11:22:46 OCA PAD INITIATION - PROJECT HEADER INFORMATION 07/10/96

Active

Project #: E-19-W18 Cost share #: Rev #: 0
Center # : 10/24-6-R0021-0A0 Center shr #: OCA file #:
Work type : RES
Contract#: LTR DTD 960619 Mod #: LTR DTD 960619 Document : SUBCONT
Prime #: 5 P60 HL48482-04 Contract entity: GTRC

Subprojects ? : N CFDA: N/A
Main project #: PE #: N/A

Project unit: CHEM ENGR Unit code: 02.010.114
Project director(s):
WICK T M CHEM ENGR (404)894-8795

Sponsor/division names: EMORY UNIVERSITY / ATLANTA, GA
Sponsor/division codes: 400 / 012

Award period: 960401 to 970331 (performance) 970331 (reports)

Sponsor amount	New this change	Total to date
Contract value	115,432.00	115,432.00
Funded	115,432.00	115,432.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: GA COMPREHENSIVE SICKLE CELL CENTER - PROJECT #1 (YEAR 04)

PROJECT ADMINISTRATION DATA

OCA contact: Ina R. Lashley 894-4820

Sponsor technical contact

JAMES R. ECKMAN, M.D.
(404)589-3572

DEPARTMENT OF MEDICINE
EMORY UNIVERSITY
69 BUTLER ST.
ATLANTA, GA 30303

Sponsor issuing office

JANE O'CONNER
(404)727-2503

OFFICE OF SPONSORED PROGRAMS
EMORY UNIVERSITY
1784 N. DECATUR ROAD, SUITE 510
ATLANTA, GA 30322

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : NA NA supplemental sheet
Equipment title vests with: Sponsor X GIT
NONE PROPOSED. (SEE SUBGRANT ARTICLE 12 FOR DETAILS.)
Administrative comments -
INITIATION OF YEAR 04 OF SUBGRANT UNDER NIH PRIME (FOLLOW-ON TO E-19-X91).

4
(1)

Closeout Notice Date 02-JUL-1997

Project Number E-19-W18 Doch Id 38700

Center Number 10/24-6-R0021-0A0

Project Director WICK, TIMOTHY

Project Unit CHEM ENGR

Sponsor EMORY UNIVERSITY/ATLANTA, GA

Division Id 5779

Contract Number LTR DTD 960619 Contract Entity GTRC

Prime Contract Number 5 P60 HL48482-04

Title GA COMPREHENSIVE SICKLE CELL CENTER - PROJECT #1 (YEAR 04)

Effective Completion Date 31-MAR-1997 (Performance) 31-MAR-1997 (Reports)

Closeout Action:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	26-JUN-1997
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory and Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments
–

Distribution Required:

Project Director/Principal Investigator	Y
Research Administrative Network	Y
Accounting	Y
Research Security Department	N
[redacted] Coordinator	Y
Research Property Team	Y
Supply Services Department	Y
Georgia Tech Research Corporation	Y
Project File	Y

E-19-W18
#1
(New)

GEORGIA TECH RESEARCH CORPORATION

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
PROGRAM INITIATION DIVISION
ATLANTA, GEORGIA 30332-0420
USA

Telex: 542507 GTRC OCA ATL
Fax: (404) 894-6956

Phone: (404) 894-4817

Refer to: CED/02.400.012.97.014
E-19-W18

13 January 1997

E-19-W18/WICK, Ch Eng
DELIVERABLE ITEM #1
ANNUAL PROGRESS RPT.

Emory University
School of Medicine
15 A Sickle Cell
80 Butler Street, N.E.
Atlanta, Georgia 30335

Attention: Dr. James R. Eckman

Subject: Research Proposal Entitled, "Georgia Comprehensive Sickle Cell Center - Project No. 1"

Dear Dr. Eckman:

GEORGIA TECH RESEARCH CORPORATION is pleased to submit for your consideration the subject proposal prepared by Dr. Timothy M. Wick, School of Chemical Engineering, Georgia Institute of Technology.

A description of the research program, the time required and estimated cost are included in the proposal. Should additional information be desired, please do not hesitate to contact Dr. Wick at (404) 894-8795 regarding technical matters or the undersigned at (404)894-4817 for administrative matters.

In the event of an award, we propose that the effort be funded by an amendment to the Subcontract under NIH Grant No. 5 P60 HL48482 drawn in the name of the GEORGIA TECH RESEARCH CORPORATION.

We appreciate the opportunity to submit this proposal and look forward to hearing from you soon.

Sincerely,

Christopher E. D'Urbano
Contracting Officer

ıclosure: Proposal

Form Approved Through 9/30/97
OMB No. 0925-0001

Department of Health and Human Services
Public Health Service

Application for Continuation Grant

Review Group	Type	Activity	Grant Number

Total Project Period
From: 15 April 1993 Through 31 March 1998

Requested Budgt Period
From: 1 April 1997 Through: 31 March 1998

1. TITLE OF PROJECT
Georgia Comprehensive Sickle Cell Center - Project #1

2. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Name and address, street, city, state, zip code)*

Timothy M. Wick, Ph.D.
School of Chemical Engineering
Georgia Institute of Technology
778 Atlantic Drive
Atlanta, GA 30332-0100

4. APPLICANT ORGANIZATION *(Name and address, street, city, state, zip code)*

Georgia Tech Research Corporation
400 10th Street, N.W.
Atlanta, GA 30332-0420

2b. E-MAIL ADDRESS
timothy.wick@che.gatech.edu

5. ENTITY IDENTIFICATION NUMBER
58-0603146

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
School

2d. MAJOR SUBDIVISION
Engineering

3. ORGANIZATIONAL CODE
20 other academic

6. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL

Contracting Officer
Georgia Tech Research Corporation
Georgia Institute of Technology
Office of Contract Administration
Atlanta, GA 30332-0420

