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14. ABSTRACT The objective of this proposal is to establish the graft preservation and immunomodulatory effects of our MP-BMPS/HBOC system in a pre-clinical large animal CTA model. We will specifically address the following specific aims in a porcine vascularized musculo-adipo-cutaneous flap model: Aim 1: Does MP-BMPS/HBOC allow prolongation of CIT without significant cellular damage to the allograft? Aim 2: Does the MP-BMPS/HBOC minimize the effects and incidence of ischemia-reperfusion injury at revascularization? Aim 3: What is the effect of MP-BMPS/HBOC on the immune profile of various flap tissues after transplantation? Our overall hypothesis is that the controlled preservation (at hypothermic and not freezing temperatures) and the continuous perfusion (with oxygen and essential nutrients) of limbs using MP-BMPS/HBOC after harvest will minimize ischemia-reperfusion injury and allow an immunomodulatory graft milieu conducive to improved immunologic, graft survival and functional outcomes. Although long term graft follow up and functional analysis are out of the scope of this project due to funding constraints, preliminary short-term ex-vivo and in-vivo insights will surely confirm the potential promise of this approach in CTA.				
15. SUBJECT TERMS Machine perfusion, limb transplantation, graft preservation, MP-BMPS/HBOC, ischemia, cold ischemia				
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a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified	24
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1. INTRODUCTION:

This study is an expansion our successful experience¹⁻⁷ with machine perfusion (MP) in combination with a newly developed hemoglobin based oxygen carrier (HBOC) solution under subnormothermic (21°C) conditions as a way to enhance organ and tissue preservation by providing effective ex-vivo oxygenation..

We have developed a new device to optimize subnormothermic MP in vascularized composite allotransplantation (VCA) by modifying our original Liver Assist Device from Organ Assist, Groningen, Netherlands. Our device for VCA as used in the current study incorporates our proprietary HBOC solution to achieve an extended duration of ex-vivo MP (over 14 hours)

The surgical procedures as proposed on this project are near completion, allowing for adequate time for correlation of ex vivo and in vivo data, analysis for statistical significance and interpretation of clinically relevant outcomes as outlined in our original application.

2. KEYWORDS:

Machine perfusion, cold static preservation, hemoglobin oxygen carrier solutions, vascularized composite allotransplants, ischemia/reperfusion, superior epigastric artery, rectus abdominal muscle, autotransplantation, heterotopic, superior epigastric vein, cold ischemia time, immunomodulation, transcriptomics, inflammatory mediators, metabolomics,

3. OVERALL PROJECT SUMMARY:

3.1. SPECIFIC AIM 1: Determine if the MP-BMPS/HBOC allow prolongation of CIT without significant cellular damage to the allograft.

Our study involving a swine vertical rectus abdominis (VRAM) myocutaneous flap VCA model was divided into 2 experimental phases: ex-vivo and in-vivo . This methodology was utilized as a way to focus on the two crucial steps involved in VCA preservation and subsequent transplantation.

3.1.1. Ex-vivo preservation of VCA – developing a new device for MP

A new MP device was initially developed for this application. Our ongoing collaboration with the leading bioengineer (Arjan van der Plaats, PhD) from Organ Assist, Groningen, Netherlands, has allowed us to modify our original Liver Assist Device. These modifications include:

3.1.2. The inception of a new mesh (medical grade PVC non-reactive to fluids) to support the graft during perfusion (Figure 1).



3.1.3. The modification of our perfusion system (initially designed as dual inflow through two different rotatory perfusion pumps) to accommodate a single perfusion port (pulsatile, arterial). New software for the MP electronic control system was also provided by Dr. van der Plaats as a way to accommodate the new range of pressures and flows needed for the VCA. The figure below shows the elimination of the portal venous inflow pump (continuous pressure) and the transformation of our system into a new single pulsatile pressure over a flat surface (Figure 2) for adequate VCA perfusion over an extended period of time.



Pump Ur

3.1.4. Preliminary data from ex-vivo studies – perfusion settings and perfusate ABGs over a 14 hour period.

Series #1 (P055-14)

Perfusion Data									Perfusate Gases/Chem					
Date	HR	Time	Min Flow Rate (ml/min)	Max Flow Rate (ml/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Peak Resistance (ru)	Temp °C	Arterial pH	Venous pH	Arterial pO2 (mmHg)	Venous pO2 (mmHg)	Arterial Lactate (mmol/L)	Venous Lactate (mmol/L)
5/19/2014	0:00	11:51	9	10	57	31		21	7.54	7.57	641	415	2.7	2.6
5/19/2014	0:15	12:06	6	30	30	10		19						
5/19/2014	0:30	12:25	20	40	50	30	3.46	21	7.56	7.55	538	357	2.7	2.6
5/19/2014	0:45	12:40	21	50	52	32	2.68	21						
5/19/2014	1:00	12:57	27	73	52	33	1.4	21	7.56	7.55	553	406	2.7	2.8
5/19/2014	1:15	13:22	32	56	52	32	1.85	21	7.56	7.56	545	410	2.8	2.8
5/19/2014	2:00	13:55	22	67	52	32	1.62	21	7.58	7.57	531	395	2.8	2.8
5/19/2014	2:30	14:25	23	66	57	32	1.21	21						
5/19/2014	3:00	14:55	33	60	55	31	1.92	21	7.57	7.56	516	424	3	3

