13:29:54 OCA PAD INITIATION - PROJECT HEADER INFORMATION 07/10/96

Active

Project #: E-25-L50 Cost share #: Rev #: 0
Center # : 10/24-6-R0020-0A0 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: MECH ENGR Unit code: 02.010.126
Project director(s):
NEREM R M MECH ENGR (404)894-2768

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	124,333.00	124,333.00
Funded	124,333.00	124,333.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: GEORGIA COMPREHENSIVE SICKLE CELL CENTER - PROJECT #2 (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., N.E.
ATLANTA, GA 30303

Sponsor issuing office

MS. JANE O'CONNER
(404)727-2503

OFFICE OF SPONSORED PROGRAMS
EMORY UNIVERSITY
1462 CLIFTON RD., NE
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.
Administrative comments -
INITIATION OF YEAR 04 UNDER NIH GRANT (CONTINUATION OF E-25-T83).

4
(1)

Closeout Notice Date 02-JUL-1997

Project Number E-25-L50 Doch Id 38682

Center Number 10/24-6-R0020-0A0

Project Director NEREM, ROBERT

Project Unit MECH 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 GEORGIA COMPREHENSIVE SICKLE CELL CENTER - PROJECT #2 (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
Reports Coordinator	Y
Research Property Team	Y
Supply Services Department	Y
Georgia Tech Research Corporation	Y
Project File	Y

E-25-L50
#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/02.400.012.97.012
E-25-L50

7 January 1997

Emory University
School of Medicine
69 Butler Street, N.E.
Atlanta, Georgia 30303

Attention: Dr. James R. Eckman

Subject: Research Proposal Entitled, "Georgia Comprehensive Sickle Cell Center - Project No. 2"

Dear Dr. Eckman:

GEORGIA TECH RESEARCH CORPORATION is pleased to submit for your consideration the subject proposal prepared by Dr. Robert M. Nerem, School of Mechanical 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. Nerem at (404) 894-2768 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

Enclosure: 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
			1P60 HL48482

Total Project Period
From: 1 April 1993 Through 31 March 1998

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

. TITLE OF PROJECT
Georgia Comprehensive Sickle Cell Center - Project #2

. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Name and address, street, city, state, zip code)*
Robert M. Nerem, Ph.D.
School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, GA 30332-0405

4. APPLICANT ORGANIZATION *(Name and address, street, city, state, zip code)*
Georgia Tech Research Corporation
OCA/PID, Room 246 CRB
Georgia Institute of Technology
Atlanta, GA 30332-0420

b. E-MAIL ADDRESS
robert.nerem@ibb.gatech.edu

5. ENTITY IDENTIFICATION NUMBER
1580603146A1

c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
School of Mechanical Engineering

d. MAJOR SUBDIVISION
College of Engineering

. ORGANIZATIONAL CODE
20 other

6. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Contracting Officer
Georgia Tech Research Corporation
OCA/PID, Room 245 CRB
Atlanta, GA 30332-0420

E-MAIL ADDRESS

. HUMAN SUBJECTS
☐ No
☒ Yes

7a. If "Yes," Exemption no. or IRB approval date
☐ Full IRB or ☐ Expedited Review

7b. Assurance of compliance no.
M1395

8. VERTEBRATE ANIMALS
☒ No
☐ Yes

8a. If "Yes," IACUC approval date

8b. Animal welfare assurance no.

COSTS REQUESTED FOR NEXT BUDGET PERIOD
a. DIRECT $ 94,359 9b. TOTAL $ 141,067

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

1. PERFORMANCE SITE(S) *(Organizations and addresses)*
Georgia Institute of Technology
Space Science and Technology Building
Room 217
Atlanta, GA 30332-0405

	AREA CODE	TELEPHONE NO. AND FAX NO.
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)*	404	894-2768 (phone)
Robert M. Nerem	404	894-2291 (fax)
12b. NAME OF ADMINISTRATIVE OFFICIAL *(Item 6)*	404	894-4817 (phone)
Christopher D'Urbano	404	894-6956 (fax)
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 15)*		
Christopher D'Urbano Contracting Officer		

E-MAIL ADDRESS

3. *Do not use this space.*

4. 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 1/6/97
5. 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 1/7/97

Progress Report Summary

1. Specific Aims

The central hypothesis underlying this project is that sickle erythrocytes contribute to arterial lesions by altering endothelial and smooth muscle cell structure and function and that endothelial alterations induced by sickle erythrocytes are modulated by the hemodynamic environment. Within this hypothesis, the overall goal is to characterize *in vitro* the alterations in endothelial cell structure and function due to contact with sickle erythrocytes, including the mechanisms involved.

The specific aims thus are as follows: (i) characterize changes in endothelial cell morphology, F-actin localization, and extracellular matrix composition due to interactions with sickle erythrocytes; (ii) determine the effect of sickle erythrocytes on endothelial cell proliferation; and (iii) quantify endothelial cell synthesis and secretion of biologically active molecules, e.g. vasoactive substances (NO, ET-1), growth factors (FGF, PDGF), and thrombotic markers (PGI_2, tissue factor), and expression of cell adhesion molecules (VCAM-1, ICAM-1, E-Selectin) as induced by sickle erythrocytes. These studies are to be performed under physiological flow conditions in order to elucidate the complex interactions between erythrocytes, endothelial cells and hemodynamics which may take place in lesion-prone vessels *in vivo*.

To date we have made significant progress on the first two of these specific aims, and the results obtained are described in the next section. We also have conducted some studies related to specific aim (iii). These were described in the progress report submitted January 1994.

2. Studies and Results

Vascular endothelial cells exhibit what may be called an elongation response, i.e. they are elongated and aligned in the presence of flow, with the alignment being in the direction of flow. This has been observed both *in vivo* and *in vitro*. If sickle erythrocytes in any way are injurious to endothelium, then there may be an influence of this "injury" on the endothelial cell elongation response. It is thus our hypothesis that sickle erythrocytes inhibit the normal elongation and alignment response of endothelial cells subjected to flow and the associated shear stress.

