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FIRST TRIANNUAL REPORT (YEAR 2)

for period February 1, 1993 to May 31, 1993

Report Date: June 28, 1993

ONR Grant No. N00014-92-J-1244

**EVALUATION OF DRIED STORAGE OF PLATELETS FOR TRANSFUSION:
PHYSIOLOGIC INTEGRITY AND HEMOSTATIC FUNCTIONALITY.**

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Attachment: Report from subcontract principal investigator, Marjorie S. Read, Ph.D.,
The University of North Carolina at Chapel Hill.

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Patents, Proposals, Papers:

In the last report period, we received a statement of rejection of claims from the U.S. Patent Office on our filing of May 1992 for use of lyophilized platelets in transfusion medicine. We have prepared and filed (4/28/93) an extensive continuation-in-part (CIP) response which included aspects of applications of lyophilized platelets in wound healing. The CIP added much new data to strengthen the prior claims and listed others related to activity of platelet-derived growth factor (PDGF) expressed by freeze-dried platelets as an adjunct application in surface dressings.

A major effort was put into preparing a proposal for augmentation of present studies to address issues of toxicity and immunogenicity and to extend the current specific aims. This application was solicited in competition for funds in the ATD for Blood Substitutes and Products, submitted 6/4/93. The proposal was entitled "Preclinical Investigation of Lyophilized Platelet Preparations" to reflect the overall goal of establishing the groundwork for an IND in '95-'96 for human trials. If funded, this proposal will greatly enhance current studies and accelerate our efforts to test the hemostatic efficacy and safety of freeze-dried preparations. A separate workplan was recently submitted by corporate and scientific representatives of Armour Pharmaceutical Corporation (Collegeville, PA) in collaboration with us to examine GMP and practical issues of scaleup for mass-producing freeze-dried human platelets.

The patent and research proposal activity has provided us with the opportunity to update review of current progress and expand plans for further experimentation. The first manuscript developed from this project was submitted to the journal Blood in the last reporting period; it is now under revision wherein in vivo data from animal experiments will be added to augment the in vitro data already presented. The original plan was to have two separate manuscripts: in vitro, then in vivo. In addition, an abstract on adhesion/activation properties of rehydrated platelets in Baumgartner vessel perfusion chambers was submitted 5/13/93 to the American Association of Blood Banks for presentation in October (see attached). Further abstracts are planned for the next reporting period to submit to the annual meeting of the American Society of Hematology.

Scientific Progress:

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Recent work at ECU with paraformaldehyde or permanganate-stabilized freeze-dried platelets has focused on adhesion and activation in a whole blood perfusion chamber, modeled after the experiments of Baumgartner et al. [Thrombos. Haemostasis 35:124, 1976]. In our adaptation, the chamber is fitted with two 1 cm. everted strips of denuded canine artery and perfused at 130 mL/min with either fresh citrated whole blood (control) or rehydrated platelets mixed with fresh packed red cells and fresh citrated platelet-poor plasma. In this reporting period, we ran 16 different platelet preparations in perfusion experiments with controls. Example results are given in the tables below. The percent coverage index was estimated by coating the vessel strips with a fluorescent monoclonal antibody to platelets (P2; AMAC Corp.) and then counting on a morphometric grid the area covered versus background in

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microphotographs under epi-fluorescence at 600x. As mentioned in our prior report, we also noted that some of the nonadherent platelets expressed activation markers (GMP-140 or GP-53). We have continued to measure neoantigens of activation on rehydrated platelets exposed to the Baumgartner chamber to demonstrate the ability of these platelet preparations to respond to physiologic stimulation.

Table 1. Adhesion of platelets to denuded blood vessel

<u>Preparation of Platelets</u>	<u>Percent Coverage of Vessel</u>
Fresh, no stabilization	53 - 76%
Rehydrated, 0.02 M permanganate, 10 min	26 - 53%
Rehydrated, 1.8% paraformaldehyde, 1 hr	23 - 43%
Rehydrated, 2.0% paraformaldehyde, 2 hrs	44 - 80%

Table 2. Activation marker expression (% platelets positive for MoAB CD62 or CD63 by flow cytometry) on nonadherent platelets exposed to denuded blood vessel; "Pre", or "Post" circulation in Baumgartner chamber.

