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14. ABSTRACT

The first ErythroMer prototype (EM-V1) was structurally stable, toroidal (size: 175±10nm; pdi: 0.26±0.0 by DLS, confirmed by TEM and AFM). CH50 (complement activation) results were indistinguishable from negative controls, and we observed no impact on plasma viscosity (1:10 and 1:5 dilution) with EM-V1. For the most recent variant of EM-V1 with increased concentration of the allosteric effector, p50 was 33.33 Torr (control RBC p50: 24.76). EM NO sequestration varied with shell crosslinking and was ≤ RBCs. Important to the future successful testing of EM in various models of prolonged-field care (PFC), we developed and fine-tuned a rabbit hemorrhagic shock model. Initial shock studies in a rodent model: 40% blood volume was removed; animals were resuscitated with EM-V1 (40wt/vol%, [Hb]=4mM) or normal saline (N=6, each). EM stabilized hemodynamics and following parameters within 1h: lactic acidosis (8.2±2.1 v 3.2±1.5 mM) [for EM and NS, respectively, throughout]; A-VO2 difference (24±11 v 67±23%) and brain pO_2 (30.5±1.4 v 17.2±1.3Torr); $p<0.05$, for all. Hemodilution model: HIF-1α(ODD)luciferase mice underwent hemodilution (70%v/v) with pentastarch, blood/autotransfusion, or EM [N=6, all; native Hb target nadir 5 mg/dL). HIF-luc radiance was higher with HES than autotransfusion and EM, which did not differ ($p<0.01$). We recently transitioned to our second prototype, EM-V2, and have preliminarily structural data (size: 320nm; pdi: 0.37 by DLS). We will endeavor to mirror the recent p50 results from our first prototype in Y2 of this grant with EM-V2. Early PK testing with EM-V2 revealed distribution t1/2=4.5hrs in rabbits (n=2). In rabbit oxygenation/acute shock studies, sufficient blood volume (BV) was removed to induce an increase in lactate, decrease in liver pO_2 and decrease in mean arterial pressure; animals were resuscitated with EM (~30% BV, N=3) or 5% Albumin or Auto-transfusion (N=5) of whole blood (N=6).

15. SUBJECT TERMS

ErythroMer (EM), Artificial Red Blood Cell (RBC), Prolonged-Field Care (PFC), PFC models, Resuscitation, Oxygenation, Hemorrhagic Shock, Pharmacokinetics, and Biocompatibility

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1. INTRODUCTION:

The overall project goal to optimized performance characteristics of the red blood cell substitute ErythroMer (EM), for resuscitation during hemorrhagic shock and subsequent prolonged field care scenarios requiring ongoing administration of an oxygen carrier when 'natural' blood products are not available. We will accomplish this goal by: developing EM prototypes with optimal oxygen (O_2) binding affinity that allow both O_2 capture in the lungs and O_2 release in other tissues and optimizing formulation and dosing to achieve stable circulation. EM will also be tested for compatibility with Thrombosomes and other hemostatic adjuncts to prevent dilutional coagulopathy via co-administration with EM. Finally, we will establish EM's efficacy and safety in modeled resuscitation of a hemorrhagic shock, with/without Thrombosomes and/or hemostatic adjuncts.

2. KEYWORDS:

ErythroMer (EM), Artificial Red Blood Cell (RBC), Prolonged-Field Care (PFC), PFC models, Resuscitation, Oxygenation, Hemorrhagic Shock, Pharmacokinetics, and Biocompatibility

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Select & Fabricate ErythroMer (EM) prototypes with high/low O_2 affinities.
Milestone #1: Select EM prototypes meeting high/low P50 targets (04/2018, completed).
Milestone #2: Fabricate selected EM prototypes for *in vivo* testing (10/2018, completed).

Major Task 2: Test efficacy *in vivo*.
Milestone #3: Obtain IACUC/ACURO approval (01/2018, completed at WUSM; 10/2019, submitted for review @ UMB).
Milestone #4: Establish O_2 delivery benefit conferred by EM prototypes with high/low O_2 affinities (defined as 20% improvement in tissue pO_2 relative to current prototype). (04/2019; completed; manuscript in preparation).

Major Task 3: Measure EM pharmacokinetics (PK).
Milestone #5: Calculate EM PK as a function of Blood Volume (BV)% replacement. (adjusted to 12/2019; 80% complete). Added PK with MPS depletion (clodronate): simulates MPS saturation by massive transfusion (Projected: 03/2020; 20% complete)

Major Task 4: Develop EM PFC dosing.
Milestone #6: Confirm EM dosing strategy for rabbit PFC models (Projected: 03/2020; 70% complete). Will be informed by PK-MPS depletion model.

Major Task 5: Determine EM:HA (hemostatic adjunct) compatibilities *ex vivo*.
Milestone #7: Obtain IRB/HRPO approval (01/2018, completed at WUSM)
Milestone #8: Confirm EM; *ex vivo* compatibility. (01/2018; completed, MS in preparation).