To test this hypothesis, confluent monolayers of bovine aortic endothelial cells (BAEC) have been statically preincubated with cell culture medium, washed normal red blood cells (NRBC), or washed sickle red blood cells (SRBC) for 5 minutes and then exposed to cell culture medium at a steady laminar shear stress of 30 dynes/cm^2 for up to 36 hours. The shape index (SI) of endothelial cells has been used as a measure of endothelial elongation, with a SI of 1 indicating rounded cells, circular in

shape, and a SI of 0 indicating the limiting case of a straight line. The SI was determined for each condition and for up to 36 hours after the onset of flow. The data obtained has been reported previously; however, it should be noted that in all experiments, endothelial viability was not less than 90% for up to 36 hours after the onset of shear stress. The SI of BAEC preexposed to SRBC retained an essentially constant value, i.e. a circular shape, this through the duration of the experiment up to 36 hours of exposure to flow and a 30 dynes/cm^2 shear stress. This is in marked contrast to BAEC preexposed to NRBC and exposed to 30 dynes/cm^2 shear stress where the SI decreased in a similar fashion to exposure to culture medium, indicating normal BAEC elongation. In fact, the SI time course of BAEC elongation after preexposure to culture medium was identical to that measured for BAEC preexposed to NRBC. Shear-induced BAEC elongation also was inhibited by preexposure to either NRBC or SRBC lysed by sonication. When BAEC were preexposed to sickle hemoglobin from lysed SRBC depleted of membrane fragments, inhibition of elongation in response to shear also was observed. This suggests that hemoglobin may be a primary contributor to the inhibition of endothelial elongation described above. Additional experiments indicate that BAEC, when preexposed to supernatant from washed, centrifuged SRBC, also exhibit an inhibition of elongation.

Since the elongation of an endothelial cell depends on its ability to reorganize its actin microfilament structure, then an impairment of this elongation response might be expected to be

associated with an alteration in the localization of F-actin. We thus also have investigated the localization of F-actin in endothelial cells using rhodamine phalloidin and fluorescent microscopy. Endothelial cells in static culture exhibit a dense peripheral band of F-actin. When subjected to flow, the F-actin in endothelial cells is reorganized into stress fibers aligned parallel to the direction of flow and thus the cell's major axis. However, if incubated first with washed SRBC, then when subjected to flow, little reorganization of the F-actin was observed to occur.

A continuing objective has been to study the mechanism(s) that might be involved in the observed inhibition of the elongation response. It was not only treatment of endothelial monolayers with SRBC, but also treatment with suspensions of sonicated SRBC and NRBC which caused the observed inhibition of elongation. Thus, there is a suggestion that free hemoglobin, released into the media when the RBC membranes are disrupted, may be a potential elongation-inhibiting substance. This hypothesis is supported by the studies of Vercelotti and coworkers[1,2] in which they examined the damaging effects of free heme on porcine aortic endothelial cells and found that it is toxic after only a one hour exposure.

To study a possible role of free hemoglobin, the concentration of hemoglobin in RBC and sonicated RBC suspensions, similar to those used previously, was first assayed

spectrophotometrically. For both cell types this was found to be in the range of 0.04-0.08 mg/mL and 6-7 mg/mL respectively. Experiments were then initiated, similar to the earlier ones, except RBC suspensions were replaced by Hb S and Hb A solutions. The initial concentrations of hemoglobin used were 6-7 mg/mL, similar to that assayed for the sonicated suspensions. The results of these experiments indicate that sickle hemoglobin causes a significant amount of endothelial denudation as compared to normal hemoglobin or DMEM control. Figure 1 is a plot of the change of shape index over time, comparing Hb S, Hb A, and DMEM control. In the Hb S experiments, the shape index represents the cells that did not shear off of the slide. These results indicate that while hemoglobin, at a concentration representative of the sonicated RBC samples, does have somewhat of an inhibitory effect, it is no where near as great as that previously observed. The denudation effect, however, is an important one which we would like to follow up.

We believe that the SRBC effects on endothelial cells also will be reflected in endothelial cell function. To test this, a variety of studies have been initiated or are planned. For our studies on cell proliferation the protocol used was described in last year's progress report. The cell proliferation results obtained to date are presented in Figures 2-4. There really are two separate questions to be addressed. First, in static culture do endothelial cells preconditioned by contact with SRBC exhibit an altered rate of proliferation? Secondly, for an endothelial

monolayer exposed to steady flow and the associated shear stress, does preconditioning with SRBC alter the effect of flow on the rate of proliferation?

In regard to the first question, the 24 hour static culture studies indicate no significant difference in the percentage of proliferating cells in monlayers pretreated with NRBC and grown in DMEM as compared to the untreated controls. For monolayers pretreated with SRBC, however, there was a 30 percent decrease. These 24 hour data are shown in Figure 1, and similar results have been obtained for 72 hours of growth, where again there is no significant difference between NRBC treated monolayers versus untreated controls.

To address the second question, flow experiments were conducted. In these untreated endothelial monolayers exposed to a 30 dynes/cm^2 steady flow of culture media exhibited a little change in cell proliferation. However, for monolayers which were SRBC treated, a 52 percent decrease in the percentage of proliferating cells was observed. Although somewhat less, there also was a decrease in cell proliferation for monolayers pretreated with NRBC. These results are shown in Figure 3-4.

From these experiments it is clear that sickle erythrocyte interactions alter both endothelial proliferation in static culture as well as the influence of flow on cell proliferation. Even though cell proliferation data indicate significant effects

on endothelial cell function, it will be important to further explore this. Plans for this are discussed in Section 4.

3. Significance

The influence of the local hemodynamic environment on vascular endothelial biology is now well recognized. This includes both *in vivo* evidence as well as results from *in vitro* cell culture studies. The latter, which is by far the more extensive, indicate that the influence of hemodynamics extends to cell function and includes gene expression.

In the studies conducted on this project we have found an alteration of the endothelial response to flow that is induced by sickle erythrocyte-endothelial interactions. This manifests itself not only in the elongation response, but also in the proliferation of endothelial cells. These findings suggest that the complex interaction between erythrocytes and endothelial cells, as modulated by the flow environment, may play an important role in vascular endothelial biology and in vascular pathobiologic complications associated with sickle cell disease.

