Nano-Oxygen Carrier for Machine Perfusion Organ Storage

G. Srinivas[*], David Eisnberg[**], and Jian Ling[***]

[*]TDA Research Inc.; gsrinivas@tda.com
[**]TDA Research Inc.; deisenberg@tda.com
[***]Southwest Research Institute; jian.ling@swri.org

ABSTRACT

Advancements in body armor combined with better combat casualty care have increased the survival rate of soldiers suffering battlefield injuries. The increased survivability, however, has resulted in an increase of wounded warriors being sent home with severe injuries that ultimately reduce their quality of life post-treatment. Recent advances in regenerative medicine offer exciting possibilities for new therapies and treatment of injuries; however, many surgical and treatment outcomes are still affected by the limited amount of time available for organ or tissue preservation following a trauma induced injury. Surgical outcomes could be improved if tissue salvage and conservation could be achieved over longer time periods. Towards this end, TDA Research, Inc. is developing a novel perfusion system for the salvage and repair of trauma injured tissue. This system will increase the preservation time for tissue, enabling advanced treatments or transplant capabilities. We are interested in collaborating with commercialization partners.

Keywords: genitourinary tissue, normothermic machine perfusion, oxygen carrying perfusate, perfusion machine

1 BACKGROUND

The standard tissue preservation technique, static cold storage, slows the cell's metabolism, and hence its ischemic injury, by cooling it to 4^{o}C. An alternative which has shown great promise in extending shelf life, repairing damaged tissues, and improving transplantation outcomes is normothermic machine perfusion (NMP). Instead of slowing the metabolism via cold temperatures, NMP prevents (and even reverses) ischemic injury by maintaining the tissue's normal metabolic function ex-vivo while providing nutrients and oxygen. NMP allows medical personnel to store transplantable tissues while maintaining metabolic activity, and to store and transport tissues for longer periods of time than for standard static cold storage. Additionally, it allows medical personnel to evaluate the tissue's viability and provide the tissue with time to undergo repair before transplantation, increasing the potential supply of transplantable tissues and organs.

Machine perfusion uses an *ex-vivo* system to deliver an oxygenated perfusate to a tissue sample or organ to preserve it; in this manner, metabolic activity, and thus potential tissue repair [1,2] can be maintained *ex-vivo* prior to transplantation of the tissue or organ. Numerous studies have shown that perfusion can preserve tissue and organs for longer and with less damage than traditional static cold storage preservation techniques, thus potentially enabling the ability to bank tissue for extended time periods while maintaining the tissue quality required for re-transplantation.

Extracorporeal machine perfusion with oxygenated perfusate offers a solution to tissue preservation by supporting the survival of all types of tissues. Normothermic and sub-normothermic *ex-vivo* machine perfusions have been demonstrated in the preservation of solid organs (lung [3], liver [4], and kidney [5]) as well as vascularized composite tissues (amputated extremities [6,7]) in porcine models. Studies have shown that machine perfusion improved tissue preservation, controlled ischemia reperfusion injury [8-10], and can extend the radius of donor procurement [11,12].

Significant progresses have been made on machine perfusion-based preservation of vital organs like the heart and lung. TransMedics has developed a transportable machine perfusion system for the heart. TransMedics, XVIVO Perfusion AB, and Vivoline Medical AB have commercial machine perfusion devices for lung preservation.

While normo- or sub-normothermic machine perfusion has shown promise for tissue preservation in the laboratory, and some limited commercial settings, there are still significant obstacles preventing the technology from being used more generally and in austere environments. For instance, the perfusion systems described in the literature are often experimental and require almost constant supervision. Additionally, they are far too large to be easily carried, and they were designed to function in a temperature controlled setting, not in extreme hot or cold. In addition to more reliable, robust perfusion systems, a portable perfusion system that can preserve trauma injured tissue without relying on blood would be a huge improvement over the currently available systems.

A critical component in a normo- or sub-normothermic machine perfusion system is the oxygen-carrying perfusate. Tissue needs nutrients and oxygen to maintain metabolism. Perfusate-dissolved oxygen is enough for monolayer cell culture, but is not enough for high density cells in a three-dimensional tissue space. Therefore, oxygen carriers that function like red blood cells are needed. For example, TransMedics uses blood as the perfusate in its heart perfusion machine. Lung perfusion is an exception, since the lung itself is a large surface oxygenator. On the

battlefield blood is not the answer, not only because of the lack of a blood supply, but also because blood type matching is needed to avoid immune-rejection. Therefore, developing a perfusate with a universal oxygen carrier is the key to a successful normo- or sub-normothermic machine perfusion system.

