital management of ALI caused by a traumatic injury in both the military and civilian environments [3,10,11]. Although tourniquet application may be a life-saving method of haemorrhage control, prolonged or inappropriate use can worsen limb ischaemia [3,12–14]. Surgical practice has demonstrated that with some degree of variability, compromised perfusion may cause nerve damage in less than 1 h, muscle damage at 2 h, permanent

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disability at 4 h, and need for amputation after 6 h. This short timeframe for restoration of perfusion is challenging regardless of the setting, but difficulty is amplified multifold in remote locations or in a combat environment, where it is unlikely that transfer and adequate reperfusion of the limb can occur quickly. Despite these urgent challenges, there currently is no pre-hospital therapy to mitigate ischaemic limb damage during that time.

To slow the progression of ischaemic damage to tissue and organs, hypothermia has long been used in cardiac surgery, transplant surgery, and neurosurgery. The benefit of local hypothermia in ischaemic injury has been validated in many animal models and continues to be an active area of research [15–20]. To address the need for an intervention that can reduce ischaemic injury after traumatic acute limb ischaemia, we conceptualised a portable limb-cooling device that can be used alone or combined with a traditional tourniquet (or REBOA) with an auto-regulating cooling sleeve and air splint. This device would be especially important in remote or austere environments where a specialised surgeon is not immediately available for limb reperfusion. Here we will describe the process of conceptualising, building, and testing the device.

2. Approach and outcomes

2.1. Conceptualisation

We envisioned this limb-cooling device as a result of several observations and ideas – the resurgence of tourniquet use in military and civilian injury, the unmet need for pre-hospital intervention for traumatic limb injury, the established benefit of induced hypothermia in multiple surgical disciplines, and anecdotal experiences of civilian vascular surgeons with inducing hypothermia of limbs after embolic or traumatic events (Figure 1). Although these observations suggested that therapeutic limb hypothermia would be helpful in the case of traumatic injury, we endeavoured to demonstrate the therapeutic benefit.

Our reasoning was as follows. To justify an intervention for traumatic ALI, we would need to demonstrate that ALI and prolonged damage control resuscitation (pDCR) would irreversibly affect the limb. We therefore generated preliminary data from a proof-of-concept study in swine showing that induction of ALI resulted in irreversible muscle injury, and that limb hypothermia altered local muscular metabolism (unpublished data). Next, to justify therapeutic limb hypothermia as an intervention, we would need to demonstrate that hypothermia limits the effects of tissue injury seen in acute limb ischaemia. Once hypothermia was established as beneficial, we would need to define the optimal hypothermic temperature and timing to achieve a clinically useful outcome.

Our goals were therefore fourfold – first, to determine the thermodynamic properties and limits of limb hypothermia along with the parameters to achieve optimal hypothermia; second, to delineate ALI and pDCR pathophysiology in a swine model; third, to determine the effect of limb hypothermia on limb viability during ALI with pDCR; and fourth, to design a novel therapeutic limb-cooling device.

2.2. Computer thermal simulations

We ran thermal simulations to establish the thermodynamic properties and limits of cooling a human limb, and to determine the external cooling parameters required to achieve optimal therapeutic limb hypothermia [21]. For this, we created a two-dimensional finite-element model using COMSOL Multiphysics® (Version 3.5 A, COMSOL AB, Stockholm, Sweden) to predict the temperature history when maintaining the surface of a human calf at 0 °C based on known thermal properties (i.e., density, heat capacity, conductivity) of different tissue types. This model demonstrated that a low surface temperature must be maintained for at least 1 h to cool the limb to less than 20 °C. Next, a three-dimensional finite-element model of an entire human leg was created, then computed using ANSYS® (ANSYS, Cannonsburg, PA, USA). Temperatures were calculated over time at several locations along the length of the simulated leg. A similar computer simulation process was completed for a swine leg. These simulations established a theoretical performance baseline and cooling