Major Task 6: Develop goal-directed HA algorithm for EM-based dilutional coagulopathy (DC). Milestone #9: Develop goal directed HA algorithm for EM-induced DC suitable for in vivo testing (rabbits). Publish manuscript and report to DoD (10/2018; 100% complete, MS in preparation).

Major Task 7: Pilot PFC Scenarios.
Milestone #10: Pilot & Optimize PFC Scenarios (A, B, C) to achieve 50% 48h mortality for colloid resuscitation controls. Publish manuscript and report to DoD (10/2018; completed, MS in preparation).
Major Task 8: Establish EM efficacy.
Milestone #11: Establish EM efficacy in comparison to reinfusion of shed blood (O_2 delivery non-inferiority yes/no) and colloid resuscitation (mortality superiority yes/no). Publish manuscript and report to DoD (Projected: 09/2020; 60% complete).

Major Task 9: Optimize PFC HA Algorithms *in vivo*.
Milestone #12: Optimize goal directed HA algorithm for DC and TIC during resuscitation in PFC Scenario B (uncontrolled hemorrhage, with dilutional coagulopathy) and PFC Scenario C (controlled hemorrhage + polytrauma, with trauma induced coagulopathy). Identify differences required (amongst colloid, blood and EM-based resuscitation) for HA administration. Publish manuscript and report to DoD (Projected: 03/2020; 60% complete).

Major Task 10: Screen EM safety.
Milestone #13: Identify laboratory and histologic evidence of EM toxicity during resuscitation from PFC Scenarios A-C, in comparison to that observed in blood re-infusion and colloid resuscitation groups. Publish manuscript and report to DoD (Projected: 09/2020, 40% complete).

What was accomplished under these goals?

1. **Select & Fabricate ErythroMer (EM) prototypes with high/low O_2 affinities.**
   - Y1: Testing of the first EM prototype (EM-V1) completed
   - Identified EM-V1 with O_2 affinities that match RBCs or are >30% and <20% that of RBCs. (Major Task 1, Milestones 1 and 2).
   - The prototype with optimal O_2 affinity was tested *in vivo* in both PK and in oxygenation studies following controlled hemorrhage (Major Task 2; Milestones 3 and 4).
   - Y2: Optimized design w/r/t biocompatibility, improved payload retention, and improved lyophilization/reconstitution (Figures 1 & 2), yielding EM-V2. (Major Task 1, Milestones 1 and 2)
   - Two complimentary proposals submitted to support additional optimization of the EM formulation:
   - DoD BA190035 (FOA USA-MRMC-BAA-2018-W81XWH18SBAA1) "Optimized Formulation, Delivery & Dosing for ErythroMer (Artificial Red Cell)"
   - NIH/NHLBI SBIR Phase I H193-004-0067 "Rapid Reconstitution of a Lyophilized, Bio-inspired, Artificial Red Blood Cell."

2. **Biocompatibility**
   Hemostasis
   - Y1-2 Biocompatibility was evaluated in *ex vivo* ROTEM analyses
   - EM-V1 and EM-V2 particles had no effect upon (*ex vivo*) hemostasis other than a correctable dilution effect, indicating need for co-administration of plasma and platelets when transfusion exceeds ~ 50% BV (e.g. massive transfusion) (see Y1 report). (Major Task 5, Milestone 7)
   - Generation of a goal-directed HA algorithm to optimize co-administration testing of hemostatic adjuncts to maintain hemostasis during resuscitation (see Y1 report). (Major Task 6, Milestone 8)
   - Extensive *ex vivo* analysis of EM biocompatibility with Thrombosomes (see Y2Q1 & Y2Q3 reports). (Major Task 5, Milestone 7)
   - EM-V2 (empty shells [enabling focused study of surface biocompatibility] and Hb-loaded EM-V2) had no effect on coagulation studies (size distribution, aggregometry, surface marker expression, thrombin generation) with Thrombosomes (desired outcome).
   - Submission of two complimentary proposals to fund additional work exploring EM biocompatibility and co-administration with:
   - Freeze dried plasma: DoD PR190685 (FOA W81XWH-19-PRMRP-TTDA) "Freeze-Dried Hemostatic O_2 Carrier for Damage Control Resuscitation)
   - Synthetic platelets: DoD/DHA SBIR Phase I H193-004-0067 "Nanoformulated Dried Whole Blood Surrogate for Hemostatic Resuscitation".