E-MAIL ADDRESS
christopher.durbano@oca.gatech.edu

7. HUMAN SUBJECTS	7a. If "Yes," Exemption no. or IRB approval date	7b. Assurance of compliance no.	8. VERTEBRATE ANIMALS	8a. If "Yes," IACUC approval date	8b. Animal welfare assurance no.
☐ No ☒ Yes	4/12/96 ☒ Full IRB or ☐ Expedited Review	M1395	☒ No ☐ Yes		

9. COSTS REQUESTED FOR NEXT BUDGET PERIOD
9a. DIRECT $ 89,460 9b. TOTAL $ 131,619

10. INVENTIONS AND PATENTS *(See instructions)*
☒ No ☐ Yes If "Yes," ☐ Previously reported ☐ Not previously reported

11. PERFORMANCE SITE(S) *(Organizations and addresses)*

Space Science and Technology Building
Room 217
Georgia Institute of Technology
Corner of Ferst and Cherry
Atlanta, GA 30332-0405

	AREA CODE	TELEPHONE NO. AND FAX NO.
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)*	404 404	894-8795 894-2866
12b. NAME OF ADMINISTRATIVE OFFICIAL *(Item 6)* Christopher D'Urbano	404 404	894-4817 894-6956
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 15)* Christopher E. D'Urbano Contracting Officer		
E-MAIL ADDRESS		

13. *Do not use this space.*

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.	SIGNATURE OF PI / PD NAMED IN 2a *(In ink. "Per" signature not acceptable.)*	DATE 11/10/97
15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.	SIGNATURE OF OFFICIAL NAMED IN 12c *(In ink. "Per" signature not acceptable.)*	DATE 1/13/98

DETAILED BUDGET FOR NEXT BUDGET PERIOD—DIRECT COSTS ONLY	FROM	THROUGH	GRANT NUMBER
	1 April 1997	31 March 1998	1 P60 HL48482-05

PERSONNEL *(Applicant organization only)*

NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Timothy M. Wick, Ph.D.	Principal Investigator	12	20	$18,040	$4,817	$22,857
James R. Eckman, M.D.	Co-Investigator	12	--	--	--	--
James P. Siano	Graduate Student	12	100	$17,000	--	$17,000
Richard Montes	Graduate Student	12	100	$17,000	--	$17,000
SUBTOTALS →				$52,040	$4,817	$56,857

CONSULTANT COSTS

None

EQUIPMENT *(Itemize)*

None

SUPPLIES *(Itemize by category)*

Tissue culture supplies (medium, serum, growth factors, etc.) attachment factors $9,000
Monoclonal antibodies, synthetic pestides, cytokins $7,000
Disposable supplies (culture flasks, pipets, filters gloves, etc.) $6,000
Elisa Reagents, FACS Supplies $2,213

$24,213

TRAVEL

Two domestic meetings for PI (ASH and Sickle Cell RFA) $ 1,900

PATIENT CARE COSTS — INPATIENT / OUTPATIENT

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*

Graduate student stipend ($2145/student/yr, not subject to indirect) $4,290
Artwork, photography, publication fees $1,200
Machine shop charges for flow chambers $1,000

$ 6,490

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD $ 89,460

~~INDIRECT COST RATE~~ — DIRECT COSTS 89,460 / INDIRECT COSTS 42,159 (49.5% of direct, excluding tuition)

TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Item 7a, Face Page)* → Direct $89,460 / $Total $131,619

BUDGET JUSTIFICATION	GRANT NUMBER 1P60 HL48482-05

Provide a detailed budget justification for those line items and amounts which represent a significant change from that previously recommended.

Personnel: Fringe benefits are 26.7% of salary. Graduate students do not receive fringe benefits. Graduate student stipend ($2145/yr/student is not subject to indirect costs.

Principal Investigator - Dr. Timothy M. Wick, Ph.D.: Funding is requested to provide time to organize the study, coordinate *in vitro* investigations with clinical studies, perform experiments, analyze data, prepare manuscripts, hold regular laboratory meetings of the investigators, and develop progress reports. It is estimated that 20% of Dr. Wick's time will be devoted to these tasks related to this project.

Graduate Student - James Siano: Mr. Siano has been working in the laboratory since September 1992. Recently, Mr. Siano has begun to characterize sickle cell adherence to pulmonary microvascular and arterial endothelium and to identify therapeutics effective at inhibiting sickle cell adherence likely to predominate *in vivo*. He will continue to systematically search for novel adherence pathways and compounds that inhibit pathological sickle cell adherence in plasma. Mr. Siano will devote 100% of his effort to this project.

Graduate Student - Richard Montes: Mr. Montes has been working in the lab since December 1993. He has developed the cone-and-plate viscometer and the protocols for measuring the kinetics of sickle cell adherence described in the Continuation Application. Mr. Montes will devote 100% of his effort to this project.

Supplies: Tissue culture costs are based upon current performance of 4 flow experiments per week at present costs. Media, serum, growth factors, buffers, and other chemicals as well as plasticware, glassware, and gloves are required for cell cultures and adhesion assays. Monoclonal antibodies to adhesion receptors will be used to identify receptors involved in sickle erythrocyte adhesion to endothelium. ELISA reagents and FACS supplies are required to identify receptors on sickle cells and endothelial cells and to measure concentrations of soluble factors which promote sickle cell adherence.

Travel: Funds are requested for Dr. Wick or an associate to attend ASH and the annual Meeting of the Sickle Cell Disease Program to present research and interact with colleagues interested in similar and related areas of hematology and sickle cell anemia.

Other Expenses: Funds are requested for photocopying, medical illustrations and page costs for presentations and publications. Machine shop charges are required to construct new adhesion systems. Georgia Tech has a glass blowing shop on campus and a machine shop in Dr. Wick's department to manufacture new flow chambers and cone-and-plate viscometers as necessary to complete this project.

CURRENT BUDGET PERIOD	FROM 1 April 1996	THROUGH 31 March 1997

Explain any estimated unobligated balance (including prior year carryover) which is greater than 25% of the current year's total budget or more than $250,000.