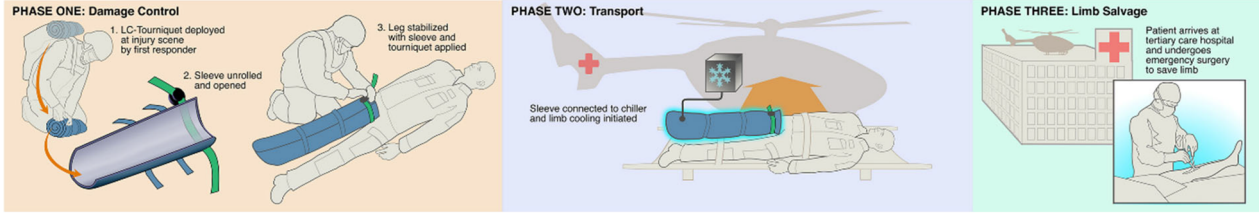

Figure 1. Example usage scenario of the limb-cooling sleeve. LC: limb-cooling.

rate on which our prototype and future tests would be based. With this preliminary data, we began designing our swine experiments and developing our limb-cooling device prototype and human-limb thermal phantom.

2.3. Laboratory experiments in swine

We performed our swine polytrauma and haemorrhagic shock experiments below in a cohort powered to demonstrate a statistically significant benefit of using therapeutic limb hypothermia. During these experiments, when we recreated an ischaemic insult, limb tourniquets and aortic cross-clamping were both required because of collateral limb perfusion, making either approach alone insufficient to produce an ischaemic limb. We also lengthened the ischaemia time and shortened the reperfusion time to produce more reliably an ischaemia-reperfusion response similar to that of a human limb.

After confirming that we could model systemic haemorrhagic shock and ischaemia-reperfusion injury in swine, we conducted a series of therapeutic limb hypothermia experiments. In all experiments, temperature histories were obtained using temperature probes placed in specific areas of the hind limbs, and tissues underwent microdialysis and histological sampling (Figure 2).

First, we conducted an experiment reproducing ALI, pDCR, and reperfusion, without limb hypothermia. This model clarified the pathophysiology of these insults by allowing us to follow metabolic changes and injury over time. We found evidence of permanent limb injury after 6 h of ischaemia and 2 h of reperfusion, similar to the assumed human limb injury pattern. Next, we obtained the same measurements in two control groups of swine: one that experienced ischaemia only, without haemorrhage or pDCR, and one that did not experience ischaemia, haemorrhage, or pDCR. With the inclusion of these control groups, our results confirmed significant metabolic differences between ischaemic and non-ischaemic limbs. Lastly, we conducted an experiment using our refined ALI/pDCR/reperfusion protocol with induced limb hypothermia. Each pig had one hind limb cooled using our device prototype to 32 °C, 15 °C, and 5 °C, and the other kept at ambient temperature as an internal control. We found that 15 °C was the most clinically useful target hypothermic temperature, below which there