As will be discussed in the next section, our attention is now being directed into two different areas. One is a continuation of our work to date focusing on the mechanism(s) related to the effects we have observed.

4. Plans

For this coming year, we have two objectives. The first objective is to continue our search for the mechanism(s) involved in the inhibition of the EC elongation and alignment response which results from pretreatment of EC by SBRC. We plan to do this by investigating the dynamics of intracellular calcium which we believe is a key second messenger involved in the EC response to flow. When BAEC respond to flow by elongating and aligning, there is an elevation in intracellular calcium. However, when intracellular calcium is blocked, then there is an inhibition of EC elongation.[3] Since EC elongation is an active response to flow, one which involves the reorganization of the F-actin microfilament cytoskeletal structure, then quite possibly this is due to the role intracellular calcium has in regulating key actin-binding proteins. In order to investigate this, alterations in intracellular calcium in EC pretreated with SRBC will be measured using Fura-2 and a fluorescenced radiometric imaging technique.[4-5] These will be compared with the response of EC pretreated with NRBC as well as that of untreated EC. The critical question being asked is whether pretreatment with SRBC, as opposed to NRBC, alters intracellular calcium signaling in EC responding to flow.

The second objective is to extend our studies to the gene expression level. Previous studies in our laboratory have provided a wide variety of evidence indicative of important effects of flow and the associated shear stress in the regulation

of gene expression. Of particular interest is nitric oxide (NO), a molecule which is a potent vasodilator and regulated by flow.[6-7] It also is linked to the nature of the vessel wall's oxidative environment. In cell culture studies of the acute response to the sudden onset of flow, there is a dramatic increase in NO release. There also is a chronic increase which appears to be the result of an upregulation in nitric oxide synthase (NOS), a catalyst in the conversion of L-arginine to NO. These studies will be extended to investigate how pretreatment of EC with SRBC influences the role of flow and the associated shear stress in the regulation of NO release and in particular NOS. In regard to this latter, NOS mRNA will be measured both in EC pretreated by SRBC and NRBC and then exposed to flow and also in control, untreated EC.

Other biologically active molecules which are candidates for our studies on the influence on EC of pretreatment with SRBC include Cu/Zn SOD, PDGF, and MCP-1. In each case we have conducted previous studies demonstrating the important role of flow in the regulation of gene expression.

We believe these planned studies will extend our knowledge of vascular endothelial biology. Equally well, this will contribute to a better understanding of the vascular complications which occur in patients with sickle cell disease.

Literature Cited

1. Balla G, H Jacob, J Eaton, J Belcher, G Vercellotti. Hemin: A possible physiological mediator of low density lipoprotein oxidation and endothelial injury. Arteriosclerosis and Thrombosis 11:1700-1711; 1991.

2. Vercellotti G, G Balla, J Balla, K Nath, J Eaton, H Jacob. Heme and the vasculature: An oxidative hazard that induces antioxidant defenses in the endothelium. Artificial Cells, Blood Substitutes, and Immobilization Biotechnology 22:207-213; 1994.

3. Malek AM, S. Izumo. Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress. Journal of Cell Science 109:713-726; 1996.

4. Geiger RV, BC Berk, RW Alexander, RM Nerem. Flow-induced calcium transients in single endothelial cells: Spatial and temporal analysis. American Journal of Physiology: Cell Physiology 262:C1411-C1417; 1992.

5. Helmlinger G, BC Berk, RM Nerem. The calcium responses of endothelial cell monolayers subjected to pulsatile and steady laminar flow differ. American Journal of Physiology: Cell Physiology 269:C367-C375; 1995.

6. Nishida K, DG Harrison, JP Navas, AA Fisher, SP Dockery, M Uematsu, RM Nerem, RW Alexander, TJ Murphy. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. Journal of Clinical Investigation 90:2092-2096; 1992.

7. Uematsu M, Y Ohara, JP Navas, K Nishida, TJ Murphy, RW Alexander, RM Nerem, DG Harrison. Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. American Journal of Physiology: Cell Physiology 269: C1371-C1378; 1996.

5. Human Subjects

a. General Guidelines

i. Proposed Use

Patients with sickle cell syndrome (HbSS, HbSC, HbS ß-thalassemia) not receiving anticoagulant therapy and without evidence of pregnancy, obvious infection, thromboembolic disease or liver disease will be eligible for this study. Patients will be studied once in pain crisis and twice during asymptomatic periods. An age and sex matched population of normal black individuals will serve as a control population. Approximately twenty patients and twenty control subjects, aged eighteen or older, will be studied annually. Ten milliliters of blood will be

drawn by venipuncture for each experiment.

ii. Specimen Usage

None of the data from the experiments will be used for diagnosis or treatment of specific individuals.

iii. Patient Recruitment

Patients from the Sickle Cell Center or the in-patient service at Grady Memorial Hospital, Atlanta, GA and hospital staff will be recruited by Dr. James R. Eckman. Subjects will agree to participate in this study by signing a consent form approved by Georgia Tech and Emory School of Medicine IRBs. The consent form explains the nature of the study, the details of blood collection, risks associated with drawing blood, the availability of personnel to discuss the results of the study, the assurance of anonymity, and the ability to withdraw from the study at any time without penalty or loss of benefits.

iv. Potential Risks

The risks of drawing blood are minimal and include slight pain, bruising, and infection at the site of the puncture. No viable alternative for drawing human blood exists.

v. Procedures to Minimize Risk

Patient confidentiality will be ensured by assigning a code to each patient studied (SS1, AA1 for sickle and normal donor, respectively) to be used when all data is reported. Blood will be drawn at Grady Hospital under the supervision of Dr. James R. Eckman, director of the Sickle Cell Clinic. Dr. Eckman will be available to answer questions and to arrange for emergency medical care if a medical problem develops during the course of this study.

vi. Justification

The risk of drawing blood is minimal compared to potential benefits of a better understanding of clotting abnormalities in sickle cell syndromes and their relationship to pain crisis.

b. Gender and Minority Inclusions

Study subjects will be patients diagnosed with sickle cell syndromes as defined above. These patients will primarily be of African descent, however no patients will be included or excluded on the basis of race. The study population will consist of approximately equal numbers of men and women. Exclusion criteria will be solely based on medical criteria as described above. Control subjects (volunteers without hemoglobinpathies) will be age, sex, and race-matched. These volunteers are recruited from

the hospital staff at Grady Memorial Hospital in Atlanta.