5/19/2	3:3	15:2																
014	0	5	30	57	57	32	1.92	21										
5/19/2	4:0	15:5																
014	0	6	23	61	57	32	1.52	21	7.58	7.57	537	424	3	3				
5/19/2	4:3	16:2																
014	0	6	24	66	52	33	1.14	21										
5/19/2	5:0	16:5																
014	0	1	33	76	52	32	1.29	21	7.58	7.57	575	414	3.1	3.1				
5/19/2	5:3	17:4																
014	0	0	29	67	52	32	1.13	21										
5/19/2	6:0	18:0																
014	0	0	29	67	57	32	1.1	21	7.58	7.58	530	404	3.2	3.2				
5/19/2	6:3	18:2																
014	0	8	26	67	52	32	1.94	21										
5/19/2	7:0	18:5																
014	0	8	25	67	51	32	1.06	21	7.58	7.57	524	407	3.2	3.3				
5/19/2	7:3	19:2																
014	0	9	31	67	51	33	1.1	21										
5/19/2	8:0	19:5																
014	0	6	31	72	51	32	1.1	21	7.58	7.58	543	404	3.3	3.3				
5/19/2	8:3	20:3																
014	0	0	36	69	51	32	1.17	20										
5/19/2	9:0	20:5																
014	0	9	34	70	52	33	1.73	20	7.58	7.58	542	388	3.4	3.5				
5/19/2	9:3	21:2																
014	0	9	25	63	52	33	1.1	20										
5/19/2	10:	21:5																
014	00	6	39	69	52	33	1.04	21	7.58	7.58	523	386	3.6	3.5				
5/19/2	10:	22:2																
014	30	5	33	68	52	32	1.13	21										
5/19/2	11:	22:5																
014	00	9	23	69	59	32	1.1	20	7.57	7.57	508	375	3.6	3.6				
5/19/2	11:	23:2																
014	30	9	28	65	59	32	1.1	20										
5/19/2	12:	23:5																
014	00	8	27	72	51	33	1.14	21	7.57	7.57	523	376	3.8	3.7				
5/20/2	12:																	
014	30	0:28	25	72	52	32	1	20										
5/20/2	13:																	
014	00	0:58	35	70	52	32	1.2	20	7.56	7.56	527	382	3.8	3.8				
5/20/2	13:																	
014	30	1:28	37	78	51	32	1.17	21	7.56	7.56	526	415	3.8	3.9				
5/20/2	14:	Syste	OFF at															
014	00	m	1:40															

Series #2 (P054-14)

Perfusion Data									Perfusate Gases/Chem									
Date	HR	Time	Min Flow Rate (ml/min)	Max Flow Rate (ml/min)	Systolic Pressure (mm Hg)	Dias-tolic Pressure (mm Hg)	Peak Resistance (ru)	Temp °C	Arteria l pH	Venous pH	Arteria l pO2 (m mHg)	Venous pO2 (m mHg)	Arteria l spO2 (%)	Venous spO2 (%)	Arteria l Methb (%)	Venous Methb (%)	Arteria l Lactate (mm ol/L)	Venous Lactate (mm ol/L)
5/21/2014	BL	8:55							7.58		552		92.4		4.1		2.9	
5/21/2014	0:00	9:55	0	12	53	23	6.5	21										
5/21/2014	0:15	10:05	2	13	56	30	6.9	21										