2 GENERAL CONCEPT

TDA and SwRI are developing a portable, ruggedized perfusion machine and a perfusate which can adequately oxygenate tissues without relying on blood, eliminating the supply chain and type matching obstacles of using human blood. The perfusate is based on proprietary technology being developed at the Southwest Research Institute (SwRI); the rest of this document will focus on the ruggedized portable perfusion machine

The perfusion machine will include a suite of sensors so that it can be automatically controlled, eliminating the need for clinician supervision. The data collected by the sensors will then be used to warn the clinician of potential problems with the perfused tissue. Further, it is well insulated and temperature controlled, allowing it to work in a wide variety of thermal environments, form -20°C to 50°C. Lastly, it is small, lightweight (<30 lbs), and battery operated so that it is easy to carry and transport under any circumstance.

3 PERFUSION MACHINE DETAILS

Figure 1 shows a variety of views of the perfusion machine: (a) The perfusion system with all doors closed, (b) front panel opened, revealing the batteries, control panel, and the insulation, (c) top door opened offering easy access to the perfused tissue, (d) the inner doors opened offering access to the main perfusion components and the additional perfusate bags, (e) the housing and insulation removed, showing the tissue container, the main perfusate components, the additional perfusate reservoir bags, and the tubing connecting them, and finally, (f) showing the size of the closed system next to a 6' tall person.

The U.S. armed forces may have to work under a wide array of weather conditions, so it is important that the system be highly insulated (we assumed a temperature range of -20°C to 50°C). Further, we designed the system so that various actions could be taken while minimizing heat leakage. For example, the outer front door can be opened without opening any insulation to allow access to the batteries and display panel. Secondly, there are separate insulated compartments for the main components, the tissue container, and the additional perfusate reservoirs. This way, if the technician has to do work on any one of those three sections, they can be accessed without exposing the others to the environment. This multi-door concept allows the user to access the various sections of the machine perfusion device without having to remove the insulation for the other sections.

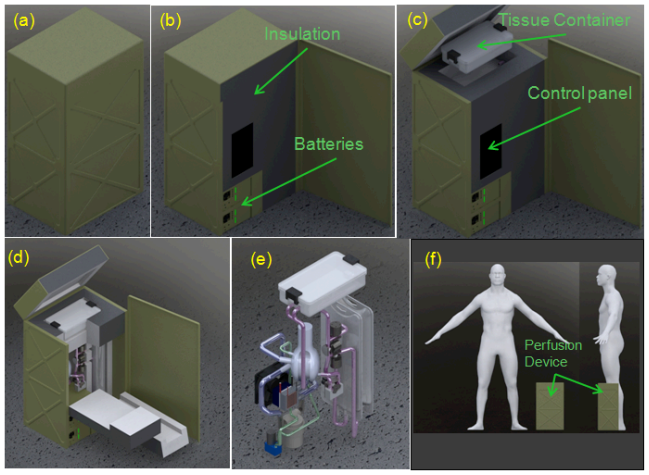

Figure 1: Image of ruggedized perfusion machine.

The dimensions of the box when closed (i.e. in the configuration shown in Figure 1a) is 9" x 12" by 20.5", which is small enough to fit into a backpack such as the MOLLE system employed by the US Army. This combined with its low weight (less than 30 lbs including water, perfusate, and two batteries) means that it can easily be transported via motor vehicle or carried by a single person.

4 FUTURE DIRECTION

TDA Research has developed a design for an improved perfusion device which is ruggedized to withstand extreme temperatures, lightweight, and portable. In future work, we intend to improve the design, with the goal of reducing the weight, making the case easier to carry, and having a longer battery life. Future work will include fabricating the machine and testing it using the perfusate being developed at SwRI on animal tissues with collaborators at the University of North Carolina at Charlotte (UNCC). To demonstrate the effectivness of our oxygenated perfusate and ruggedized portable perfusion machine. We are interested in collaborating with potential customers or commercialization partners to advance this technology.

REFERENCES

[1] Nassar, Ahmed et. al *Surgical Innovation,* 2014, DOI: 10.1177/1553350614528383

[2] Hosgood, S.A. et al. (2015) J. Transl. Med., 13, 329.

[3] Cypel, M., et. al. *American Journal of Transplantation*, 2009; 9: 2262-2269

[4] Adham, M., *et al.* (1997) *Transpl Int*, 10(4), p. 299-311.

[5] Shah, A.P., *et al.* (2008) *Transplantation*, 86(7), p. 1006-9.

[6] Constantinescu, M.A., *et al.* (2011) *J Surg Res*, 171(1), p. 291-9.

[7] Ozer, K., Pena-Rojas, A., Mendias, C.L., Toomasian, C., and Bartlett, R., *Transplantation* 2015

[8] Xu, H., *et al.* (2012) *J Surg Res*, 173(2), p. e83-8.

[9] Karaoz, E. (2013) *Journal of Transplantation Technologies & Research*, 04(01).
[10] Bon, D., *et al.* (2014) *Progrès en Urologie*, 24, p. S44-S50.
[11] Erasmus, M.E., et. al. *European Society for Organ Transplantation* **19** (2006) 589-593
[12] Graham, J.A. and J.V. Guarrera (2014) J Hepatol, 61(2), p. 418-31.