6. Vertebrate Animals

None.

7. Publications (from this project)

a. Journal Articles

Sherrill, A.W., Williams, J.J., Eckman, J.R., Wick, T.M., and Nerem, R.M., "Washed Sickle Cells Inhibit Arterial Endothelial Cell Elongation and Alignment in Response to Shear Stress," ASME Journal of Biomechanical Engineering (submitted for publication).

Williams, J.J., Wick, T.M., and Nerem, R.M., "Short-Term Contact with Sickle Erythrocytes Inhibits Endothelial Cell Proliferative Activity" (in preparation).

b. Abstracts and Meeting Presentations

Sherrill, A.W., Williams, J., Wick, T.M., and Nerem, R.M., "Short-Term Contact with Sickle Erythrocytes Inhibits Shear-Induced Elongation of Arterial Endothelium," Annual Meeting of the American Society of Hematology, Nashville, TN, December 2-6, 1994.

7. Inventions and Patents

None.

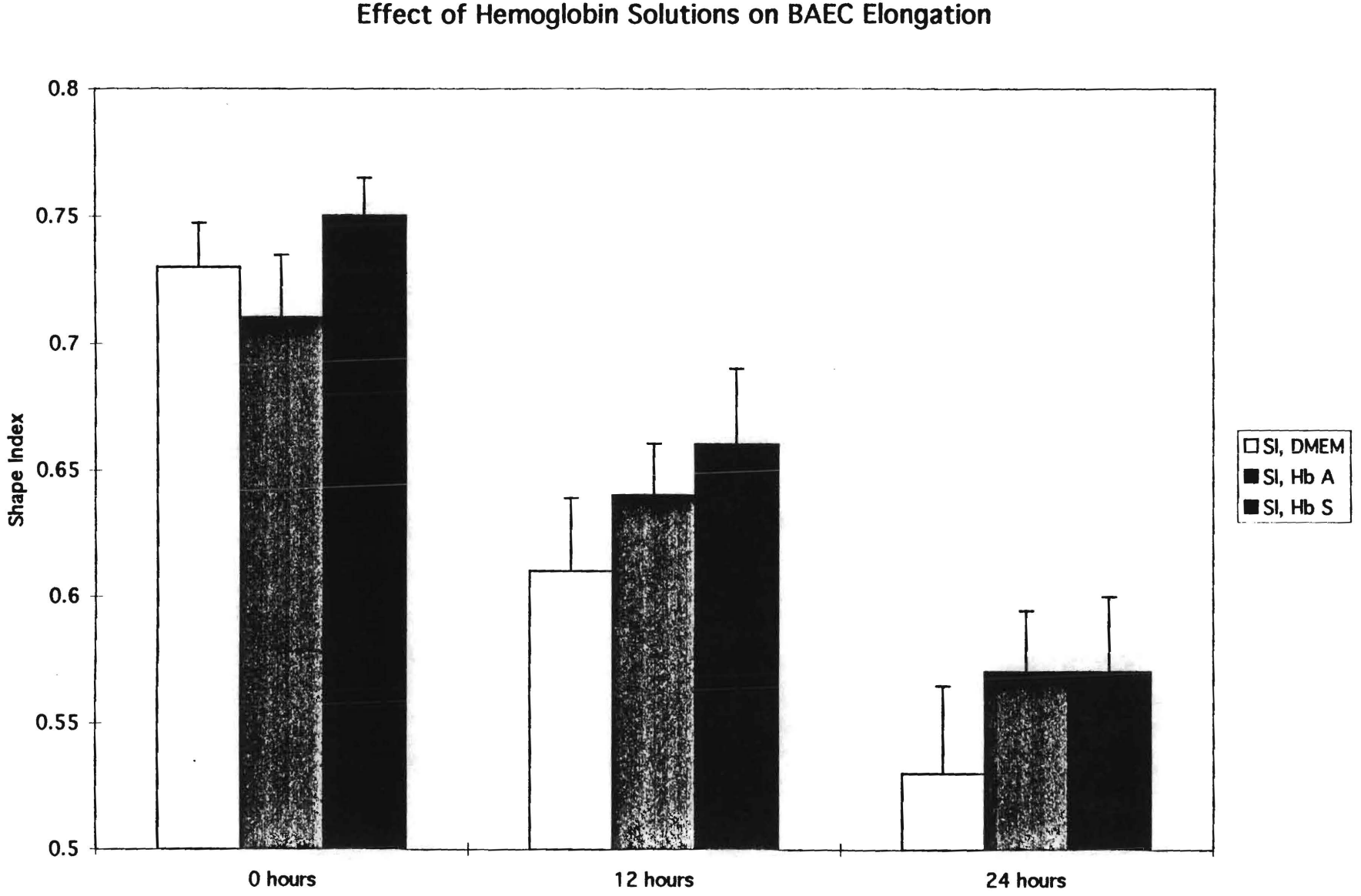
Effect of Hemoglobin Solutions on BAEC Elongation
Shape Index
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0 hours
12 hours
24 hours
SI, DMEM
SI, Hb A
SI, Hb S