5/21/ 2014	0: 30	10 :2 0	6	19	55	29	9.99	21	7.5 6	7.5 4	537	384	92. 9	90. 2	4.2	4.3	2.7	2.7
5/21/ 2014	0: 45	10 :3 5	9	30	54	29	5	21	7.5 6	7.5 5	543	379	93. 1	90. 6	4.5	3.8	2.7	2.7
5/21/ 2014	1: 00	10 :5 3	11	30	56	30	5.6	21										
5/21/ 2014	1: 30	11 :2 9	8	30	56	29	2.94	21										
5/21/ 2014	2: 00	11 :5 3	11	36	57	29	6.75	21	7.5 8	7.5 7	544	411	92. 8	90. 5	4.9	5	2.7	2.7
5/21/ 2014	2: 30	12 :2 0	6	35	57	28	7.25	21										
5/21/ 2014	3: 00	12 :5 3	13	36	55	29	6.87	21	7.5 9	7.5 9	506	408	92. 5	91. 2	5.2	5.7	2.7	2.7
5/21/ 2014	3: 30	13 :2 6	7	39	57	29	4.83	21										
5/21/ 2014	4: 00	13 :5 9	12	43	57	28	6	21	7.6 1	7.6	529	404	93	90. 9	4.9	5.1	2.8	2.8
5/21/ 2014	4: 30	14 :2 5	7	41	57	29	3.22	21										
5/21/ 2014	5: 00	14 :5 3	18	41	57	28	3.37	21	7.6 1	7.6	534	415	92. 7	90. 1	4.7	5.1	2.9	2.9
5/21/ 2014	5: 30	15 :2 0	16	40	56	30	2.45	21										
5/21/ 2014	6: 00	15 :5 1	11	45	57	29	2.9	21	7.6 1	7.6 1	518	397	92. 4	90. 3	6	5.6	3	2.9
5/21/ 2014	6: 30	16 :2 8	13	43	54	29	3.22	21										
5/21/ 2014	7: 00	16 :5 0	9	43	57	28	2.9	21	7.6 1	7.6	528	392	93	89. 7	5.7	6.2	3.1	3
5/21/ 2014	7: 30	17 :2 8	10	49	57	29	2.63	21										
5/21/ 2014	8: 00	17 :5 5	16	39	57	29	2.43	21	7.6 1	7.6 1	531	373	92. 4	89. 9	6.2	6.1	3.1	3.1
5/21/ 2014	8: 30	18 :2 5	16	40	56	30	2.59	21										
5/21/ 2014	9: 00	18 :5 5	15	43	54	29	2.54	21	7.6 1	7.6	524	378	92. 3	90	6.5	6.7	3.1	3.2
5/21/ 2014	9: 30	19 :2 7	8	45	55	29	2.63	20										
5/21/ 2014	10: 0	19 :5 7	8	41	56	29	2.41	21	7.6 1	7.6 1	537	388	92. 7	90. 5	6.4	6.7	3.2	3.3
5/21/ 2014	10: 0	20 :2 8	20	42	54	29	2.27	21										

5/21/2014	11:00	20:05	8	46	55	28	3.75	20	7.61	7.6	533	381	93.1	90.8	6.5	7	3.3	3.3
5/21/2014	11:03	21:02	13	47	55	29	3.11	21	7.61	7.6	532	373	92.9	90.1	7.2	7.3	3.4	3.4
5/21/2014	12:00	21:08	10	48	55	29	2.41	21	7.54	7.5	531	385	93.4	91	7.1	7	3.6	3.6
5/21/2014	13:00	23:00	26	50	56	29	2.14	21	7.6	7.6	523	384	93.1	90.8	7.6	7.6	3.6	3.6
5/21/2014	14:00	23:05	18	53		30	2.38	21										
					56													

Series #3 (P053-14)

Perfusion Data									Perfusate Gasses/Chem												
Date	H	R	Time	Min Flow Rate (ml/min)	Max Flow Rate (ml/min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Peak Resistance (ru)	Temp °C	Arterial pH	Venous pH	Arterial pO2 (mmHg)	Venous pO2 (mmHg)	Arterial SpO2 (%)	Venous SpO2 (%)	Arterial Methb (%)	Venous Methb (%)	Arterial Glucose (mmol/L)	Venous Glucose (mmol/L)	Arterial Lactate (mmol/L)	Venous Lactate (mmol/L)
5/27/2014	BL		9:05							7.353	7.349	524	507	93.1	92	1.4	4.2	7.5	7.4	2.7	2.7
5/27/2014	00		10:08							7.58	7.55	500	356	92.2	89.4	3.8	4.3	7.5	7.1	2.7	2.6
5/27/2014	15		10:01	0	11	54	32	5.2	20												
5/27/2014	30		10:08	7	15	54	31	9.99	21	7.57	7.33	515	362	92.8	89.4	4.6	4.5	7.2	7.2	2.6	2.6
5/27/2014	45		11:00																		
5/27/2014	00		11:02	8	19	53	31	9.99	21	7.57	7.56	514	336	92.5	88.5	3.5	4.8	7.2	7.1	2.6	2.7
5/27/2014	10		12:00																		
5/27/2014	20		12:01	4	21	54	21	9.99	21	7.58	7.57	513	349	93.2	89	4.5	4.9	7.2	7	2.7	2.7
5/27/2014	30		12:04	5	15	53	31	9.99	21												