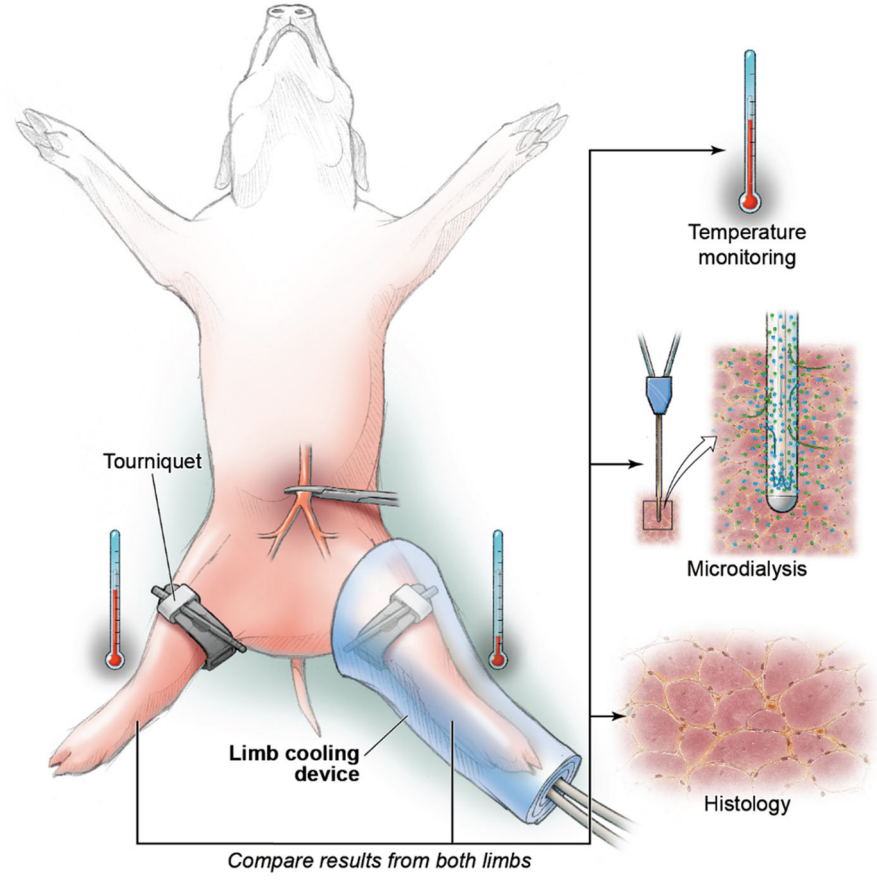

Figure 2. Depiction of swine lab experimental setup.

did not appear to be any physiologic benefit to local hypothermia and the risk of skin injury increased, as did the power requirements for the device to function. We noted that all pigs maintained core body temperature despite limb hypothermia and traumatic injury, and none sustained skin damage from induced hypothermia. These studies further elucidated the required cooling time to achieve target limb temperature and confirmed that hypothermia did in fact impact metabolism.

Collectively, our unpublished data from the non-survival swine experiments described above (submitted for publication January 2024) demonstrate a metabolic benefit associated with limb hypothermia in the setting of traumatic limb ischaemia. To support additional swine experiments that will compare the functional differences due to limb hypothermia in traumatic ALI we have secured further funding from the Department of Defense.

2.4. Prototype development

Our preliminary thermal simulation results demonstrating heat transfer behaviour of the human lower leg formed the basis for devising our first experimental setup. Using a metal human limb model, we attached multiple Peltier solid-state thermoelectric coolers (TECs) with a counter-current heat sink circulating water system. We found that the heat transfer rate of the TECs to the ambient air was inadequate to remove enough heat to induce therapeutic hypothermia of a human limb [22]; we moved from TECs to a more conventional circulating-liquid external cooling system. We started by looking into available cooling pads on the market, but after basic trials on swine and human thermal phantom limbs, we realised that existing styles would not fit our purpose for several reasons. Our device required a sleeve larger than what was commercially produced and would require a custom shape to avoid restriction of coolant flow. Moreover, considering the intended use of our device in the combat environment, we required an efficient system to minimise chiller (cooling machine unit) size, and a durable base material. We concluded that we would need to generate our own designs and began concurrent development of human and swine cooling-sleeve prototypes.

We determined that a sleeve with multiple dot welds that formed channels would minimise coolant volume (and thus minimise chiller size) while maximising coolant flow and surface area of limb contact (Figure 3). The sleeve material had to be flexible while still able to maintain the dot welds and withstand the pressure of flowing coolant. Next, for the outer base layer we considered several fabrics and conducted ballistics impact testing to assess puncture resistance. Ballistics-grade nylon fabric was selected given its favourable balance of impact testing results and cost. To augment efficiency of heat transfer, a hydrogel thermal contact layer was used.