None

BIOGRAPHICAL SKETCH

Give the following information for all *new* key personnel.
Copy this page for each person.

NAME	POSITION TITLE
Timothy M. Wick	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Colorado (Boulder, CO)	B.S.	1983	Chemical Engineering
Rice University (Houston, TX)	Ph.D.	1988	Chemical Engineering
Rice University (Houston, TX)	Post-Doc	1988	Chemical Engineering and Biochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

Professional Experience

7/94- Associate Professor, School of Chemical Engineering, Georgia Tech, Atlanta, GA
7/94- Associate Professor, School of Mechanical Engineering, Georgia Tech, Atlanta, GA
4/93-6/94 Assistant Professor, School of Mechanical Engineering, Georgia Tech, Atlanta, GA
9/88-6/94 Assistant Professor, School of Chemical Engineering, Georgia Tech, Atlanta, GA
2/88-9/88 Post-doc, Chemical Engineering and Biology Departments, Rice University, Houston, TX

Honors and Awards

1996 Reviewer, Special Emphasis Panel for RFA "Sickle Cell Disease Therapy," NIH, NHLBI.
1995 Reviewer, for RFA "Coagulation, Platelets, and Thrombosis in Sickle Disease Pathophysiology," NIH, NHLBI.
1993 Member, American Society of Hematology (1993-present).
1993 Ad-Hoc Reviewer. Biomaterials Panel, Biomedical Engineering and Research to Aid Persons with Disabilities Program, National Science Foundation.
1993 Reviewer, Special Emphasis Panel for RFA "NIH Collaborative Projects (RO1s) on Minority Health."
1993 Outstanding Chemical Engineering Professor. Omega Chi Epsilon.
1992 Lilly Foundation Teaching Fellowship.
1991 American Heart Association-Georgia Affiliate, Grant-In-Aid (1991-1993).
1991 The Whitaker Foundation, Biomedical Engineering Research Grant (1992-1995).
1991 NIH-First Independent Research and Transition (FIRST) Award (1991-1996).
1990&1992 Du Pont Young Faculty Award.
1989 American Heart Association-Georgia Affiliate, Grant-In-Aid (1990-1991).
1987 Beecham Award for outstanding original research presented at annual meeting of the SSCI, the Southern Section of the AFCR and the Southern Society for Pediatric Research.
1986 Omega Chi Epsilon (National Chemical Engineering Honor Society).

Original Articles

1. Wick, T.M., J.L. Moake, M.M. Udden, S.G. Eskin, D.A. Sears and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow." Journal of Clinical Investigation, 80:905-910 (1987).
2. Wick, T.M., S.D. Doty, and R.M. Nerem. "Influence of Fluid Mechanical Stresses on Vascular Cell Adhesion." In: *Biomechanical Transport Processes*, F. Mosora, C. Caro, E. Krause, H. Schmid-Schönbein, C. Baquey, and R. Pelissier, eds, Plenum, New York, pp. 283-292, 1990.
3. Wick, T.M. and V. Louis. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Human Umbilical Vein and Human Dermal Microvascular Endothelial Cells under Shear Conditions." American Journal of Tropical Medicine and Hygiene 45: 578-586 (1991).
4. Swerlick, R.A., K. Lee, T.M. Wick, and T.J. Lawley. "Human Dermal Microvascular Endothelial but not Human Umbilical Vein Endothelial Cells Express CD36 *In Vivo* and *In Vitro*." Journal of Immunology, 148:78-83 (1992).