   Immune System Using a CH50 and quantitative western blotting for C3/C3a assay, we assessed activation of the complement system by EM. Limited, but detectable, complement activation, was observed with EM-V1, evaluation of V2 is planned in Y3 (evaluation of C' activation and Ab generation) (Major Task # 5, Milestone 7)

3. Pharmacokinetics

- Y1: Top-loading (10% BV replacement) PK studies were completed with both EM-V1 and EM-V2. Analysis of EM-V2 in rabbits indicated a $t_{1/2}$ of ~4.5h. (Major Task 3, Milestone 5).
- Y2: Confirmation of findings, in the context of 20 & 40% BV replacement (data in Y2Q3 report). We anticipate that PK in the setting of higher EM dosing (>40% BV replacement) may exhibit complex multi-phase elimination due to saturation of the mononuclear phagocytic system (MPS). Designed experiments to test this hypothesis, employing an established liposomal clodronate model for MPS depletion (see Text below) (Major Task 4, Milestone 6).

4. Safety/Toxicity

- Y2: We performed exploratory work evaluating the rheologic impact of the "Nanocrit" (Blood Volume comprised by EM particles) in relation to Hematocrit (Blood Volume comprised by RBCs).
  - Circulating EM # (50, 75, 125, 150, 300 x 10^9 particles/mL) was tested in murine & rabbit models. With 20% Blood Volume replacement (Hematocrit ~ 30%), we found physiologic evidence of right ventricular strain and slight impairment of O_2 transport (liver pO_2, lactate) when 'NanoCrit' exceeded 150 x 10^9 particles/mL (data in Y2Q1, Q2 and Q3 reports). (MT 4, Milestone 6)
  - While this is important safety information – and analogous to rheologic impact of elevated hematocrit (blood flow is impaired with Hct > 65%); given the generous [Hb]/particle, maintaining circulating EM # < 150 x 10^9 will NOT limit O_2 carrying capacity during resuscitation.
  - Note: Given the differences in flow dynamics and vessel caliber, we anticipate that the therapeutic window (for the NanoCrit) will (1) be broader in humans and (2) dependent on the degree of hemorrhage (# residual RBCs circulating after resuscitation), allowing for greater concentrations/higher particle abundance to be tolerated. *Defining this relationship will be a focus of a related project*: DoD BA190035 Field Optimized Composition and Use for ErythroMer (Artificial Red Cell) submitted in response to: (USA-MRMC-BAA-2018-W81XWH18SBAA1)

5. Efficacy

- Y2: Rabbit hemorrhagic shock model pilot (Major Task 7, Milestone 9)
  - Initial studies indicated non-inferiority of EM-based resuscitation to re-infused shed blood and superiority of EM- to Colloid (MAP, lactate, tissue pO_2), (Data reported in Y2Q1-3).
  - Several model elements have been evaluated to optimize our shock model and establish appropriate conditions for further PK analysis with volume replacement.
  - Initial studies were performed using ventilation with 100% oxygen. However, to realistically simulate hemorrhagic shock under field conditions, we reduced FiO_2 to 21% oxygen.
  - Additionally, we were concerned about auto-resuscitation (release of sequestered RBCs) due to splenic contraction. Empiric evaluation (studies +/- splenectomy) indicated limited impact upon outcome. As such, we have determined that splenectomy is not necessary, which more accurately models hemorrhagic shock in field.
  - We have now completed initial pilot work to optimize our hemorrhagic shock + polytrauma model (with pseudofracture, crush injury and liver laceration).

6. Team Transition to Baltimore

- Y2: Drs. Doctor, Pan and the KaloCyte Team transitioned from WU, UIUC and the St. Louis Cortex District (respectively) to join the newly formed Center for Blood Oxygen Transport and Hemostasis (CBOTH) at the University of Maryland, Baltimore – which Dr. Doctor directs. CBOTH is located in the new Health Sciences Facility (HSF) III and includes resources that will accelerate project task completion (10,000 sf labs, with six core labs: RBC and Hematology; Nanofabrication and Characterization; Imaging; Small Animal Surgery and Physiology; Analytical Chemistry; and Biospecimen Repository and Clinical Research).
  - *Work stopped on this grant at WUSM on 6/7/2019. Work continued with KaloCyte, however.*
  - Due to personnel turnovers at the sponsoring agency, the transition of this grant was delayed.

Optimization of EMv2 preparation (MT 1, Milestones 1 and 2). From the perspective of sample preparation and quality, formation of EM nanosuspensions were studied using various techniques such sonication, membrane hydration, ethanol injection and high pressure microfluidization. Interestingly some notable differences were still noticed (**Figure 1**). The generation of EM involved self-assembly of their constituent parts from a mixture of amphiphile in micellar state. The constituent parts are as follows: i) phospholipids (20 mole%); ii) mixture of hemoglobin and allosteric effector RSR13 and iii) lipid-amphiphile-precursor (80 mole%). EMv2 has been designed with a biocompatible lipid-oligomeric amphiphile chains across the surface, producing a net negative zeta potential, excellent payload retention and differential gas permeability. Moreover, unlike phospholipid bilayers in liposomal-based HBOCs, EM has a tunable membrane offering greater integrity due to counterionic Hb and precursor interaction and pH responsiveness. EM V2 can be classified as a hybrid-vesicles resulting from the combined self-assembly of both amphiphilic lipid-oligomer into an advanced vesicular structure. To afford such a design, the different parameters controlling both self-assembly and membrane structure must be tuned. Compared to EM V2, the cell biomimetic character of EM V1 is rather limited as polymeric amphiphile in EM V1 is entirely synthetic in nature, while lipid-oligomeric amphiphiles used in EM V2 are mostly natural components of the cell membrane. Optimization studies revealed that hybrid vesicular structures can be obtained according to the molar composition and thermodynamic phase of the phospholipids and precursor mixture. In the composition optimization experiment, the effect of precursor and cholesterol concentration on particle size was investigated by varying the concentration of precursor and cholesterol. All the samples were prepared by following the EMv2 procedure of probe sonication followed by purification using tangential field flow fractionation (TFF). DLS and zeta potential measurements were obtained in triplicate for each measurement. The average mean and their induvial std. dev. are calculated for each measurement and the results were compared to each other. Results indicated that increasing the cholesterol concentration will increase the stability and Hb encapsulation. (**Figure 2a**)