Basic technical specifications of a suitable chiller were estimated based on thermal simulation results,

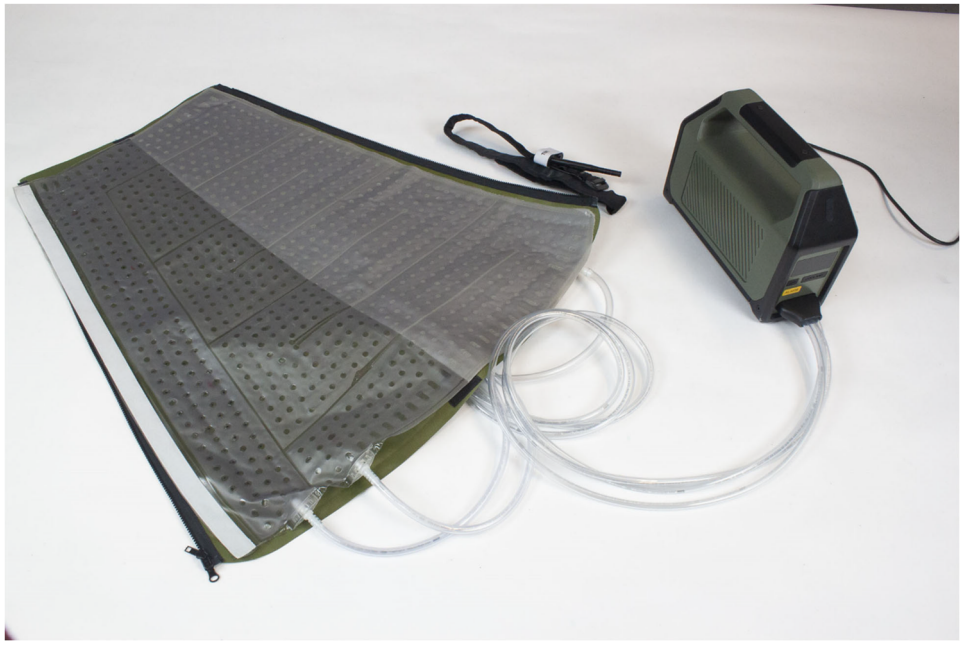

Figure 3. Photo of limb-cooling device chiller and sleeve with dot welds.

and air pump control was added for the integrated air-splint component of our device. Through testing on the human thermal limb phantom, the development of which is described below, we verified that the optimal wattage and output temperature were 800 W and 0 °C, respectively.

2.5. Development of the limb phantom and testing

While our prototype was under development, we worked on creating a human thermal limb phantom. Because medical devices cannot be tested on humans in such preliminary stages, our phantom allowed for early prototype testing. We also created a swine thermal phantom.

Using the software suite Simpleware (Synopsys, Inc., Mountain View, USA), we created a 3D digital structure on which our thermal phantom would be based by inputting the CT scan of a normal leg of a young male patient who had a body mass index in the 50th percentile. Next, we assessed the suitability of different building materials. At first, we experimented with silicone layers containing varying percentages of graphite that modified the conductivity of each layer and thus simulated different tissue types. A heating element with thermal properties of bone was placed in the centre of the thermal phantom to heat it to physiologic limb temperature. However, we discovered that in this thermal phantom iteration, the conductivity and centric heating of the layers was inconsistent. A literature search indicated that water-based gels could be used as thermal tissue phantoms due to their similar thermal properties [23,24]. We thus revised our thermal phantom by 3D-printing a silicone and graphite shell and filling this with a commercially available water-based ultrasound gel and placing thermocouples wired along a structural 3D-printed "bone" for temperature sensing in the calf, thigh, and skin (Figure 4). To heat the thermal phantom to physiologic limb temperature, it was placed in a heated water bath until steady state was reached. Initial testing of the thermal limb phantom involved cooling with an ice bath to establish a baseline cooling speed. After a usable prototype was available, subsequent cooling tests were done with the cooling sleeve prototype (Figure 5).

In our formal prototype cooling experiment, we heated the thermal phantom to physiologic limb temperature, then applied our prototype attached to an 800 W chiller. The temperature histories of the thermocouples within the phantom's calf, thigh, and