5. Yoganathan A.P., T.M. Wick, & H. Reul. "The Influence of Flow Characteristics of Prosthetic Valves on Thrombus Formation." In: Thrombosis, Embolism, and Bleeding, EG Butchart & E Bodnar, eds, ICR Publishers, London, pp 123-48, 1992.
6. Wick, T.M. and V. Louis. "*Plasmodium fragile*: Cytoadherence of Parasitized Rhesus Monkey Erythrocytes to Human Endothelial Cells under Shear Flow Conditions." Experimental Parasitology, **74**:228-231 (1992).
7. Brittain, H.A., J.R. Eckman, and T.M. Wick. "Sickle Erythrocyte Adherence to Large Vessel and Microvascular Endothelium under Physiologic Flow is Qualitatively Different." Journal of Laboratory and Clinical Medicine, **19**:538-545 (1992).
8. Wick, T.M., J.L. Moake, M.M. Udden, and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Preferentially Promote Young Sickle and Non-sickle Erythrocyte Adhesion to Endothelial Cells," American Journal of Hematology, **42**:284-292 (1993).
9. Johnson, J.K., R.A. Swerlick, P. Millet, K. Grady, T.M. Wick. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Microvascular Endothelium is Regulatable by Cytokines and Phorbol Ester," Journal of Infectious Diseases, **167**:698-703 (1993).
10. Brittain, H.A., J.R. Eckman, R.J. Howard, and T.M. Wick. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelium under Physiologic Flow: A Potential Role for Platelet Activation in Sickle Cell Vaso-occlusion," Blood, **81**:2137-2143 (1993).
11. Flaherty, A.L. and T.M. Wick. "Prolonged Contact with Blood Alters Surgical Gown Permeability," The American Journal of Infection Control, **21**:249-256 1993.
12. Swerlick, R.A., J.R. Eckman, A. Kumar, M. Jeitler, and T.M. Wick. "$\alpha_4\beta_1$-Integrin Expression on Sickle Reticulocytes: Vascular Cell Adhesion Molecule-1-Dependent Binding to Endothelium," Blood, **82**:1891-99 (1993).
13. Wick, T.M., H.A. Brittain, R. Howard, and J.R. Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelial Cells via CD36 and integrin receptors," In: *Vascular Endothelium: Physiological Basis of Clinical Problems II*, J. Catravas, A. Callow, U. Ryan, eds, Plenum Press, New York, pp. 213-214, 1993.
14. Wick, T.M., J.K. Johnson, R.A. Swerlick, K.K. Grady, and P. Millet. "Cytokine Upregulation of CD36, but not ICAM-1, Increases *Plasmodium falciparum* Infected Erythrocyte Adherence to Microvascular Endothelial Cells under Shear Conditions," In: *Vascular Endothelium: Physiological Basis of Clinical Problems II*, J. Catravas, A. Callow, U. Ryan, eds, Plenum Press, New York, pp. 211-212, 1993.
15. Smolinski, P.A., M.K. Offermann, J.R. Eckman, and T.M. Wick. "Double Stranded RNA Induces Sickle Erythrocyte Adherence to Endothelium: A Potential Role for Viral Infection in Vaso-Occlusive Pain Episodes in Sickle Cell Anemia," Blood **85**:2945-2950 (1995).
16. Gonzales, R.S. and T.M. Wick. "Hemodynamic Modulation of Monocyte Adherence to Vascular Endothelium," Annals of Biomedical Engineering **24**:382-293 (1996).
17. Wick, T.M. and J.R. Eckman. "Molecular Basis of Sickle Cell-Endothelial Cell Interactions," Current Opinion in Hematology **3**:118-124 (1996).
18. Wick, T.M. and A.L. Flaherty. "A Novel Method for Quantification of Surgical Gown Permeability," In: *Performance of Protective Clothing: Fifth Volume*, ASTM STP 1237, J. S. Johnson and S. Z. Mansdorf, Eds., American Society for Testing and Materials, Philadelphia, PA, pp. 123-130 (1996).
19. Kumar, A., R.A. Swerlick, J.R. Eckman, and T.M. Wick. "Phorbol Ester Stimulation Increases Sickle Erythrocyte Adherence to Endothelium: A Novel Pathway Involving $\alpha_4\beta_1$ Integrin Receptors on Sickle Reticulocytes and Fibronectin," Blood **88**:4348-4358 (1996).
20. Kumar, A., J.R. Eckman, and T.M. Wick. "Inhibition of Plasma-Mediated Adherence of Sickle Erythrocytes to Microvascular Endothelium by Conformationally Constrained RGD-Containing Peptides," American Journal of Hematology **53**:92-98 (1996)
21. Smolinski, P.A., J.R. Eckman, T.M. Wick. "Tenacity of Receptor Mediated Sickle Erythrocyte Adherence to Vascular Endothelium: Implications for Microvascular Occlusion in Sickle Cell Anemia," Blood **(In review)**.
22. Siano, J.P., K.K. Grady, P. Millet, R.A. Swerlick, T.M. Wick. "*Plasmodium falciparum*: Cytoadherence of HB3 Parasitized Erythrocytes to Human Microvascular Endothelium under Shear Flow Conditions," Experimental Parasitology **(In review)**.
23. Siano, J.P., K.K. Grady, P. Millet, T.M. Wick. "*Plasmodium falciparum*: Cytoadherence of Parasitized Erythrocytes to Microvascular Endothelium Under Shear is Mediated by $\alpha_v\beta_3$," Experimental Parasitology **(In review)**.

OTHER SUPPORT

Wick, Timothy M.

ACTIVE

1 R29 HL44960-05 (Wick) NIH.NHLBI Mechanisms of Sickle Erythrocyte/Endothelial Adherence	9/25/91-6/30/97 $349,994 (direct)	50%

This project elucidated the mechanisms of sickle cell/endothelial cell adherence under flow conditions. This project expired 6/30/96 and NIH has granted a 1-year no-cost extension to complete studies in progress.

NAG 9-836 (Freed, MIT) NASA Microgravity Tissue Engineering	9/1/95-12/31/98 $2,984,472 (direct)	8.33%

To develop bioreactor systems for culture of custom-engineered tissues for clinical applications.

NIH-NRSA NIGMS GM08433 (Nerem) NIH/NIGMS Cellular Engineering Training Grant	7/1/96-6/30/01 $75,368 (annual direct)	5%

This project provides funds for 4 predoctoral students studying Cellular Engineering. Dr. Wick supervises one of these students working on sickle cell/endothelial cell interactions.

The Whitaker Foundation (Nerem) Biomedical Engineering Education: An Interdisciplinary Tissue Engineering Education and Research Program	7/1/96-6/30/97 $3,000,000	0%

This grant provides funds for laboratory space renovation, the hiring of six new faculty in Tissue Engineering, and a limited number of graduate student stipends.

Emory/Georgia Tech Biomedical Technology Research Center Mechanisms of Lung Allograft Rejection	7/1/96-6/30/97 $27,000	5%

This project studies the adherence of lymphocytes and monocytes to human lung microvascular endothelial cells. Dr. Wick is working in collaboration with Dr. Clinton Lawrence on this project. Dr. Wick is responsible for studying cell adherence using the dynamic flow models he has developed.

OTHER SUPPORT (continued)

American Heart Association - Georgia Affiliate — 7/1/96-6/30/98 — 5%
In Vitro Flow Characterization in the Hinge Regions of Bileaflet Mechanical Valves: Relevance to Thrombogenesis — $60,000

This grant investigates the mechanisms of thrombosis in artificial hart valves. Dr. Wick was appointed PI when the original PI left Georgia Tech, in order to keep the project funded at Georgia Tech. The research is carried out under the direction of Dr. A.P. Yoganathan. Dr. Wick provides input into the research and assists in supervising the graduate student working on the project.

PENDING

1-PO1-HL48482-06 (Eckman) — 4/1/98-3/31/03 — 30%
NIH/NHLBI
Georgia Comprehensive Sickle Cell Center

This is a Competing Continuation Renewal application of the NIH funded Sickle Cell SCORE.