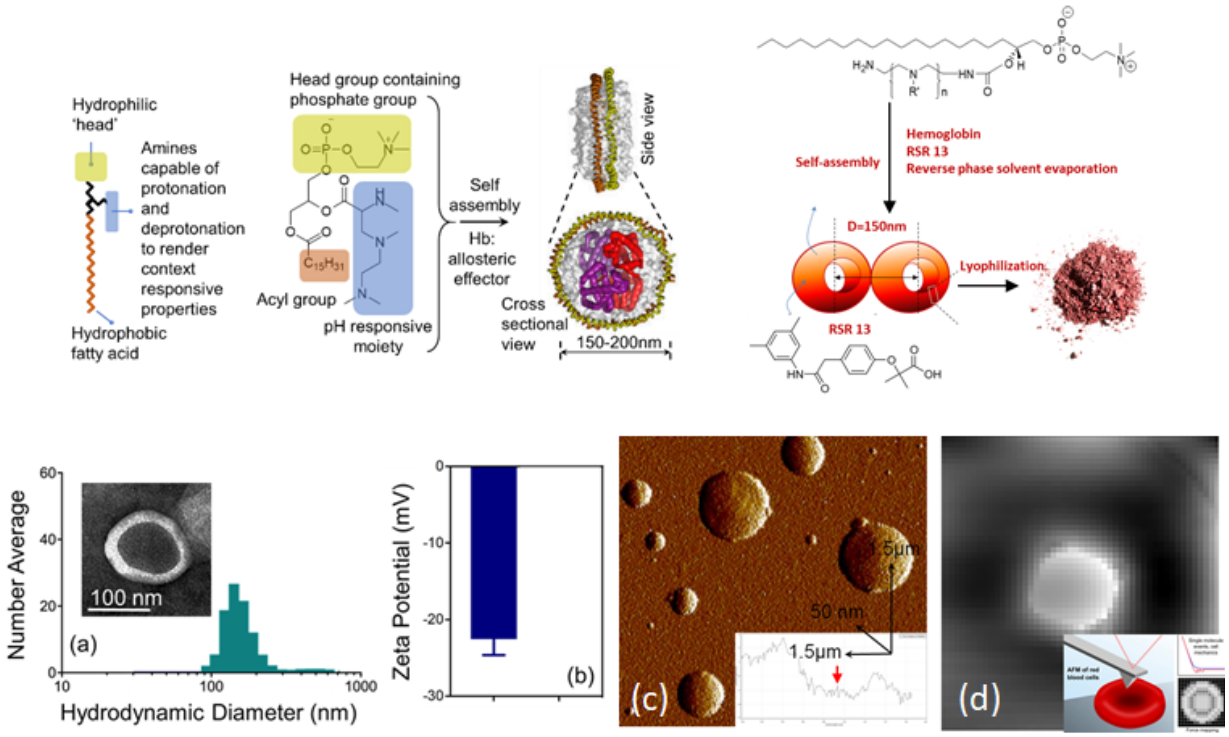

Figure 1. Schematic representation of the composition and self-assembly; chemical structure of the precursor and the process. (a) Hydrodynamic diameter and TEM image (inset) and (b) electrophoretic potential results. Tapping

Lyophilization and resuspension (MT 1, milestones 1 and 2). Once lyophilized, the EM nanoparticles form easy-to-handle fluffy cakes (**Figure 1**) which require manual shaking and short resuspension times and maintain the size characteristics of the origin suspensions (**Figure 2b**). Indeed, it appears that most particles retained their initial size and surface properties during the lyophilization/resuspension process, and that aggregation is not an encumbering issue. The respective electrophoretic potential values (mV), was employed here as indicators of the stability of EM colloidal dispersions, also remained mostly unaffected by the lyophilization and resuspension procedure and may even improve marginally. Unlike earlier liquid preparations, these particles also incorporated RSR13 as allosteric effector. Results indicated that the presence of RSR13 helps with the stability of the particles (**Figure 2c**). For characterization, we have employed nanoparticle tracking analysis (NTA) to measure particle size by video tracking, simultaneously, many individual particles. This results in a particle size distribution of high resolution, particle concentration. Results indicated the presence of multiple population in the suspension which is very typical for a 'soft' particle. (**Figure 2d**). Overall, the results obtained as part of this study support the concept that suspensions of stabilized EM-nanoparticles can be lyophilized to yield ready-to-use powders which can be resuspended on demand without notable loss of particle quality (i.e., no significant aggregation).