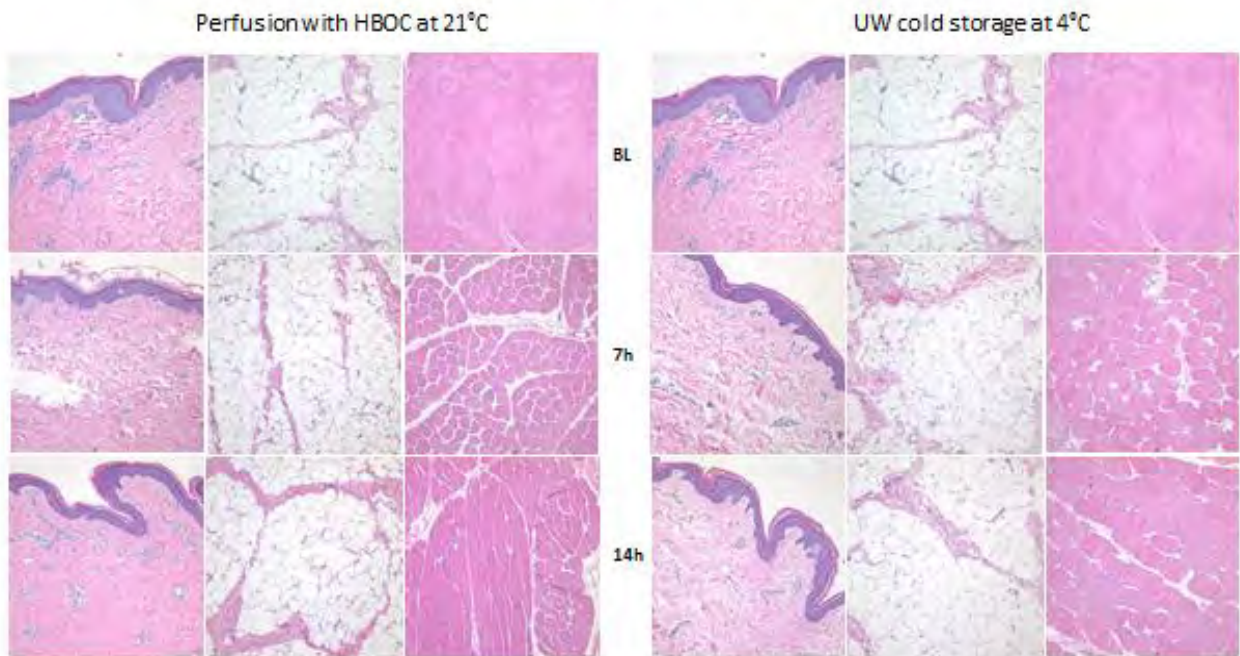
Series #4 (P057-14)

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3.1.5. Preliminary data from ex-vivo studies – histology

Tissue samples from both groups (Cold static perfusion with UW and MP with HBOC) were H&E stained and blindly reviewed by an experienced transplant pathologist. All the swine flaps (n=4) perfused with MP-HBOC did not show any evidence of apoptosis (TUNEL or caspase) and were normal on histology after a 14 hour period. There were no signs on endothelial cell damage in the MP/HBOC group (n=4) while being perfused with parameters outlined above.

MP/HBOC compared to CSP – Ex vivo studies (H&E staining, 10x magnification)