1-RO1-44906-06 — 7/1/97-6/30/01 — 30%
N1H/NHLBI
Biophysics of Sickle Cell /Endothelial Cell Adherence

This application represents a Competing Continuation Renewal of Dr. Wick's R29 grant. In this application, Dr. Wick hypothesizes that *in vivo*, adherence biophysics (primarily strength) will regulate the extent of sickle cell adherence and vaso-occlusion. Conditions which lead to extensive and strong adherence will be most relevant to the pathophysiology of sickle cell adherence and vaso-occlusion *in vivo*. In order to address this hypothesis, experiments have been designed with the following **specific aims:** (1) Demonstrate that sickle cell adherence under flow is stronger than adherence under static conditions and that adherence under flow selects for cells expressing more adhesion receptors; (2) Quantify the strength of sickle cell/endothelial adherence in response to red cell agonists, endothelial activators, and adhesive plasma proteins for cell populations and individual cells in the range of 0 - 10 dynes/cm^2 shear stress. Particular emphasis will be placed on conditions which invoke multiple adherence pathways to mimic clinical conditions which may precipitate pain episodes *in vivo*, such as thrombosis or infection; (3) Develop flow channels with venular dimensions (20-100mm) and demonstrate that sickle cell adherence in confined channels is higher and stronger.

OVERLAP

There is no overlap with the present application and other funded or pending projects.

PROGRESS REPORT SUMMARY

GRANT NUMBER: 1P60 HL48482-05

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR: James R. Eckman, M.D.

APPLICANT ORGANIZATION: Emory University School of Medicine

PERIOD COVERED BY THIS REPORT: FROM 1 April 1996 THROUGH 31 March 1997

TITLE OF PROJECT *(Repeat title shown in Item 1 on first page)*: Georgia Comprehensive Sickle CEll Center - Project 1

a. Human Subjects *(Complete Item 7 on the Face Page)*
Use of Human Subjects ☐ Change ☒ No Change Since Previous Submission

b. Vertebrate Animals *(Complete Item 8 on the Face Page)*
Use of Vertebrate Animals ☐ Change ☒ No Change Since Previous Submission

(SEE INSTRUCTIONS)

GENDER AND MINORITY INCLUSION
Provide the number of subjects enrolled in the study *to date* (cumulatively since the most recent competitive award) according to the following categories. (See Page 8 for definitions.) If there is more than one study, provide a separate table for each study. In addition, report on the subpopulations which are included in the study.

Study Title: The mechanisms of Sickle Erythrocyte/endothelial Adhesion

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	TOTAL
Female			56		7	1	64
Male			40		5	1	46
Unknown							
TOTAL			96		12	2	110

Center Director:	James R. Eckman, M.D.
Subproject Principal Investigator:	Timothy M. Wick, Ph.D. Associate Professor
Participating Investigator:	James R. Eckman, M.D. Professor of Medicine Adjunct Professor of Pediatrics
Title of Project:	The Mechanisms of Sickle Erythrocyte/Endothelial Adhesion

PROGRESS REPORT: FEBRUARY 3, 1997

A. Narrative

1. Ultimate Goals of the Project

Although polymerization of sickle hemoglobin at low oxygen tension is assumed to be the dominant problem in sickle cell anemia, the abnormal adherence of sickle erythrocytes to endothelium likely initiates or propagates microvascular occlusive pain episodes. Adherence is mediated by receptors on sickle reticulocytes, endothelial cell adhesion molecules, and adhesive plasma proteins. Adherence can be upregulated during inflammation or thrombosis. Adherence *in vivo* likely involves multiple adherence pathways and modulated by local hemodynamic conditions. The **specific aims** are to (i) demonstrate that infection induces endothelial cell alterations which increase sickle cell adherence to endothelium, (ii) identify the dominant adherence mechanism(s) in the plasma milieu and factors which inhibit plasma-mediated sickle cell adherence, and (iii) develop novel adherence systems to study the interrelationship between adherence, endothelial cell function, and fluid mechanics under conditions which more closely mimic those *in vivo*. The **ultimate goals of this project** are to (i) identify receptors, ligands, and adhesive proteins involved in extensive and strong sickle cell adherence capable of initiating or propagating microvascular occlusion and pain; and (ii) identify agents which inhibit or reverse sickle cell/endothelial cell adherence as potential therapeutics to reduce microvascular occlusion and tissue ischemia.

2. Intermediate objectives in attaining goals

In the current budget period the aims were to (i) evaluate the ability of a non-ionic surfactant poloxamer compound (RheothRx) to inhibit sickle cell adherence to microvascular endothelium mediated by plasma, (ii) develop an adherence reversal assay and test the ability of RheothRx to dislodge adherent sickle cells, and (iii) to determine whether continuous contact of endothelium with sickle cells under flow increases adherence in the absence of addition of adhesion proteins of agonists.

3. Activities, procedures, or methods employed to achieve objectives

We have developed *in vitro* flow models to study sickle cell adherence to human endothelium under conditions mimicking the hemodynamic and biochemical environment in microvessels *in vivo*. Using these models, we have identified receptors, ligands, and adhesive proteins involved in adherence. More recent investigations have also focused on the biophysics of sickle cell adherence. In these studies, we are attempting to identify conditions which lead to extensive and strong adherence. We speculate that strongly adherent sickle cells will not be detached by flowing blood and will be able to initiate or propagate microvascular occlusion. For these studies we utilize parallel-plate flow chambers and cone-and-plate viscometers to quantify the number and strength of sickle cell adherence promoted by different adherence pathways or combinations of adherence pathways.

4. Project accomplishments during the past year

Inhibition of plasma-mediated sickle cell adherence

In vivo, sickle cell adherence occurs in plasma. We speculate that adherence in plasma will involve multiple receptor/ligand adhesion pathways simultaneously (1). Previous attempts to block sickle cell adherence mediated by plasma with monoclonal antibodies or agonists that inhibit adherence only a single receptor-ligand pathway have met with limited success (2). Possibly, inhibition of adherence promoted by plasma requires agonists capable of blocking multiple receptor-ligand interactions. We have recently reported that a conformationally constrained arginine-glycine-aspartic acid (RGD) peptide is capable of inhibiting sickle cell adherence to microvascular endothelium mediated by autologous plasma under conditions in which linear or cyclical RGD constructs were ineffective (3).