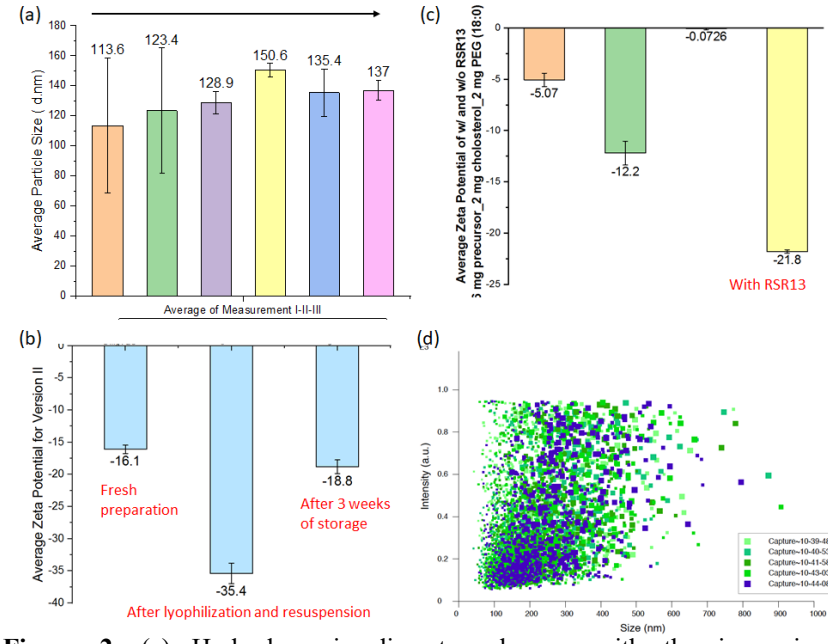

Figure 2. (a) Hydrodynamic diameter changes with the increasing concentration of cholesterol; (b) stability measurements immediately after preparation, after lyophilization and resuspension and after 3 weeks of shelf life study; (c) NTA study measures mean square displacement in two dimensions.

Sterility testing (MT 1, milestone 1 and 2). The EMv2 samples were subjected to membrane filtration 0.45µm cellulosic membrane filter. To investigate the presence of the microorganism in the EM sample, a bacterial culture test was done. LB agar plate was used as a nutrition media to culture the bacteria from EM unsterile sample. In a sterile environment, an aliquot of the EM sample was streaked on the plate and incubated at 37°C for 48h. Around 10 separate colonies were grown indicating the contamination of the sample with microorganisms (**Figure 3**). The number of colonies was found to be negligible indicating a very low concentration of microorganism's presence in the sample. The number of colonies in the region where the sample is diluted because of the streaking (low concentration region) showed no colonies which further confirmed the low bacterial concentration in the original sample (**Figure 3**).

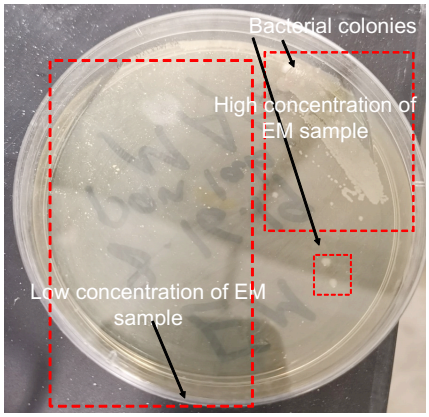

Figure 3. LB agar plate image after 48h incubation, 37°C after EM sample streaking. The lack of bacterial colonies is evident.

Clodronate based depletion of the Mononuclear Phagocytic System (MPS) to simulate MPS saturation during massive transfusion (MT 4, milestone 6). Studies to date continue to support our hypothesis that EM clearance from the circulation is driven by the MPS, which may become saturated during massive blood volume replacement with EM. MPS saturation will result in a reduced EM clearance rate (extended circulation time). However, in our rabbit model of acute hemorrhagic shock/resuscitation, the ability to evaluate the extent to which this saturation will affect EM half-life is complicated by ischemia-reperfusion (which alters MPS saturation thresholds). To address this, we have recently employed selective macrophages depletion (and

depletion of circulating monocytes/macrophage-precursors) with systemic injection of liposomal encapsulated clodronate (dichloromethylene bisphosphonate) prior to infusion with EM. This approach will enable us to fully appreciate the extent to which MPS saturation will increase the half-life/circulation time for EM. In order to evaluate the effect of tissue macrophages on EM circulation, we are first using dose escalation studies with clodronate-liposome 24 hours prior to collection of tissues (liver and spleen). In the next quarter, once dosing is established for maximal MPS depletion, we will employ fluorescently-labeled liposomes (empty/no clodronate but same composition as clodronate-liposomes) as an EM-surrogate (control), as these particles are known to be cleared by the MPS, to further validate our PK studies with EM. Testing the effect of MPS depletion 24 hours prior to administration of either EM or fluorescently-labeled liposomes will help us model the circulation time for EM when the MPS is severely or totally saturated or otherwise affected (ischemia-reperfusion injury).