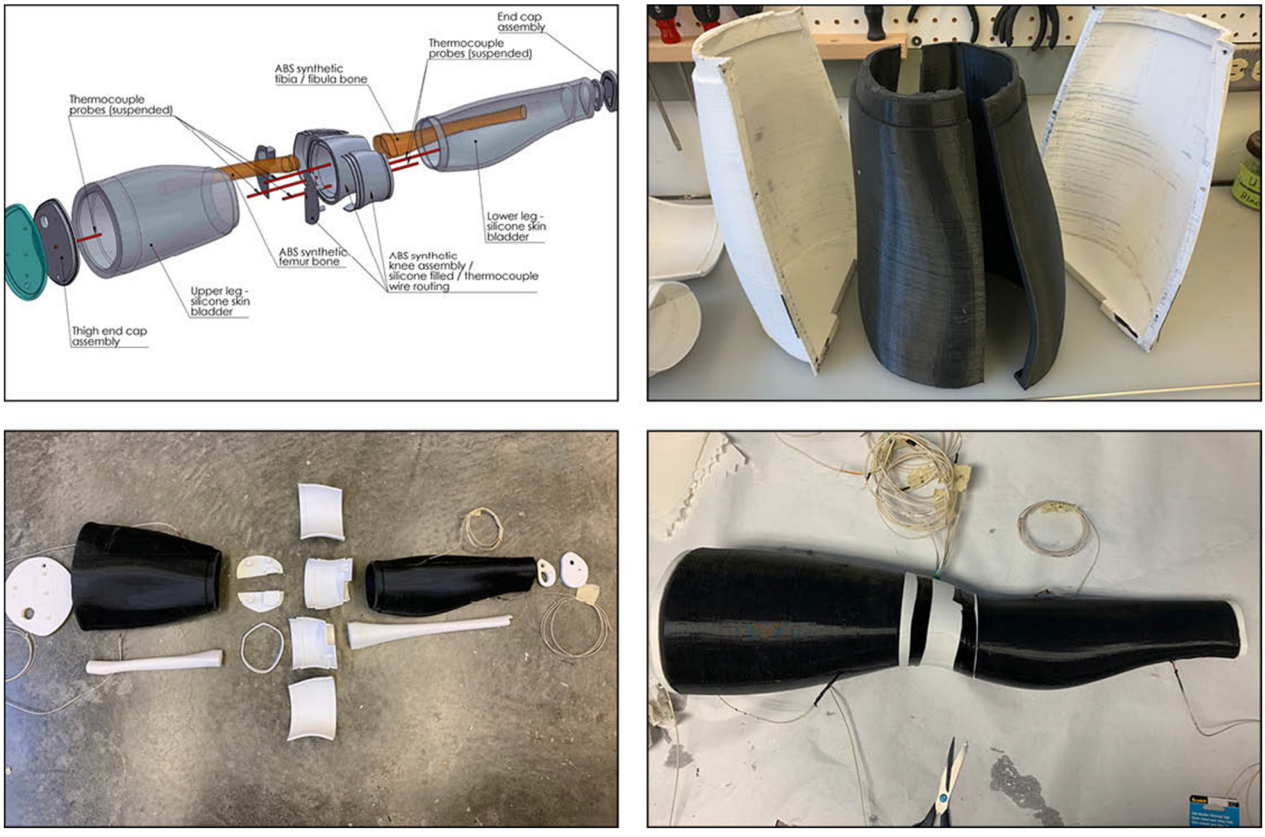

Figure 4. Human phantom limb with labelled components. ABS, acrylonitrile butadiene styrene.