More recently, we have tested the ability of a nonionic poloxamer surfactant (RheothRx, generously provided by CytRx Corp., Norcross, GA) to inhibit sickle cell adherence to microvascular endothelium mediated by plasma based on the observation that these compounds effectively reduce analgesic use, pain intensity, and crisis duration in sickle patients during acute pain episodes (4,5). As shown below, preincubation of washed sickle red cells with RheothRx reduces adherence to microvascular endothelium

in a dose-dependent manner. For these studies, RheothRx, at the indicated concentration, was incubated with sickle cells suspended in 30% autologous sickle plasma. Then, sickle cells adherence was quantified in a parallel-plate flow chamber adherence assays under continuous a shear stress of 1.0 dyne/cm^2 (2,3). All experiments were done with blood collected during asymptomatic periods. As shown in Table, 1, maximal adherence inhibition was 37±7% (range: 21-59%) at 1.0 mg/ml poloxamer.

Table 1: Inhibition of Plasma-Mediated Sickle Cell Adherence by RheothRx

	Adherence (SRBC/mm^2)			
Patient	Serum-Free Medium	30% Plasma	30% Plasma + 0.5 mg/ml CRL 85178	30% Plasma + 1.0 mg/ml CRL 85178
SS-1	7±1	107±10	127±10	60±6
SS-2	9±1	129±12	68±5	53±3
SS-3	3±1	163±14	144±14	116±10
SS-4	199±21	534±37	432±28	360±21
SS-5	3±1	104±13	70±8	82±9

Data are mean±SEM adherence sickle cells/mm^2 endothelium for 20 fields of view counted on each endothelial monolayer.

In vivo adherence may be an early step in vaso-occlusion. Thus, compounds may need to *reverse* sickle cell adherence to restore tissue blood perfusion. To test the ability of poloxamer to reverse sickle cell adherence, poloxamer at 1.0 mg/ml in serum-free medium was perfused through the flow chamber at 1.0 dyne/cm^2 shear stress following attachment of sickle cells in 30% plasma to the endothelium. Adherent sickle cells were quantified in 3 minute intervals after poloxamer perfusion to determine the ability of poloxamer to reverse sickle cell adherence. For experiments where the initial adherence (e.g. adherence after red cells in plasma without poloxamer were attached to the endothelium at 1.0 dyne/cm^2 shear stress) ranged from 104±14 to 534±37 SRBC/mm^2, perfusion of RheothRx at 1.0 mg/ml reversed sickle cell adherence mediated by plasma by 38-65% (n=5) (Figure 2). Adherence reversal appears to be maximal within 30 minutes (Figure 2).

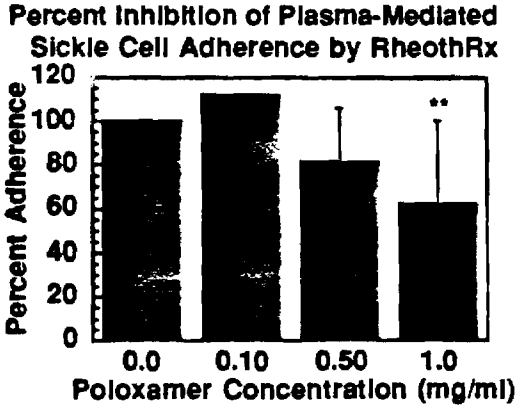


Figure 1: Data plotted from Table 1 to show mean percent adherence of sickle cells suspended in plasma to microvascular endothelium as a function of poloxamer concentration.

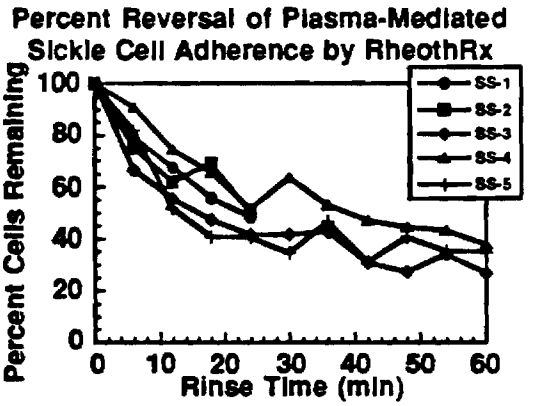


Figure 2: Data show reduction in sickle cell adherence to endothelium when RheothRx is perfused under continuous flow at 1.0 dyne/cm^2 shear stress through the flow chamber at 1.0 mg/ml concentration.

The data of Figures 1& 2 and Table 1 were generated with a formulation of RheothRx (CRL 85178) that may be associated with reversible renal toxicity (4). According to the manufacturer, purified RheothRx (designated CRL 5861) appears to be associated with reduced renal complications (unpublished communication from Dr. R. M. Emanuele, CytRx Corporation). Since CRL 5861 may be tested clinically, we also determined whether this compound could inhibit or reverse sickle cell adherence mediated by plasma. In studies similar to those outlined above, inhibition of plasma-mediated sickle cell adherence by CRL 5861 ranged from 0-40% (ave: 19±9%, n=5) at 0.5 mg/ml concentration (figure 3). In an additional 5 experiments, CRL 5861 reversed plasma-mediated sickle cell adherence from 40-73% (Figure 4).

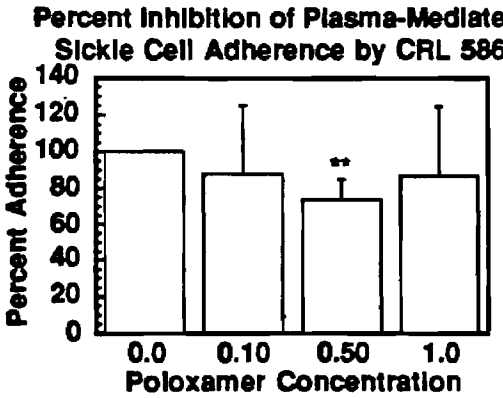


Figure 3: Experimental conditions are identical to those described in Figure 1, except poloxamer CRL 5861 was used to inhibit sickle cell adherence. *p<0.05