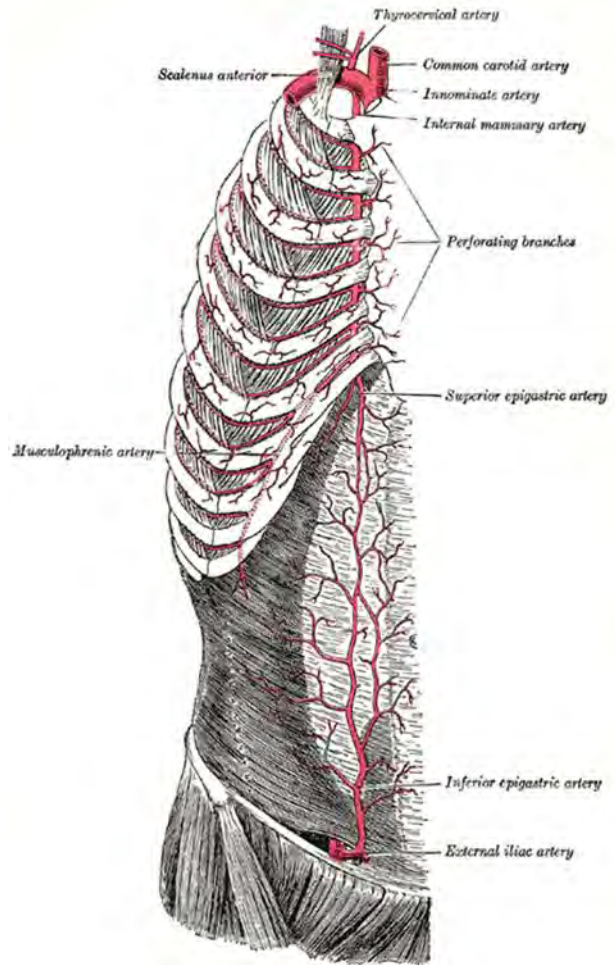


3.1.6. In-vivo stage – the pre-clinical large animal (swine) surgical model for VCA

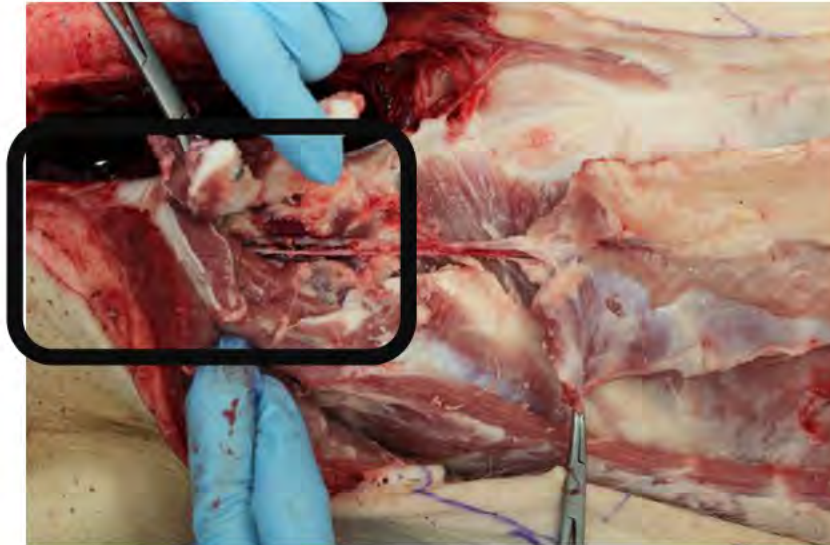
We have developed a reliable VCA surgical model with the utilization of a composite flap (muscle, adipose tissue and skin) from the whole rectus abdominal muscle (RAM). This model was maximized through extensive anatomical studies performed by our group in our lab at the Center for Pre-Clinical Services (CPCS), McGowan Institute of Regenerative Medicine.

3.1.7. Anatomical studies

The RAM flap had all anatomical features reinforced by additional studies performed with swine cadavers at no cost for the current project. The anatomical studies were initially focused on the RAM vascular pedicles. Through our anatomical dissections in cadaveric swine tissue it became very clear that the best vascular pedicle to be utilized should be the one arising from the superior epigastric artery. Figure 3 has an anatomical display of both vascular pedicles perfusing the RAM.



Since the superior epigastric artery (SEA) is a terminal branch on the internal mammary artery (IMA) an additional dissection within the chest was added to our procedure in order to obtain a lengthy vascular pedicle for subsequent implantation. Figure 4 shows the SEA arising from the IMA after two ribs have been transected.



The RAM grafts were recovered through a meticulous dissection of the entire anterior upper portion of the abdominal wall, after careful ligation of all vascular tributaries, leaving the SEA as the only inflow route for arterial blood supply to the RAM. Figure 5 shows the initial dissection of the RAM from adjacent planes.



Figure 6 shows the RAM fully isolated from the adjacent tissue within the upper portion of the abdominal wall. The overall dimensions of the RAM grafts were kept within 15 x 6 cm to ensure full arterial supply from the SEA.



Figure 7 shows the RAM graft after full removal from the donor site. Skin and adipose tissue paddles were kept intact with the muscular tissue.



3.1.8. Functional studies – vascular cannulation of the RAM flap

Since this is a new approach to preserve VCA, we also developed a protocol to properly cannulate the SEA prior to flap recovery and preservation. The SEA was cannulated on site with a 20-22 gauge cannula and perfused with University of Wisconsin (UW) solution after the animal has received 20,000 U of heparin. All these microsurgical procedures were performed with surgical microscopes and surgical loupes.

3.1.9. Functional studies – autotransplantation (heterotopic) of the RAM into the cervical area

In order to streamline our results and eliminate additional variables, a series of 3 animals underwent a full surgical procedure (RAM recovery followed by implantation within the animal's own cervical region). These procedures were intended to establish the best technical features for the vascular anastomosis to be performed after VCA (heterotopic RAM graft) implantation. The arterial inflow was established through an end-to-end anastomosis between the carotid artery and the RAM's SEA. The venous drainage was established by an end-to-end anastomosis between the RAM's superior epigastric vein (SEV) and the external jugular vein (EJV).

Three animals underwent heterotopic (cervical implantation) autotransplants of their RAM grafts. There were no technical complications from these procedures and the surgical model became firmly established for the in-vivo portion of our studies involving VCA and the use of immunosuppressive therapy.

3.2. SPECIFIC AIM 2: Determine if the MP-BMPS/HBOC minimize the effects and incidence of I/R injury at revascularization.