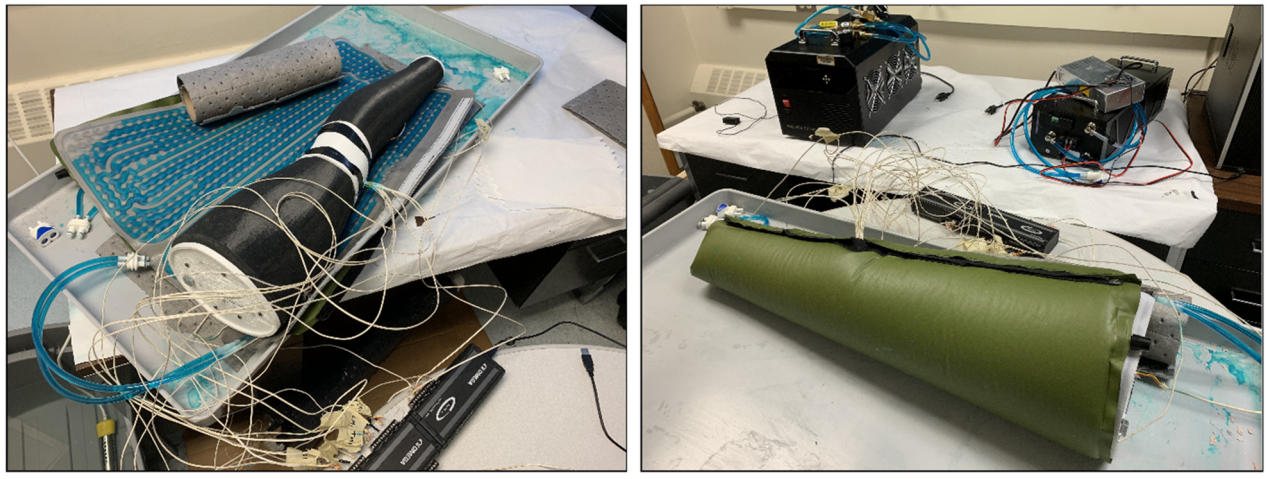

Figure 5. Cooling test performed by applying the cooling sleeve to the human phantom limb.

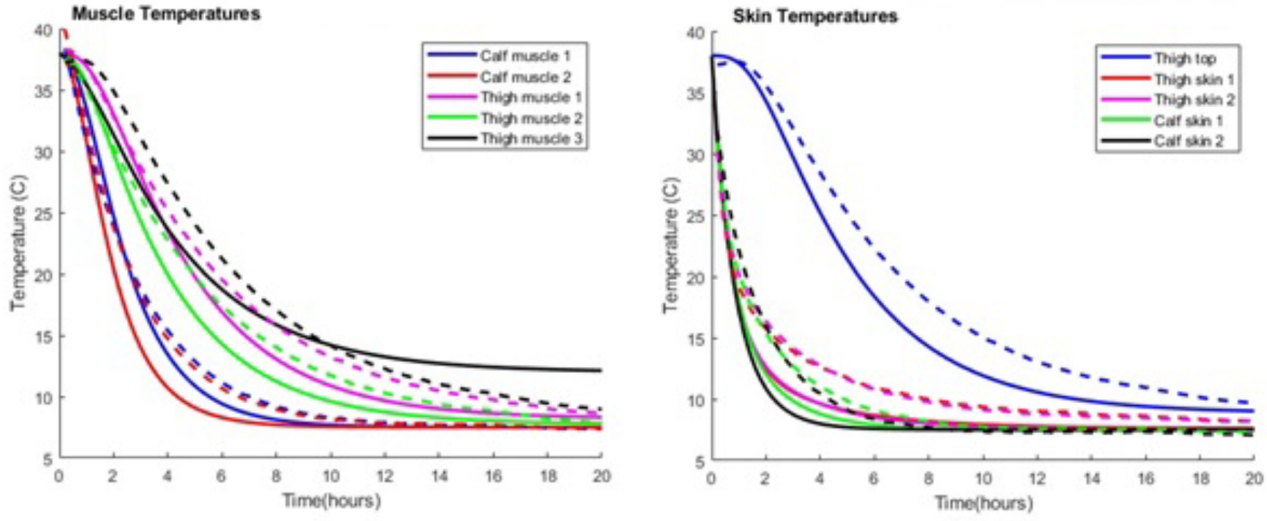

Figure 6. Comparison of cooling test results between human limb phantom testing and thermal simulation. Solid lines represent temperature curves from thermal simulation. Dashed lines represent temperature curves from limb phantom testing. Temperatures of the muscle and skin are shown.

skin were recorded over 20 h of cooling. These results were congruent with those from a thermal simulation that modelled cooling with a set surface temperature of 0 °C (Figure 6). When the prototype coolant temperature was set to 0 °C, the cooling speed of the thermal limb phantom was also consistent with the ice bath baseline. Because we had established that the phantom reflected the thermal simulation results, we then did additional tests on specific prototype components with the intention of improving the device. We discovered that an 800 W chiller cooled the thermal limb phantom down much faster than a 450 W chiller due to the initial heat load, but that the 450 W chiller could then maintain the low temperature. The cooling rate was greater with a higher pump level. Insulation of the splint only minimally improved chiller performance, which did not justify the trade-off of increased packaging size and weight. Insulation of the tubing increased cooling rate and decreased the time to reach 15 °C by about 2 h. The use of gel applied to the inner surface of the cooling sleeve as a conductive medium had almost no effect on the cooling rate.