Clodronate dose escalation studies (MT 4, milestone 6). The conditions for tissue macrophage identification in dissociated and collagenase-digested liver and spleen tissue had been previously established using mouse tissues (separate experiments), so these studies were optimized based on this prior work. To confirm the level of macrophage depletion, flow cytometry was used to evaluate the presence of tissue macrophages in the liver and spleen. We dissociated and collagenase-digested spleen, and liver tissue from the control rabbits (received empty liposomes) and the clodronate liposome-infused rabbits (0.75ml/kg, 1.5ml/kg, 3.0ml/kg, or 6.0ml/kg) for flow cytometry analysis at 24h post-infusion. However, during our preliminary testing, we found that interpretation of the spleen and liver data is complicated as clodronate treatment appears to cause an influx of neutrophils into the tissues. As this major neutrophil population makes identification of macrophages more challenging, we are optimizing conditions with more specific macrophage-targeting antibodies, which have demonstrated promising results. Using the macrophage-specific antibodies, flow cytometry on the liver, spleen, and blood samples revealed a near-complete depletion of monocytes from the blood in the clodronate liposomes-treated rabbits as well as a near-complete depletion of tissue macrophages with the higher concentrations (3.0ml/kg, or 6.0ml/kg) of clodronate liposomes. (We will confirm these results by histology, using paraffin-fixed some spleen and liver tissue from these animals.)

Rabbit Polytrauma Pilot (MT 7, milestone 9). As outlined in our proposal, in addition to testing EM in a model of acute hemorrhage/resuscitation, we also are testing EM in a model of polytrauma – trauma induced coagulopathy (TIC) in which we can realistically evaluate utility of EM-based resuscitation in a setting requiring simultaneous correction of oxygen delivery impairment and abnormal hemostasis. CBC and ROTEM data are presented for the albumin groups in **Figures 4-7**, demonstrating progressive coagulopathy over time. In the plots below, we show anticipated superiority of Blood based resuscitation (v albumin). This model will be transitioned to the UMB Center for Blood Oxygen Transport and Hemostasis, for ErythroMer efficacy and safety testing.

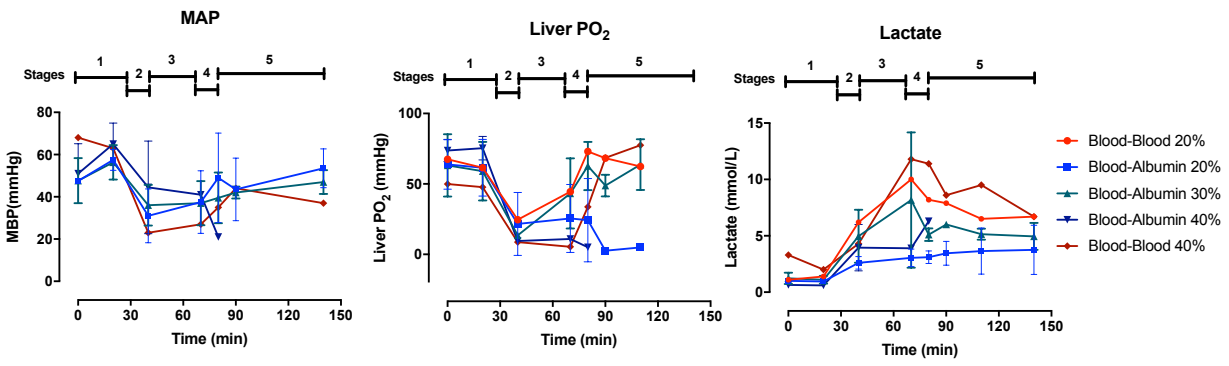

Figure 4 Recorded Values of Parameters Measured During Polytrauma Model Pilot. After establishing baseline conditions (Stage 1), hemorrhagic shock was induced (by removal of 20, 30 or 40% BV estimated by weight) and polytrauma was simulated (thigh crush with femur pseudo-fracture + liver laceration), animals were allowed to stabilize (20 min), then resuscitated by infusion (by returning removed volume over 10 minutes) of either: shed blood or human albumin (blue) The figure captures (from left to right): mean arterial pressure (MAP), liver tissue pO2, and venous lactate (mmol/L), which demonstrate: anticipated superiority of blood in this context. We are further calibrating this model to identify the blood volume removal %age which achieves optimum separation of the blood and albumin groups, to enable testing of EM (non-inferiority to blood and superiority to albumin).