Our initial group assignment was the following:

Group	Intervention	Description
1 (Ex Vivo Control, Standard of Care)	14 hours ex-vivo cold perfusion of VRAM flap with UW	2 donors (2 flaps raised from each animal = 4 flaps total)
2 (Ex Vivo Study Group, MP-BMPS/HBOC)	14 hours ex-vivo MP-BMPS/HBOC perfusion of VRAM flap	2 donors (2 flaps raised from each animal = 4 flaps total)
3 (In Vivo Control) Standard of Care - CSP	Transplantation of 14 hours ex-vivo UW perfused VRAM flaps	4 donors = 4 recipients 4 flaps total
4 (In Vivo Study Group) MP-BMPS/HBOC	Transplantation of 14 hours ex-vivo MP-BMPS/HBOC perfused VRAM flaps	4 donors = 4 recipients 4 flaps total

The following protocol was developed for tissue biopsy and perfusate analyses during the in-vivo experiments (groups 3 and 4 highlighted in the table above):

3.2.1. Machine Perfusion

We performed 4 machine perfusions of the skin flaps for 12 hours prior to transplantation. Perfusion of the skin flaps was performed using the prototype Organ Assist Liver Device (OALD) (Figure 8) and the VIR-1 solution, which contains a hemoglobin-based oxygen carrier and BMPS mixed 1:3 ratio, respectively. The starting hemoglobin as measured by an ABL800flex (Radiometer, Copenhagen) blood gas analyzer was 3.4 g/dL. The baseline OALD settings were : 60 mmHg pressure, 21 Celsius, FiO2 60%, sweet gas 0.3 L/min. Perfusion was initiated with an inlet pressure of 60mmHg at 1Hz pulse pressure, achieving a flow of ~10mL/min. Initial blood gas values were ~93% saturated VIR-1 solution at a pO2 of ~400 mmHg. As brief period of vasoconstriction was observed immediately after graft procurement and flushing. This transient vasospasm was treated with a short dripping of Lidocaine 2% around the vascular pedicles. The flows increased (the OALD alters centrifugal pump speed to maintain a set pressure) progressively after this. After 2 hours, with flows exceeding 25ml/min, the pressure set point was lowered to 45 mmHg where it was maintained throughout the remainder of the perfusion. After 14 hours, the skin flap was removed from the OALD, flushed with cold LR and implanted into the recipient.



3.2.2. Tissue fixation methods and analyses:

a. Samples for H&E staining and histomorphometry.

Formalin fixation or Bouin's solution fixation

Every sample was evaluated for I/R injury–induced alterations

b. Samples for Immunohistochemistry were stored at - 80°C.

Fixation media: OCT or gelatin 5% + sucrose 5% in PBS

2.1. Immunohistochemical analyses in the tissue:

Test	Complement	Cytokines	Growth factors
	C3a	IL-1 β	VEGF
	C4d	IL-6,	PDGF
		IL-8,	FGF
		IL-10	
		TNF- α	

Endothelial activation – damage

-vWF

-Heparan sulfate proteoglycans (HSPG)

-HIF-1 α

Apoptosis

- TUNEL/caspase

c. Samples for Gene Expression studies

Fixation media:

Stored in RNA later solution

Gene expression analyses in the tissue

-HIF-1 α

3.2.3. Tissue biopsies for ex vivo study:

Three tissue samples (for three different tissue fixation methods) from three different regions (proximal – mid – distal flap regions) of the flap were obtained. The biopsies included Skin - Subcutaneous Fat – Muscle. (9 tissue samples per each biopsy time point for each flap) The biopsies were taken by punches.

There were 11 different biopsy time points per flap. The total number of the flaps was 8 for ex vivo study (4 flaps per groups).

3.2.4. Ex vivo study biopsy time points:

a. Baseline (before ischemia)

b. Ex vivo perfusion biopsy time points

0 – 1 – 5 – 7 – 9 – 11 – 13 – 17 – 21 hours / 9 time points

c. Total flap tissue (including nerve and vessel) at 24th hour (end point).

d. Tissue Sample Size (Ex-vivo groups):

Total tissue sample size: 792 (264 formalin or bouin's / 264 fresh frozen / 264 RNA Later)

3.2.5. Perfusate / Serum Analyses sample take time points:

Groups 1 and 2: Baseline blood sample before harvest - Hourly perfusion solution samples starting from hour 0.

0 – 1 – 3 – 5 – 7 – 9 – 12 – 14 hours / 8 time points

- 11 samples per flap

- 4 flaps per group

- 2 groups

3.2.6. Blood Gas Analysis

Enzymes (AST, LDH,CK,MPO,SOD,MDA,GSH)