<u>Platelet Preparation</u>	<u>GMP-140</u>		<u>GMP53</u>	
	<u>Pre</u>	<u>Post</u>	<u>Pre</u>	<u>Post</u>
Rehydrated, 1% Para/60 min.	12%	16%	18%	22%
Rehydrated, 1.8% Para/60 min.	6%	12%	14%	21%
	3%	7%	19%	30%
Rehydrated, 2% Para/2 hrs *	11%	6%	1%	1%
Rehydrated, 0.02M permanganate,	39%	86%	-	-
10 mins.	31%	60%	-	-
Rehydrated, 0.01M permanganate,	-	37%	-	-
10 mins.				

*This preparation showed no increase in signal of activation as a result of circulation in the Baumgartner chamber.

The long-term storage experiment is continuing as planned, with only MoAB SZ-1 (GPIbIX complex) showing any loss of surface staining over the first 6 months. The one year workup will be performed in July, 1993. Also, another preparation of para-platelets, and a preparation of perm-platelets will be entered into a new long-term storage study in July. In additional studies on current preparations, we have obtained evidence of PDGF expression by flow cytometry on rehydrated platelets increasing after circulation in a Baumgartner chamber. This provides support for the notion that freeze-dried platelets may be an effective treatment (topical or IV) capable of releasing PDGF upon activation in wound sites to promote healing. In vivo experimentation is described in the attached subcontract report from Dr. Read at UNC-Chapel Hill.

Report Period February 1, 1993 - May 31, 1993
University of North Carolina at Chapel Hill

Contract: UNC/ECU
Grant No. N0014-92-J-1244
The Office of Naval Research
Department of the Navy

Performance Site: University of North Carolina at Chapel Hill
Principal Investigator: Marjorie S. Read, Ph.D.
Co-PI: Robert Reddick, MD
Submitted: June 29, 1993

Studies Conducted during the reporting period.

1. Antibody determination of platelet surface antigens and the effect of antibodies against those antigens on clotting and aggregation. Platelets that are saline washed have less plasma protein reactions but have been reported to have a lower recovery rate than ACD washed or unwashed control platelets (Pineda et al Transfusion, 1989). Surface bound antigens and the relationship of bound antigen to clotting, agglutination and the ability of platelets to circulate are being studied. We are comparing the surfaces of rehydrated platelets and fresh platelets for the presence of bound protein, either plasma proteins or platelet released proteins. We have examined the platelet surface for bound plasmin, Factor XIII, and fibrinogen. Preparations of washed platelets, ACD and citrate-ACD washed platelets present all three antigens on the surface. So far no difference has been seen between platelets stabilized with paraformaldehyde and fresh washed platelets with the bank of antibodies being used. Bound antigens had no effect on the agglutinating and clotting activity as determined in agglutinating and clotting tests in the presence of specific antibodies.

Studies of surface antigens and the effect on circulation are not complete.

2. Infusion of rehydrated pig platelets into a von Willebrand deficient pig with a spontaneous clinical bleed. In addition to the above study we have ongoing studies with rehydrated platelets in animals. Correction of the bleeding had not been accomplished with cryoprecipitate alone. This was consistent with previously observed response in the bleeder pigs. Likewise, correction was not seen with rehydrated platelets alone. Rehydrated platelets were then given along with cryoprecipitate. The bleeding time shortened to 12 minutes and the clinical bleeding stopped.

Studies of the post infusion platelets showed few labeled platelets present. This may have been due to use of the platelets at bleeding sites. Since this was not a terminal experiment, biopsies were not taken.

Further studies with infused rehydrated platelets in the bleeder pig indicate that circulation of labeled rehydrated platelets may be enhanced by the addition of cryoprecipitate or plasma at the time of infusion.

Why plasma or cryoprecipitate will enhance rehydrated platelet circulation in the pig is unknown to us at present. Whether the effect is related to surface bound protein is being studied in conjunction with the above antibody study.

A third infusion of rehydrated platelets into a vWD pig again showed increased circulation following the second infusion.