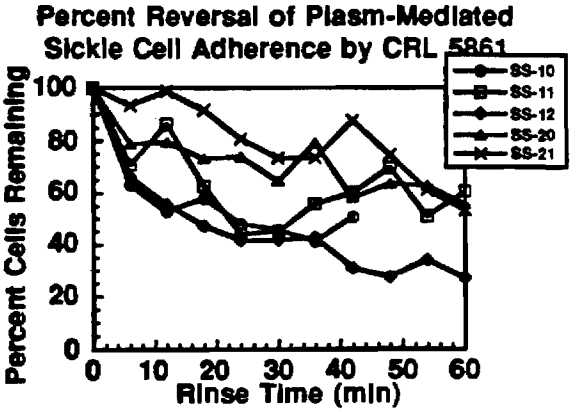


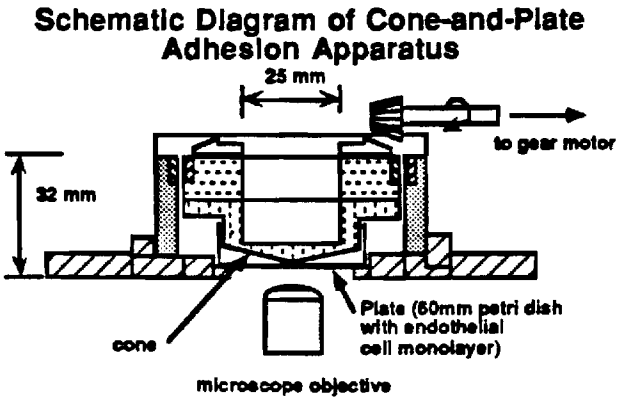
Figure 4: Experimental conditions are identical to those described in Figure 2, except poloxamer CRL 5861 was used to reverse sickle cell adherence.

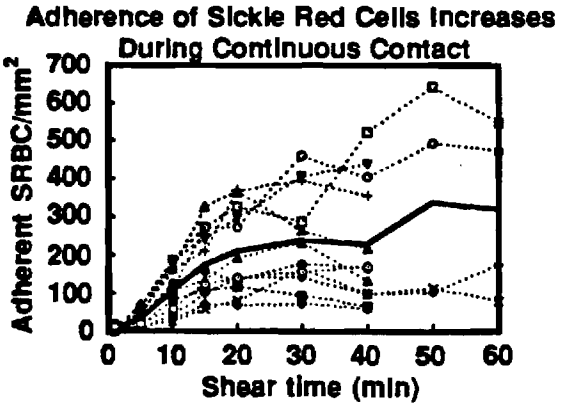
Thus, in addition to the conformationally constrained RGD peptides which have been shown by us to significantly inhibit sickle cell adherence mediated by plasma under physiologic flow (3), the present data demonstrate that two different formulations of the non-ionic surfactant RheothRx also effectively inhibit plasma-mediated sickle cell adherence to microvascular endothelium. Since our assay system is designed to mimic the biochemical and biophysical environment in microvessels *in vivo*, we anticipate that the adherence observed is similar to that in occluded vessels during pain episodes and that these compounds may reduce the duration or severity of sickle cell pain episodes by inhibiting or reversing pathological red cell adherence to microvascular endothelium.

Continuous interaction between sickle cells and endothelium increases adherence

In vivo, sickle red cells are in near continuous contact with the endothelium, particularly in the microcirculation. This situation is not well reproduced in parallel-plate flow chamber assays, where red cells make only one pass through the flow chamber. Our previous studies demonstrated that prolonged contact (≥ 8 hrs) induces expression of endothelial cell adhesion molecules, including VCAM-1 (6).

In order to determine whether prolonged continuous contact of endothelium with red cells increases adherence, we developed a cone-and-plate viscometer to quntify adherence when sickle cells are continuously circulated over confluent endothelial monolayers at 1.0 dyne/cm^2. In this device, confluent endothelial cell monolayers cultured in 60 mm petri dishes form the planar base (or plate) ofthe viscometer (Figure 5). The cone is machined from transparent polycarbonate to allow visualization of the endothelial cell monolayers via epifluorescent microscopy. For a cone angle of 1.0° and a rotation rate of 23 rpm, the shear stress is 1.0 dyne/cm^2 everywhere on the endothelium. For our initial studies, 2 ml of washed sickle cells suspended to 0.05% hematocrit in serum free medium were placed in the petri dish, the assembly was placed on the stage of an inverted phase-constrast microscope and the cone was rotated. The number of adherent sickle cells was counted in 3-minute intervals





5. Plans for the coming year

Range of shear stresses, etc. in cone -and-plate.

Literature cited

1. Wick TM and JR Eckman, 1996. Molecular basis of sickle cell-endothelial interactions. Current Opinion in Hematology 3:118-124.
2. Brittain HA, JR Eckman, and TM Wick. 1992. Sickle erythrocyte adherence to large vessel and microvascular endothelium under physiologic flow is qualitatively different. The Journal of Laboratory and Clinical Medicine, 19:538-545.
3. Kumar A, JR Eckman, and TM Wick. 1996. Inhibition of plasma mediated adherence of sickle erythrocytes to microvascular endothelium by conformationally constrained RGD-containing peptides. American Journal of Hematology 53:92-98.
4. Adams-Graves P, et al. 1994. RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease. Blood 84:410a.
5. Emanuele M, P Adams-Graves, A Kedar, M Koshy, M Steinberg, R Veith. 1996. RheothRx (poloxamer 188) lowers serum LDH during an acute painful episode of sickle cell disease. Blood 88:13a
6. Wick TM, MD Brown, and JR Eckman. 1993. Sickle red blood cells induce expression of cell adhesion molecules on human umbilical vein endothelial cells. Blood, 82:352a.