Myoglobin

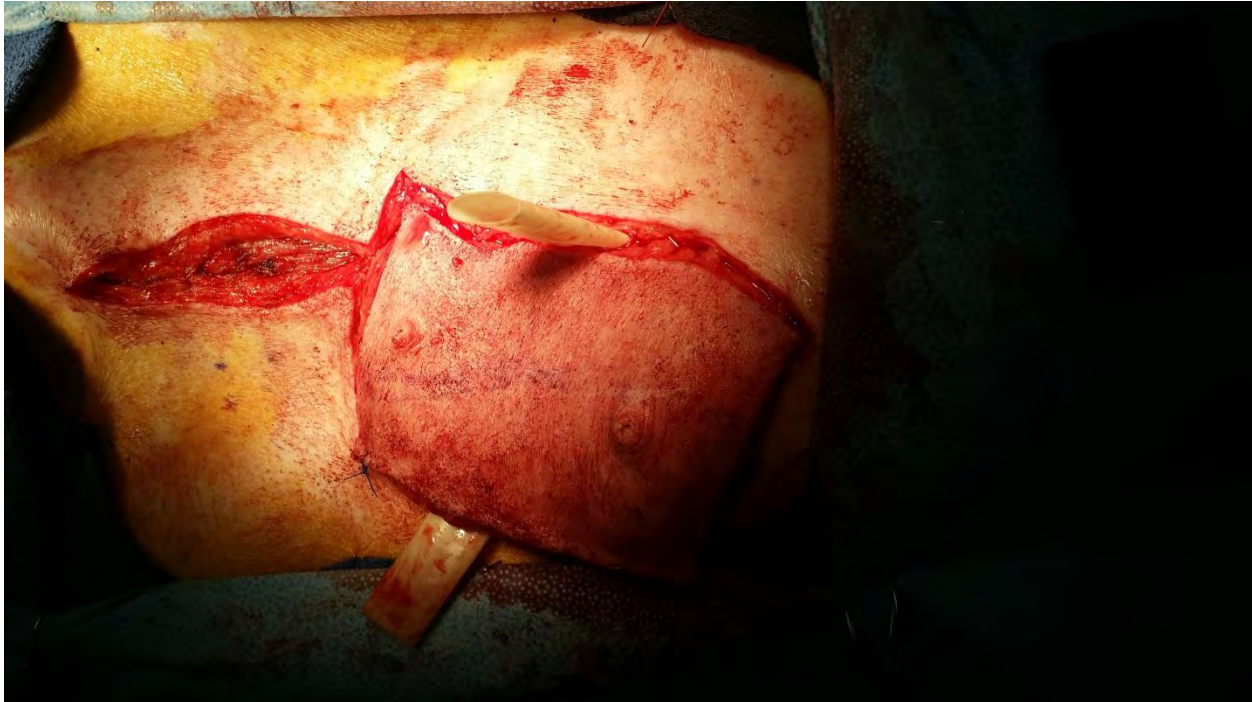
3.2.7. Flowcytometer (for granulocytes CD11a, CD18)

3.2.8. In-vivo studies

According to our original plan, almost all surgical procedures have been already performed (e.g. all ex-vivo experiments and 6/8 in-vivo experiments).

All the samples have been properly collected, labeled and stored for subsequent analyses. There were no technical complications or any other medical issues with the recipients of the VCA in the post-operative period (7 day follow up). All the VCA recipients tolerated well our immune suppressive therapeutic regimen.

The RAM grafts showed excellent viability after 14 hours of machine perfusion. Since all the animals were female, the RAM grafts had nipples as part of the superficial tissues. Figure 8 shows the heterotopic cervically implanted RAM flap prior to completion of surgical inset.



3.3. SPECIFIC AIM 3: Determine the effect of MP-BMPS/HBOC on the immune profile of various flap tissues after transplantation.

All tissue and perfusate samples collected from these experiments will undergo extensive immune histochemical analyses over the next months. According to our initial proposal, these samples will be additionally tested for transcriptomics, inflammatory mediators and metabolomics.

We have 10 months to complete all these additional tests prior to proceed with our final analyses of the entire data set.

4. KEY RESEARCH ACCOMPLISHMENTS:

- Successful development of a pre-clinical large animal model for VCA
- Successful development of the RAM graft as a reliable VCAVCA model for I/R injuries
- Successful development of a new MP device for VCAVCA perfusion ex-vivo
- Successful implantation of the heterotopic (cervical) RAM grafts in all surgical experiments.

- Successful implementation of the MP/HBOC method for VCAVCA preservation based on the initial clinical results.

5. CONCLUSION:

This has been a very productive year for this project, which brings a major innovative development to our ability to treat military and civilian patients in need for limb transplantation. The average cold ischemia time (CIT) for VCA remains around 4-8 hours under the current standard of care using cold storage as the only reliable method for graft preservation. This remains a major limiting factor for the expansion of VCA as a reliable therapeutic option for limb replacement across wider geographic regions and better suitable skin and size matching requirements for a larger population. Our new technology involving MP/HBOC can be a groundbreaking development for the field if capable to extend the CIT and improve the standards for graft and patient survival based on the minimization of IR injuries.

These preliminary studies show exciting results with MP/HBOC preservation of the RAM grafts over an extended period of ex-vivo perfusion (14 hours) when compared to the current standard of care (CSP).