Figure 1

Transient SRBC contact inhibits endothelial DNA synthesis in static culture

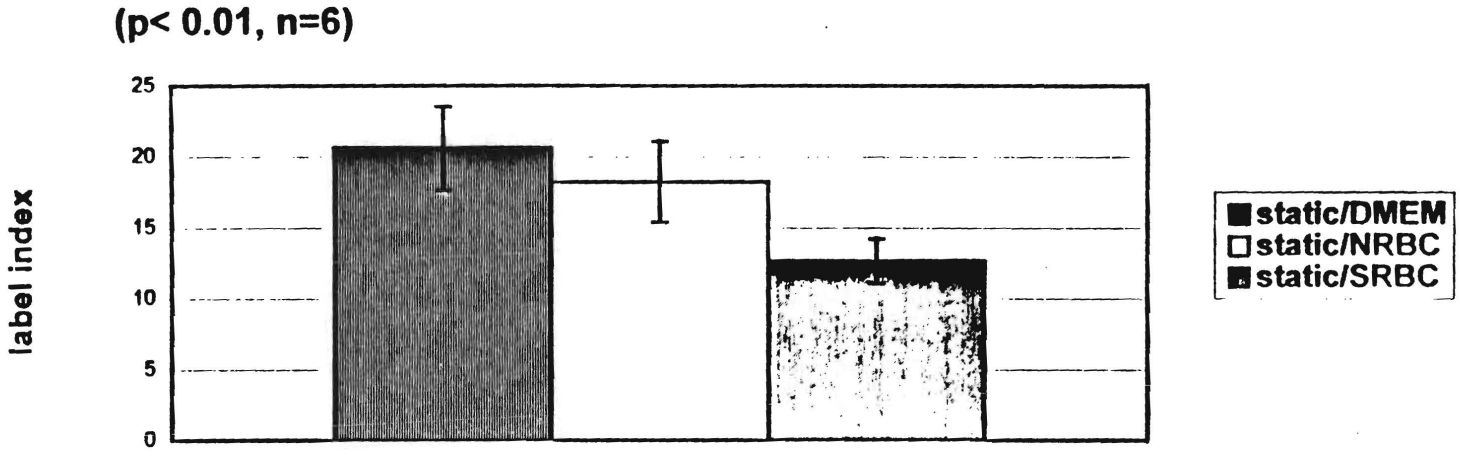

Figure 2

Shear stress inhibits DNA synthesis of monolayers pre-treated with NRBC

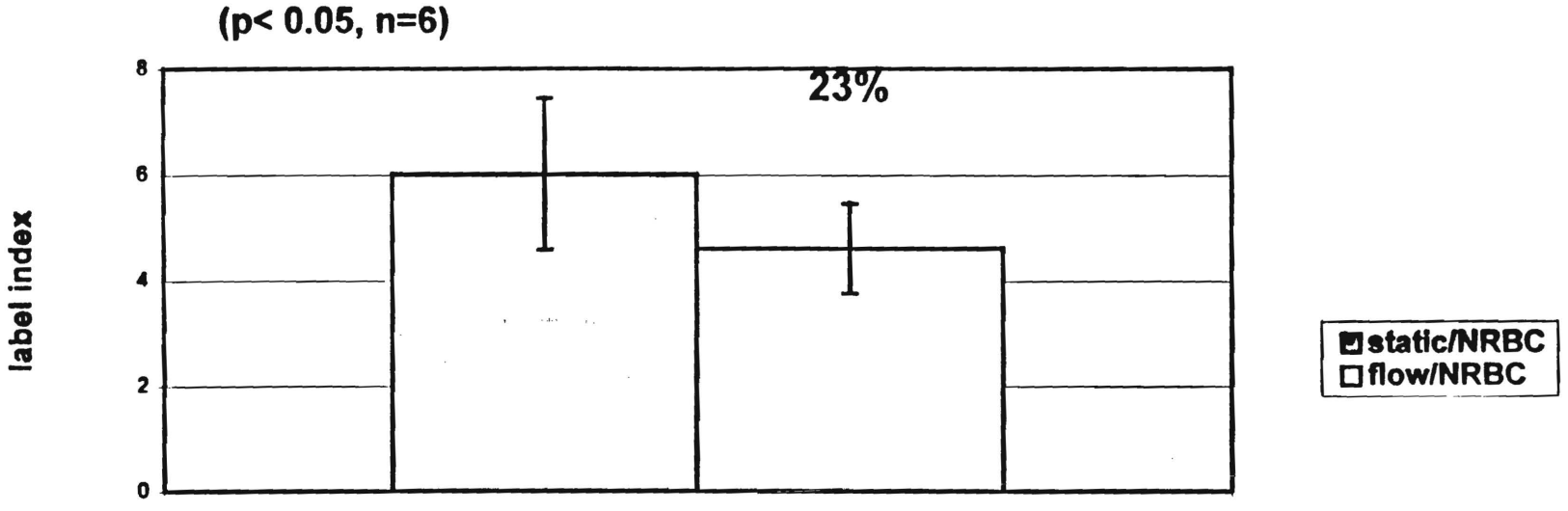

Figure 3

Shear stress inhibits DNA synthesis of monolayers pre-treated with SRBC

(p<= 0.05, n=6)

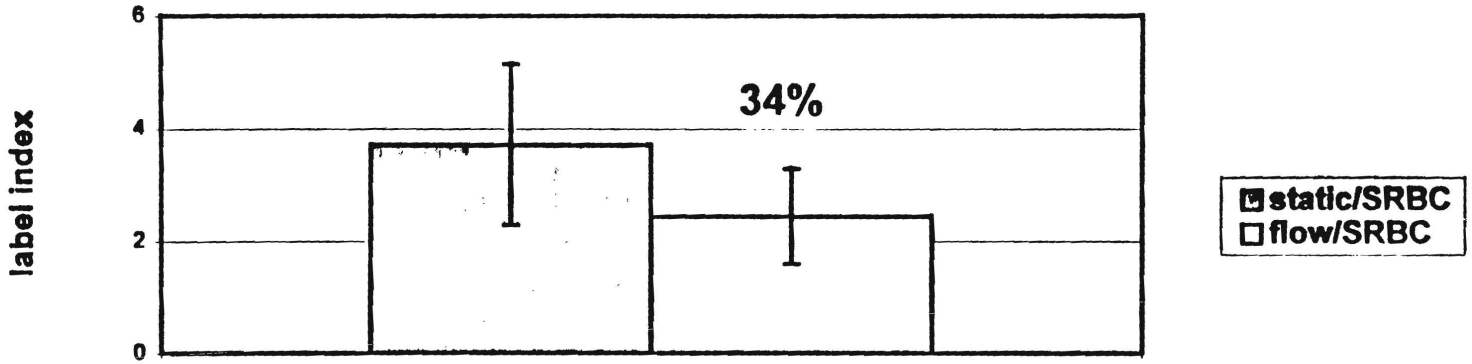

Figure 4

D Principal Investigator/Program Director *(Last, first, middle)*:

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 1 April 1997 THROUGH: 31 March 1998

ERSONNEL *(Applicant organization only)*

NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Robert M. Nerem, Ph.D.	Principal Investigator	12	15	125,000	18,750	5,006	23,756
Timothy M. Wick	Co-Invest.	12	10	87,600	8,760	2,339	11,099
Jane E. Thomas	Research Assistant	12	50	37,996	18,998	5,072	24,070
Christine Pflederer	Grad. Student	12	100	18,720	18,820	---	18,720
	SUBTOTALS →				65,328	12,417	77,645

ONSULTANT COSTS

UIPMENT *(Itemize)*

PPLIES *(Itemize by category)*

Cell Culture Supplies	$5,000
Disposable Plastics	$2,000
Chemicals, Immunochemicals	$3,000
Radioisotopes	$2,000
Photographic Supplies	$1,250
Miscellaneous	$1,200

14,450

AVEL
Travel to one scientific meeting for investigators — 1,000

TIENT CARE COSTS — INPATIENT
OUTPATIENT

ERATIONS AND RENOVATIONS *(Itemize by category)*

IER EXPENSES *(Itemize by category)*

Publication expenses, artwork, photography ($1,264) — 1,264

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD — $ 94,359

SORTIUM/CONTRACTUAL TS	DIRECT COSTS $94,359	94,359
	INDIRECT COSTS 46,708 (49.5%)	46,708

AL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* → $ 141,067

BUDGET JUSTIFICATION

1. Funding is requested for both the Principal Investigator and the Co-Investigator, Dr. Wick, to provide time to organize and coordinate the study, perform experiments and analyze the data, prepare manuscripts, presentations, and progress reports, and participate in regular laboratory meetings of the investigators and in other meetings of the Georgia Comprehensive Sickle Cell Center. The Fringe Benefit Rate is 26.7% of salaries.

2. Research Assistant: Ms. Thomas has been working in the laboratory for 2.5 years and is primarily responsible for maintaining endothelial cell cultures and for performing western blots, and other protein identification assays. She will also be responsible for obtaining supplies and reagents required for the experiments. Her efforts will be crucial in maintaining smooth, continuous progress on the project. Ms. Thomas will spend 50% of her effort on this project and 50% on other projects in the laboratory.

3. Graduate Students: Ms. Christine Pflederer is an M.S. graduate student in the laboratory who has been working on this project for the past two years and will be finishing up.

4. Supplies: Tissue culture costs are based upon anticipated performance of 3 flow experiments per week as well as current usage and costs. Media, serum, growth factors, buffers, and other chemicals as well as plasticware, glassware, and gloves are required for cell cultures and adhesion assays.

5. Travel funds are requested to attend one scientific meeting.

6. Miscellaneous: Funds are requested to cover the costs of phone, copying, fax supplies, and postage related to the transfer of data and data forms between Emory, Grady and Georgia Tech.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel, consultants, and collaborators listed on page 2.
Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (MO. DAY. YR)
Robert M. Nerem	Professor	July 20, 1937

EDUCATION (*Begin with baccalaureate or other initial professional education, such as nursing, and include post doctoral training.*)

INSTITUTION AND LOCATION	DEGREE CONFERRED	YEAR	FIELD OF STUDY
University of Oklahoma, Norman, OK	B.S.	1959	Aeronautical Engr.
The Ohio State University, Columbus, OH	M.Sc.	1961	Aero-Astro. Engr.
The Ohio State University, Columbus, OH	Ph.D.	1964	Aero-Astro. Engr.

RESEARCH AND/OR 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 and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1964-1968	Assistant Professor, Dept.of Aeronautical and Astronautical Engineering, The Ohio State University, Columbus, OH
1968-1972	Associate Professor, Dept. of Aeronautical and Astronautical Engineering, The Ohio State University, Columbus, OH
1970	Visiting Professor, Physiological Flow Studies Unit, Imperial College, London, England
1972-1979	Professor, Dept. of Aeronautical and Astronautical Engineering, The Ohio State University and Professor, Dept. of Veterinary Physiology and Pharmacology (1975-1979)
1975-1979	Associate Dean, Graduate School, The Ohio State University; primary responsibilities in area of research
1979-1986	Professor and Chairman, Dept. of Mechanical Engineering, University of Houston, Houston, TX
1980-1981	Visiting Professor, Institute of Biomedical Engineering, Swiss Federal Institute of Technology (ETH) and University of Zurich, Zurich, Switzerland
1980-present	Foundation Scientist, Southwest Foundation for Biomedical Research, San Antonio, TX
1980-present	Professor, Dept. of Pathology (Adjunct), The University of Texas Health Science Center at San Antonio, San Antonio, TX
1986-1987	Visiting Professor, Bioengineering Division, Dept. of Applied Mechanics and Engineering Sciences, University of California, San Diego, CA
1987-present	Parker H. Petit Distinguished Chair for Engineering in Medicine, George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA
1991-present	Institute Professor, Georgia Institute of Technology, Atlanta, GA
1995-present	Director, Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA

HONORS:
Fellow, Council on Arteriosclerosis, American Heart Association, 1976
Fellow, American Physical Society, 1982
Fellow, American Society of Mechanical Engineers, 1984
Konrad Witzig Memorial Lecture, Cardiovascular System Dynamics Society, 1986
National Academy of Engineering, Elected 1988
Senior Visiting Fellow, Japan Society for the Promotion of Science, 1989
ASME Lissner Award, 1989
Fellow, American Association for the Advancement of Science, 1990
Honorary Doctorate (Docteur Honoris Causa), University of Paris, 1990
Institute of Medicine, National Academy of Sciences, Elected 1992
Foreign Member, Polish Academy of Sciences, Elected 1994

PUBLICATIONS: (selected)