B. Publications:

Journal Articles

1. Smolinski, P.A., M.K. Offermann, J.R. Eckman, and T.M. Wick. "Double-Stranded RNA Induces Sickle Erythrocyte Adherence to Endothelium: A Potential Role for Viral Infection in Vaso-Occlusive Pain Episodes in Sickle Cell Anemia," *Blood* 85:2945-2950 (1995).
2. Wick, T.M. and J.R. Eckman. "Molecular Basis of Sickle Cell-Endothelial Interactions," *Current Opinion in Hematology* 3:118-124 (1996).
3. Kumar, A., J.R. Eckman, and T.M. Wick. "Inhibition of Plasma Mediated Adherence of Sickle Erythrocytes to Microvascular Endothelium by Conformationally Constrained RGD-Containing Peptides," *American Journal of Hematology* 53:92-98 (1996).
4. Kumar, A., J.R. Eckman, R.A. Swerlick, and T.M. Wick. "Phorbol Ester Stimulation Increases Sickle Erythrocyte Adherence to Endothelium: A Novel Pathway involving $\alpha_4\beta_1$ Integrin Receptors on Sickle Reticulocytes and Fibronectin," *Blood* 88:4348-4358.
5. Smolinski, P.A., J.R. Eckman, and T.M. Wick. "Tenacity of Receptor-Mediated Sickle Erythrocyte Adherence to Vascular Endothelium: Implications for Microvascular Occlusion in Sickle Cell Anemia," *Blood* (In review).

Abstracts and Meeting Presentation (last year only)

1. Montes R.A.O, J.R. Eckman, T.M. Wick. "Kinetic Studies on Transient Interactions and Firm Adherence of Sickle Erythrocytes to Endothelium Under Shear Stress," 21st Annual Meeting of the National Sickle Cell Disease Program, Mobile, AL (March 1996).
2. P.A. Smolinski, J.R. Eckman, T.M. Wick. "The Tenacity of Sickle Erythrocyte Adherence to Vascular Endothelium Depends upon the Receptor-Ligand Pairs Involved," 21st Annual Meeting of the National Sickle Cell Disease Program, Mobile, AL (March 1996).
3. Wick, T.M. and J.R. Eckman. "Modulation of Sickle Cell/Endothelial Cell Adherence," Workshop on Approaches to the Treatment of Sickle Cell Disease," Sickle Cell Disease Scientific Research Group, National Heart Lung and Blood Institute, NIH, Washington DC (20 August 1996).
4. Smolinski, P.A., J.R. Eckman, T.M. Wick, "Biophysics of Sickle Erythrocyte-Endothelial Cell Adhesion," *Annals of Biomedical Engineering* 24:5-33, 1996 Annual Fall Meeting of the Biomedical Engineering Society, State College, PA, (October 1996).
5. Montes R.A.O., J.R. Eckman, T.M. Wick, "Continuous Recirculating Contact Enhances Adhesion of Sickle Red Blood Cells to Endothelial Cells," *Blood*, 88:10a (1996). 1996 Annual Meeting of the American Society of Hematology, Orlando, FL (December 1996).

6. Vassy W.M., J.R. Eckman, T.M. Wick, "Inhibition of Plasma-Mediated Sickle Erythrocyte Adherence to Microvascular Endothelium by Poloxamer Compounds," *Blood*, **88**:9a (1996). 1996 Annual Meeting of the American Society of Hematology, Orlando, FL (December 1996).
7. McNaull, S.A., Eckman, J.R., T.M. Wick, "Sickle Red Blood Cell Adherence to Vascular Endothelium is More Tenacious in Confined Flow Channels," *Blood*, **88**:9a (1996). 1996 Annual Meeting of the American Society of Hematology, Orlando, FL (December 1996).
8. Smolinski, P.A., J.R. Eckman, T.M. Wick, "Tenacity of Sickle Red Blood Cell Endothelial Cell Adherence is Augmented under Hemodynamic Shear and by Involvement of Multiple Adhesion Pathways," *Blood*, **88**:649 a (1996). 1996 Annual Meeting of the American Society of Hematology, Orlando, FL (December 1996).

CHECKLIST	GRANT NUMBER 1P60 HL48482-05

1. ASSURANCES/CERTIFICATIONS *(See Instructions, Page 9)*

The following assurances/certifications are made and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application. If unable to certify compliance where applicable, provide an explanation and place it after this page.

• Human Subjects; • Vertebrate Animals; • Debarment and Suspension; • Lobbying; • Delinquent Federal Debt; • Research Misconduct; • Civil Rights (Form HHS 441 or HHS 690); • Handicapped Individuals (Form HHS 641 or HHS 690); • Sex Discrimination (Form HHS 639-A or HHS 690); • Age Discrimination (Form HHS 680 or 690); • Financial Conflict of Interest.

2. PROGRAM INCOME *(See Instructions, Page 10)*

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
NONE		

3. INDIRECT COSTS

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of forprofit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will *not* be paid on foreign grants, construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, and specialized grant applications.

☐ DHHS Agreement dated: _____ ☐ No Indirect Costs Requested.

☒ No DHHS Agreement, but rate established with Office of Naval Research Date 7/10/96

CALCULATION*

Entire proposed budget period:

Amount of base $ 85,170* x Rate applied 49.5 % = Indirect costs $ 42,159

Add to total direct costs from form page 2 and enter new total on FACE PAGE, Item 9b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct costs base ☐ Other base *(Explain below)*

☐ Off-site, other special rate, or more than one rate involved *(Explain below)*

Explanation *(Attach separate sheet, if necessary.):*

*Base does not include graduate student tuition, since this is not subject to indirect costs.

PERSONNEL REPORT	GRANT NUMBER 1P60 HL48482-05

All Personnel for the Current Budget Period

Name	Degree(s)	SSN	Role on Project *e. g., PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Current Personnel					
Timothy M. Wick	Ph.D.	505-94-2891	PI	07/09/61	20%
James R. Eckman	M.D.	474-48-8946	Co-Investigator	08/25/43	5%
Richard Montes	M.S.	274-66-1040	Graduate Student	12/02/69	100%
James Siano	M.S.	261-83-5016	Graduate Student	04/26/68	100%
Planned Changes					
Deletions					
Paula Smolinski graduated with Ph.D. degree December 1996					