A new device has been developed for this VCA application, which has been covered by our initial patent application in 2012 (WO2014059316 A1). This new device will allow us to proceed with subsequent experiments utilizing deceased donor human upper extremities for ex vivo MP assessment.

Our future plans include the completion of these preliminary studies with the RAM graft and the submission of a subsequent proposal to determine the role of our MP/HBOC system in mitigating IR injuries while allowing neuroprotection of VCAs.

Our initial results comparing the MP/HBOC system with autotransplants are also very important for future developments in limb replantation at the battle field for patients capable to recover the severed limb. These patients might be able to be properly resuscitated and treated in the supporting military hospitals within the region while their limbs could be fully oxygenated ex-vivo by our technique. This would allow “elective” limb replantation procedures at these locations, which might enhance the outcomes of these rather challenging injuries to our troops.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

- a. No manuscripts have been submitted for publication since the project is not yet completed.
- b. List presentations made during the last year

1. Invited speaker – Summer School, McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA. The new ex-vivo world; perfusing human organs outside of the body. July 2014.
2. Invited speaker – Starzl Transplantation Institute, University of Pittsburgh Medical center, Pittsburgh, PA. The new ex-vivo world; perfusing human organs outside of the body. August 2014.
3. Invited speaker – Meeting with the McGowan Foundation, McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA. The new ex-vivo world; perfusing human organs outside of the body. August 2014.
4. Invited speaker – Meeting with PA legislators, McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA. The new ex-vivo world; perfusing human organs outside of the body. September 2014
5. Invited speaker – Meeting with CORE representatives, McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA. The new ex-vivo world; perfusing human organs outside of the body. September 2014
6. Invited speaker – Carnegie Mellon University, Undergraduate course for bioengineers, Pittsburgh, PA. The new ex-vivo world; perfusing human organs outside of the body. September 2014

7. INVENTIONS, PATENTS AND LICENSES:

Not applicable at this time

8. REPORTABLE OUTCOMES:

- 8.1. Development of a new machine perfusion device for CVA preservation

9. OTHER ACHIEVEMENTS:

All achievements are highlighted above. No additional achievements.

10. REFERENCES:

- 1. **Fontes PA**, Marsh JW, Lopez RC, Soltys K, Cruz RJ, van der Plaats A, Light WR, Scott VL, Damiani D, Venkatmaran R, Sruti S, Minnervini M, Demetris AJ. Machine perfusion with a new oxygen-carrier solution: the future of liver preservation. *Hepatology* 2012; 56:1524A.
- 2. **Fontes PA**, Marsh W, Lopez R, Soltys K, Scott V, A van der Plaats, W Light, S Shiva, M Minnervinni, Demetris AJ. Liver preservation with machine perfusion under full oxygenation using a new cell free oxygen carrier solution. *American Journal of Transplantation* 2013; 13:119.
- 3. **Fontes PA**, Marsh JW, Lopez RC, van der Plaats A, Light WR, Paranjpe S, Shiva S, Vodovotz Y, Michaelopoulos G. Machine perfusion with a new cell-free oxygen carrier solution provides effective ex-vivo oxygenation, minimizes

ischemia reperfusion injuries and downregulates genes associated with liver damage. 16th Congress of the European Society for Organ Transplantation, Vienna, Austria, September 2013.

- 4. **Fontes PA**, Vodovotz Y, Marsh JW, Lopez RC, van der Plaats A, Light WR, Shiva S, Stolz D, Minnervini M, Barclay D, Handler G, Sadowsky D, Paranjpe S, Michalopoulos G. Ex-vivo hepatic perfusion with a new cell-free oxygen carrier solution upregulates hepatocyte associated gene response against ischemia-reperfusion injury and triggers protective and regenerative pathways. *Hepatology* 2013, 58; 4(S):211-A.
- 5. **Fontes PA**, Marsh W, Vodovotz Y, Lopez R. Van Der Plaats A, Light WR, Michalopoulos G. Metabolomic profile of perfusate and bile comparing machine perfusion with a new cell free oxygen carrier solution and cold storage preservation in a porcine model of liver transplantation. *Transplant International* 2014, 27(Suppl.1):12.
- 6. Paranjpe S, **Fontes P**, Vodovotz Y, Michalopoulos G. Gene network analysis of liver allografts preserved with machine perfusion. *FASEB Journal* 2014; 28:649.10 http://www.fasebj.org/content/28/1_Supplement/649.10.abstract
- 7. **Fontes P**, Lopez R, van der Plaats A, Vodovotz Y, Minervini M, Scott V, Soltys K, Shiva S, Paranjpe S, Sadowsky D, Barclay D, Zamora R, Stolz D, Demetris AJ, Michalopoulos G, Marsh JW. Liver preservation with machine perfusion and a newly developed cell free oxygen carrier solution under subnormothermic conditions. *American Journal of Transplantation* 2014, in press.

11. APPENDICES: n/a