Evaluation of Functional Hemostasis in our Polytrauma Model: The purpose of adding polytrauma injuries to our hemorrhagic shock model is to more accurately model (1) dilutional and (2) trauma-induced coagulopathies. The liver laceration and pseudofracture introduce exposure of tissue factor, collagen and matrix – this is expected to trigger the consumptive features of trauma induced coagulopathy. Dilutional features of the model are demonstrated in Figure 5; showing ~ 40% reduction in platelet count. Battlefield TIC is typically associated with > 50% reduction. The consumptive features of the coagulopathy are demonstrated in Figures 6 & 7; which show a modest (but detectable) increase in clotting time (CT) and notable reduction in maximum clot firmness (MCF). While the reduction in MCF is a reasonable approximation of TIC findings under battlefield conditions, we typically see a more significant increase in CT. *We will work to optimize this feature after the model is transitioned to UMB.*

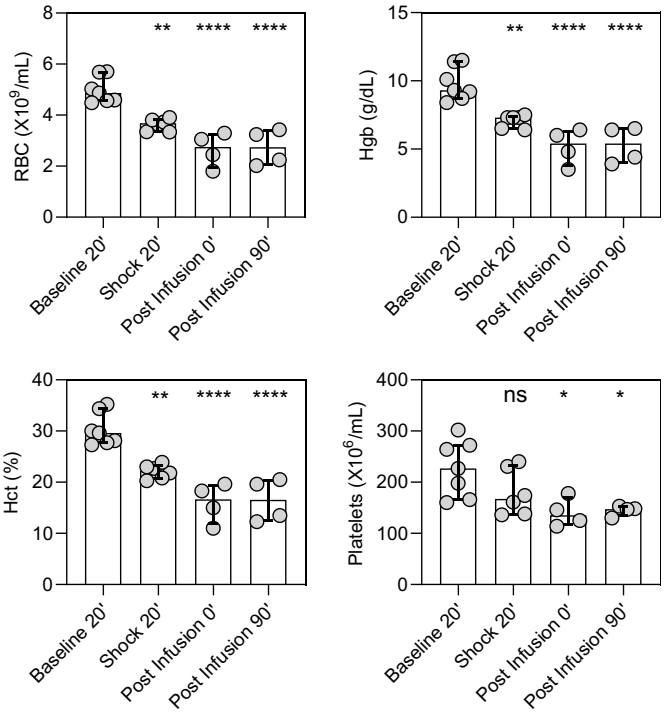

Figure 5 Data presented as median (IQR); we compared time point vaues back to baseline 20' values using an un-paired one way ANOVA. *, p<0.05; **, p<0.01; ****, p<0.0001

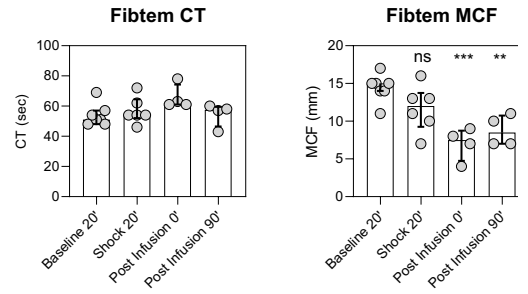

Figure 6 ROTEM FibTEM data presented as median (IQR); statistical analyses comparing time points back to baseline 20' values using an un-paired one way ANOVA. **, p<0.01; ***, p<0.001

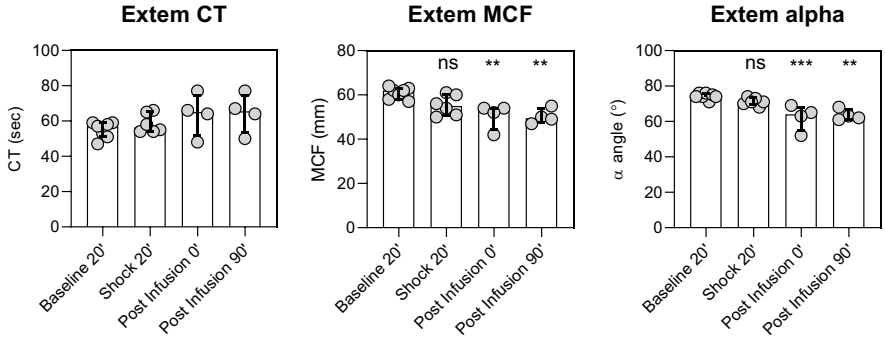

Figure 7 ROTEM ExTEM data presented as median (IQR); statistical analyses comparing time points back to baseline 20' values using an un-paired one way ANOVA. **, p<0.01; ***, p<0.001

What opportunities for training and professional development has the project provided?