1. Nerem RM and Seed WA. An *in vivo* study of aortic flow disturbances. Cardiovasc. Res 6:1-14, 1972
2. Caro CG and Nerem RM. Transport of ^{14}C-4-cholesterol between serum and wall in perfused dog common carotid artery. Circ Res 32:187-205, 1973
3. Nerem RM, Rumberger JA, Gross DR, Hamlin RL and Geiger GL. Hot-film anenometer velocity measurement of arterial flow in horses. Circ Res 34:193-203, 1974
4. Nerem RM, Rumberger JA, Gross DR, Muir WW and Geiger GL. Hot-film coronary artery velocity measurements in horses. Cardiovasc Res, 10:301-313, 1976
5. Rumberger JA, Jr, and Nerem RM. A method of characteristics calculation of coronary blood flow. J Fluid Mech 82:429-448, 1977
6. Rittgers SE, Karayannacos PE, Guy JF, Nerem RM, Shaw GW, Mostetler JR and Vasko JS. Velocity distribution and intimal proliferation in autologous vein grafts in dogs. Circ Res 42:729-800, 1978

7. Cornhill JF, Levesque MJ and Nerem RM. Quantitative study of the localization of sudanophilic coeliac lesions in the white carneau pigeon. Atherosclerosis 35:103-110, 1980
8. Nerem RM, Levesque MJ and Cornhill JF. Social environment as a factor in diet-induced atherosclerosis. Science 208:1475-1476, 1980
9. Nerem RM, Levesque MJ and Cornhill JF. Vascular endothelial morphology as an indicator of the pattern of blood flow. ASME J Biomech Engr 103:172-176, 1981
10. Batten JR and Nerem. Model study of flow in curved and planar bifurcations. Cardiovasc Res 16:178-186, 1982
11. Holenstein R, Nerem RM and Niederer P. On the propagation of a wave front in viscoelastic arteries. ASME J of Biomech Engr 106:2, 115-122, 1984
12. Levesque MJ and Nerem RM. The elongation and orientation of cultured endothelial cells in response to shear stress. ASME J Biomech Engr 107:4, 341-347, 1985
13. Levesque MJ, Liepsch D, Moravec S and Nerem RM. Correlation of endothelial cell shape and wall shear stress in a stenosed dog aorta. Arteriosclerosis 6:2, 220-229, 1986
14. Sato M, Levesque MJ and Nerem RM. Application of the micropipette technique to the measurement of the mechanical properties of cultured bovine aortic endothelial cells. ASME J. Biomech Engr, 109:1, 27-34, 1987
15. Sato M, Levesque MJ and Nerem RM. Micropipette aspiration of cultured bovine aortic endothelial cells exposed to shear stress. Arteriosclerosis 7:276-286, 1987
16. Sprague EA, Steinbach BL, Nerem RM and Schwartz CJ. Influence of a laminar steady state fluid-imposed shear stress on the binding, internalization, and degradation of low density liporoteins (LDL) by cultured arterial endothelium. Circulation 76:3, 648-656, 1987
17. Theret DP, Levesque MJ, Sato M, Nerem RM and Wheeler LT. The application of a homogeneous half-space model in the analysis of endothelial cell micropipette measurements. ASME J Biomech Engr 110:3, 190-199, 1988
18. Levesque MJ, Sprague EA, Schwartz CJ and Nerem RM. The influence of shear stress on cultured vascular endothelial cells: the stress response of an anchorage-dependent mammalian cells. Biotech Prog 5:1, 1-8, 1989
19. Sato M, Theret DP, Wheeler LT, Ohshima N and Nerem RM. Application of the micropipette technique to the measurement of cultured porcine aortic endothelial cell viscoelastic properties. ASME J Biomech Engr 112:263-268, 1990
20. Levesque MJ, Nerem RM, Sprague EA. Vascular Endothelial Cell Proliferation in Culture and the Influence of Flow. Biomaterials 11:702-707, 1990
21. Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Nerem RM. The Pathogenesis of Atherosclerosis: An Overview. Clin Cardiol 14:1-16, 1991
22. Helmlinger G, Geiger RV, Schreck S, Nerem RM. Effects of Pulsatile Flow on Cultured Vascular Endothelial Cell Morphology. ASME J Biomech Engr 113:123-131, 1991
23. Geiger RV, Berk BC, Alexander, RW, Nerem RM. Flow-induced calcium transients in single endothelial cells: spatial and temporal analysis. Amer J Phys 262: C1411-C1417 (Cell Physiology 31), 1992
24. Nishida, K., Harrison, D.G., Navas, J.P., Fisher, A.A., Dockery, S.P., Uematsu, M., Nerem, R.M., Alexander R.W., Murphy, T.J., "Molecular Cloning and Characterization of the Constitutive Bovine Aortic Endothelial Cell Nitric Oxide Synthase," J. Clinical Investigation, Vol. 90, pp. 2092-2096, 1992
25. Prasad, A.R.S., Logan, S.A., Nerem, R.M., Schwartz, C.J., Sprague, E.A., "Flow-Related Responses of Intracellular Inosital Phosphate Levels in Cultured Aortic Endothelial Cells," Circulation Res., Vol. 72, No. 4, pp. 827-836, 1993
26. Taylor, W.R., Nerem, R.M., Alexander, R.W., "Polarized Secretion of IGF-I and IGF-I Binding Protein Activity by Cultured Aortic Endothelial Cells," J. Cellular Physiology, Vol. 154, pp. 139-142, 1993
27. Mitsumata, M., Fishel, R.S., Nerem, R.M., Alexander, R.W., Berk, B.C., "Fluid Shear Stress Stimulates Platelet-Derived Growth Factor Expression in Endothelial Cells," American Journal of Physiology: Heart Circ. Physiol., Vol. 265, pp. H3-H8, 1993
28. Ziegler, T. and Nerem, R.M., "The Effect of Flow on the Process of Endothelial Cell Division," Arteriosclerosis and Thrombosis, Vol. 14, pp. 636-643, 1994
29. Nerem, R.M. and Sambanis, A., "Tissue Engineering: From Biology to Biological Substitute," Tissue Engineering, Vol 1, No. 1, pp. 3-13, 1995
30. Thoumine, O., Nerem, R.M., and Girard, P.R., "Oscillatory Shear Stress and Hydrostatic Pressure Modulate Cell-Matrix Attachment Proteins in Cultured Endothelial Cells," In Vitro Cell. Dev. Biol., Vol. 31, No. 1, pp. 45-54, 1995
31. Ziegler, T., Alexander, R.W., and Nerem, R.M., "An Endothelial Cell-Smooth Muscle Cell Co-Culture Model for Use in the Investigation of Flow Effects on Vascular Biology," Annals of Biomedical Engineering, Vol. 23, pp. 216-225, 1995.
32. Helmlinger, G., Berk, B.C., and Nerem, R.M., "The Calcium Responses of Endothelial Cell Monolayers Subjected to Pulsatile and Steady Laminar Flow Differ," American Journal of Physiology: Cell Physiology , Vol. 269, No. 2, pp C367-C375, 1995.
33. Helmlinger, G., Berk, B.C., and Nerem, R.M., "Flow-Induced Calcium Responses in Endothelial Cells are Synergistically Modulated by Serum," J. Cell. Engr., Vol. 1, No. 1, pp. 13-20, 1995.
34. Thoumine, O., Nerem, R.M., and Girard, P.R., "Changes in Organization and Composition of the Extracellular Matrix Underlying Cultured Endothelial Cells Exposed to Laminar Shear Stress," Laboratory Investigation, Vol. 73, No. 4, pp 565-576, 1995.
35. Wiesner, T.F., Berk, B.C., and Nerem, R.M., "A Mathematical Model of Cytosolic Calcium Dynamics in Humar Umbilical Vein Endothelial Cells," American Journal of Physiology: Cell Physiology, Vol. 270, pp. C1556-C1569, 1996.
36. Ziegler, T., Alexander, R.W., and Nerem, R.M., "Co-Culture of Vascular Cells with Collagen Gels: Assessment of the Cell Morphology, Cytoskeleton, and Growth Rate," J. Cell. Engr., Vol. 1, No. 2, pp. 75-83, 1996.
37. Inoue, N., Ramassami, S., Fukai, T., Nerem, R.M., and Harrison, D.G., "Shear Stress Modulates Expression of Cu/Zr Superoxide Dismutase in Human Aortic Endothelial Cells," Circulation Research, Vol. 79, pp. 32-37, 1996.