After the results of the human thermal limb phantom were validated against its corresponding thermal simulation, we created a swine thermal limb phantom using the same technique. We aimed to prove that the human thermal limb phantom was a valid substitute for a true human limb by demonstrating that a swine thermal phantom accurately represented a true swine limb in terms of cooling test results. Using the swine thermal phantom and cooling sleeve designed for a

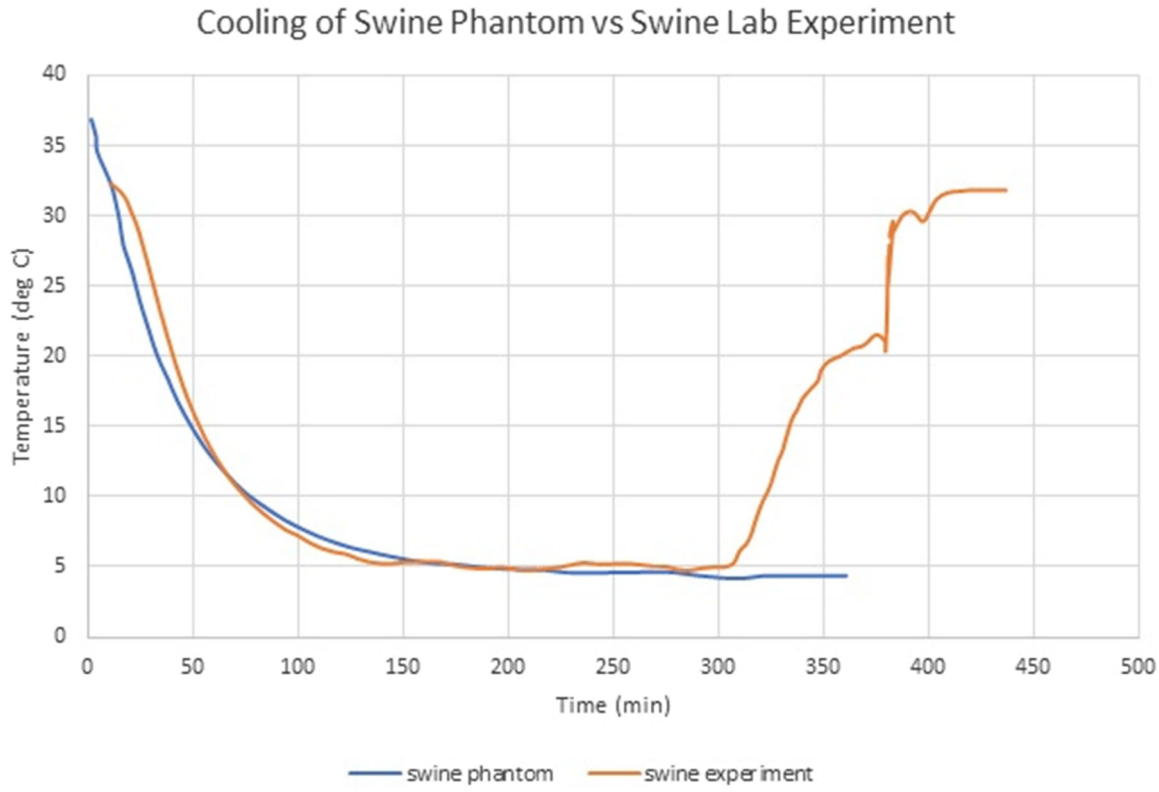

Figure 7. Comparison of cooling test results between swine phantom limb testing and swine lab experimental testing.

swine limb, a cooling test was carried out for 6 h at an ambient temperature of 27 °C. Temperature readings were similar between the swine thermal phantom cooling test and our laboratory swine limb hypothermia experiments (Figure 7). This suggested that our human thermal phantom was an appropriate surrogate for a true human limb.