Multiple team members attended and presented at MHSRS, at the Intl Society of Blood Substitutes (ISBS), International Society of Oxygen Transport to Tissue (ISOTT) and AABB conferences. Multiple team members are at various stages in their research training; this project has greatly enhanced their education.

How were the results disseminated to communities of interest?

Selected data with EM-V1 was disseminated in poster form and an oral presentation to members of the military community at the 2019 MHSRS conference. Additionally, ErythroMer data was presented at a special THOR-AABB symposium on pre-hospital resuscitation and at the International Society of Oxygen Transport to Tissue (ISOTT) (see above). ErythroMer data was also presented at the 2019 International Artificial Cell Symposium (Nara, Japan).

What do you plan to do during the next reporting period to accomplish the goals?

As outlined in our SOW, further PK and oxygenation/acute shock studies are planned for Y3 in our PFC (48h survival models) – which will be the focus of Y3 activity; this set of PFC models will be exploited to further our understanding of dosing (PK), biocompatibility, safety and efficacy. We also plan to use this set of experiments to optimize our hemostasis/oxygenation algorithm in concert with Cellphire's platelet derived product, to determine compatibility of these products in *in vivo*.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

This project involves significant partnership with KaloCyte, Inc. – a startup created to commercialize ErythroMer. KaloCyte continues to thrive as a result of this partnership – competing successfully for additional Federal grants (SBIR) and private equity.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

The Team transitioned to the Center for Blood Oxygen Transport and Hemostasis at the University of Maryland, Baltimore as indicated above and in the Y2Q3 report. Otherwise, nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Fabrication scaling of EM version 2.0 was delayed by inability to obtain purified polymer precursor – after a competitive search we have entered into partnership with Southwest Research Institute (San Antonio, TX) to address this issue, which is not expected to persist in Y3.

Changes that had a significant impact on expenditures

Team Transition to Baltimore

- *Work stopped on this grant at WUSM on 6/7/2019. Work continued with KaloCyte, however.*
- Due to personnel turnovers at the sponsoring agency, the transition of this grant was delayed; as such, work by the UMB team did not resume before the end of this reporting period (10/29/19).

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

There have been no significant changes in the use/care of human subjects. No changes to our IRB protocol submission have been made; we are still approved to enroll 50 Healthy Adults. There have been no "significant deviations" or protocol violations for human subjects.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

There have been no significant changes in the use of biohazards and/or select agents.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Journal publications.

Abstract submitted and published for the 2019 MHSRS conference with both a poster and oral presentation at the conference (previously reported and shared).

Books or other non-periodical, one-time publications.

Sen Gupta A and Doctor A. Oxygen Carriers. Chapter 11, pp 197-222. in: Damage Control Resuscitation: Identification and Treatment of Life-Threatening Hemorrhage. Editor: Phillip C Spinella. Springer Nature. 2019.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

https://www.kalocyte.com/ – this is the website for KaloCyte, Inc. the spinoff startup that was created to develop ErythroMer. KaloCyte is a subawardee on this project.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

During Y1, an exclusive license was obtained by KaloCyte from Washington University for all relevant patent rights for EM-V1.

A provisional patent application is in process for filing by KaloCyte for its development of the novel composition (EM-V2).

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

WU (individuals who transitioned to University of Maryland are identified below with an asterisk)
Name: Allan Doctor*
Project Role: PI
Researcher Identifier (ORCID): https://orcid.org/0000-0002-6096-6400
Nearest person month worked: 2
Contribution to Project: As PI, Dr. Doctor has directed all aspects of the project thus far.

Name: Stephen Rogers*
Project Role: Senior Scientist
Researcher Identifier: N/A
Nearest person month worked: 3
Contribution to Project: Dr. Rogers assists in animal physiology experiments and is responsible for bench top characterization of ErythroMer.

Name: Xue Lin – No Change
Name: Jose Aldana – No Change
Name: Rohit Rasane – No Change
Name: Sarbani Ghosh – No Change
Name: Anja Fuchs – No Change
Name: Sarbani Ghosh – No Change

KaloCyte
Name: Jennifer Richards*
Project Role: Product Development Lead Scientist
Researcher Identifier: N/A
Nearest person month worked: 5
Contribution to Project: Dr. Richards is responsible for supervision, implementation and refinement of the development program for ErythroMer.

Name: Zhuozhi Wang*
Project Role: Synthetic Chemist
Researcher Identifier: N/A
Nearest person month worked: 5
Contribution to Project: Dr. Wang is responsible for fabrication of ErythroMer and evaluating bench top characterization of ErythroMer.

UIUC
Name: Dipanjan Pan* – No Change
Name: Dinabandhu Sar
Project Role: Postdoctoral Associate
Researcher Identifier: N/A
Nearest person month worked: 3
Contribution to Project: Dr. Sar has conducted method development and in vitro studies required to chemically and biologically characterize nanoparticles.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

None

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: *Attached separately*

None