10/1/96

CURRENT AND PENDING SUPPORT -- R.M. NEREM

Current Support --

Source and identifying no. - **National Institutes of Health Award for a Comprehensive Sickle Cell Center (subcontract from Emory)**

P.I. - James Eckman

Title - "Comprehensive Sickle Cell Center"

Role - Director of Project 2

% Effort - 10%

Dates/costs entire project - 4/1/93 - 3/31/98; $460,831

Dates/costs third year - 4/1/96 - 3/31/97; $124,333

Specific aims of project - To study alteractions in vascular endothelial biology due to interactions with sickle red blood cells.

Source and identifying no. - **Whitaker Foundation**

P.I. - Robert M. Nerem

Title - "Biomedical Engineering Development: Cellular Engineering at Emory and Georgia Tech"

Role - Project Director

% Effort - 20%

Dates/costs entire project - 9/1/93 - 8/31/98; $3,000,000

Dates/costs third year - 9/1/99 - 8/31/97; $500,000

Specific aims of project - To expand biomedical engineering program at Georgia Tech

Scientific and budgetary overlap - None

Source and identifying no. - **National Science Foundation Grant BCS-9412010**

P.I. - Robert M. Nerem

Title - "Tissue Engineering a Blood Vessel"

Role - P.I.

% Effort - 20%

Dates/costs entire project - 9/1/94 - 2/28/97; $341,014

Dates/costs current year - 9/1/95 - 8/31/96; $173,613

Specific aims of project - To develop a tissue-engineered blood vessel substitute.

Scientific and budgetary overlap - None

Source and identifying no. - **National Institutes of Health Grant, GM08433** (a training grant)

P.I. - Robert M. Nerem

Title - "Cellular Engineering"

Role - P.I.

% Effort - 5%

Dates/costs entire project - 9/26/91 - 6/30/01; $814,183

Dates/costs current year - 7/1/96 - 6/30/97; $79,163

Specific aims of project - To train pre-doctoral students in cellular engineering.

Scientific and budgetary overlap - None

Source and identifying no. - **National Institutes of Health Grant No. 1 PO1 HL48667-01** (subcontract from Emory University)

P.I. - R. Wayne Alexander

Title - "Initiating Events in Vascular Lesion Formation"

Role - Collaborator on Project 2

% Effort - 10%

Dates/costs entire project - 9/30/92 - 9/29/97; $1,274,908

Dates/costs current year - 9/30/95 - 9/29/96; $59,135

Specific aims of project - To study the role of shear stress in regulation by the oxidative environment of the oxidative adhesion molecule expression and monocyte adherence.

Scientific and budgetary overlap - None

Source and identifying no. - **National Institutes of Health Grant HL52218** (Subcontract from University of Texas Health Science Center at San Antonio)

P.I. - E.A. Sprague

Title - "Flow Regulation of Monocyte-Endothelial Interaction"

Role - Co-Investigator

% Effort - 10%

Dates/costs entire project - 5/1/95 - 4/30/00; $520,795

Dates/costs first year - 5/1/96 - 4/30/97; $101,281

Specific aims of project - To investigate the flow regulation of monocyte-endothelial adherence.

Scientific and budgetary overlap - None

Source and Identifying no. - **NASA NAG 9-836** (subcontract from MIT)

P.I. - Lisa E. Freed

Title - "Microgravity Tissue Engineering"

Role - Co-Investigator and Director, Georgia Tech Effort

% Effort - 8.33%

Dates/costs entire project - 7/1/95 - 6/30/99; $1,284,445

Dates/costs current year - 9/1/95 - 8/31/96; $293,227

Specific aims of project - To study the influence of fluid forces on the tissue engineering of 3-D constructs.

Scientific and budgetary overlap - None