3. Conclusion and next steps

Though our prototype has yet to undergo live human testing, the results of our swine and limb thermal phantom studies suggest that our limb cooling device would be beneficial in cases of limb ischaemia. We determined that ischaemia does indeed result in irreversible injury, but that inducing limb hypothermia to 15 °C within a short time frame is possible and can alter tissue metabolism to mitigate this. Although we considered other methods of local limb hypothermia that were based on external cooling (e.g., TECs, liquid nitrogen) or on internal cooling (e.g., using instilled coolant materials, endovascular cooling techniques), we felt the safety concerns and logistical challenges of using these methods in the field or during transport from the field were insurmountable. We opted for a simple and safe approach. The preponderance of our efforts has been focused on devising a pre-hospital ALI intervention of the right power and size in a useful form factor that would cool quickly enough without causing systemic hypothermia or skin damage. Through multiple rounds of testing and revision, we have developed an effective and relatively portable method of inducing therapeutic limb hypothermia. Based on our work, we will be able to ultimately achieve a form factor of 15–20 pounds for the entire device based on a direct (vehicle- or tent hospital- based) power source with battery backup to maintain limb temperature during patient transport.

Though our focus has been on optimising the device to address traumatic ALI in the combat environment, we anticipate applicability in many other circumstances. As a limb cooling device, it could serve as a pre-hospital intervention for embolic or thrombotic ALI or civilian traumatic ALI. And given that we have preliminary data demonstrating the benefit of therapeutic hypothermia towards metabolism in ischaemic limbs, our device could be a crucial adjunct in procedures that alter, reduce, or halt limb perfusion, such as REBOA or vascular shunt placement, or in severe limb injuries without direct arterial injury. As a general portable temperature control solution, our device could be suitable in the field for burn management or as a temporary blood cooler. Furthermore, the design of our sleeve itself offers versatility. With its maximised

ratio of contact surface area to circulating liquid, it could be part of a portable and efficient warming system for systemic hypothermia after traumatic shock or submersion. These potential secondary usages are diverse and will require additional investigation.

While the physiologic benefits of our cooling sleeve and limb hypothermia in the setting of ALI are currently being established by our past and future experiments, there are several barriers to its acceptance. Without demonstrating functional benefit or meaningful clinical improvement, our intervention would not be useful despite promising preliminary data. To address this, we next intend to compare the ambulatory ability of swine that received therapeutic limb hypothermia for ischaemic injury using our established ALI and pDCR protocol to that of control swine. Functional benefit would be demonstrated if the ambulatory ability of the swine who received therapeutic limb ischaemia were significantly better, as determined by a validated gait scoring system. And despite the lack of any pre-hospital intervention for ALI, the addition of a new medical device to the first responder toolbox will face numerous administrative, funding, and training hurdles. As we prepare for a future human trial, we are continuing to optimise the prototype, deliberating methods of functional benefit assessment, and considering the practical secondary uses of our device to extend its relevance. Our hope is that our limb cooling device will successfully fill the unmet need of a pre-hospital ALI intervention, thereby reducing this burden to patients and the healthcare system by preventing amputation and reducing functional disability.

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Author contributions

SA conceived the device idea and obtained research funding. JG and SA conceived and refined the device design. AE, KM, and AS developed the computer simulations, thermal phantoms, and thermal phantom tests. SA, NW, XW, KR, and EMB designed and supervised the animal studies and analysed the data. EK analysed the data. EK, SP, and SA drafted and edited the manuscript. All authors contributed substantially to its revision.

Disclosure statement

SA is a shareholder of TourniTek Inc., a Washington State company. SA is an inventor on the pending patent application PCT/US2017/022442 "Cooling Sleeve and Tourniquet" SA, JG, and AS are inventors on the pending patent application PCT/US2020/037153 "Feedback-Controlled Pressure Monitoring System for Limb-Stabilizing Medical Pressure Splints". The other authors have no conflicts of interests to report.

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