08:29:21 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 07/17/95

Active

Project #: E-25-T03 Cost share #: E-25-370 Rev #: 2
Center # : 10/24-6-R8262-0A0 Center shr #: 10/22-1-F8262-0A0 OCA file #:
Work type : RES
Contract#: BES-9412010 Mod #: AMENDMENT 001 Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA: 47.041
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: NATL SCIENCE FOUNDATION / GENERAL
Sponsor/division codes: 107 / 000

Award period: 940901 to 960831 (performance) 961130 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	343,172.00
Funded	173,613.00	341,014.00
Cost sharing amount		10,000.00

Does subcontracting plan apply ?: N

Title: TISSUE ENGINEERING A BLOOD VESSEL

PROJECT ADMINISTRATION DATA

OCA contact: Jacquelyn L. Bendall 894-4820

Sponsor technical contact

FRED G. HEINEKEN
(703)306-1319

NATIONAL SCIENCE FOUNDATION
4201 WILSON BLVD.
ARLINGTON, VA 22230

Sponsor issuing office

DIANE P. WASHINGTON
(703)306-1217

NATIONAL SCIENCE FOUNDATION
4201 WILSON BLVD.
ARLINGTON, VA 22230

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NSF supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
AMENDMENT NO. 1 ADDS $173,613 TO PROJECT.

U

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 12/10/96

Project No. E-25-T03_____ Center No. 10/24-6-R8262-0A0_

Project Director NEREM R M_____ School/Lab MECH ENGR_____

Sponsor NATL SCIENCE FOUNDATION/GENERAL_____

Contract/Grant No. BES-9412010_____ Contract Entity GTRC

Prime Contract No. _____

Title TISSUE ENGINEERING A BLOOD VESSEL_____

Effective Completion Date 960831 (Performance) 961130 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	_____
Final Report of Inventions and/or Subcontracts	N	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

Comments_____
LETTER OF CREDIT APPLIES. 98A SATISFIES PATENT REPORT._____

Subproject Under Main Project No. _____

Continues Project No. _____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other _____	N
_____	N

E-25-703 #1

(New)

ANNUAL PROGRESS REPORT ON NSF GRANT BES-9412010 ENTITLED "TISSUE ENGINEERING A BLOOD VESSEL"

NSF Program: Bioengineering and Environmental Systems

NSF Award Number: BES-9412010

PI Name: Robert M. Nerem

Period Covered By This Report: January 1, 1994 - June 30, 1995

PI Institution: Georgia Institute of Technology

Date: June 21, 1995

PI Address: School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, GA 30332-0405

☒ Check if Continued Funding is Requested

To test our hypothesis and to achieve our overall long term objective, the *in vitro* cell culture studies to be conducted as part of this two-year effort are designed to continue the development of a tissue-engineered, living arterial wall model and to use this to study vascular EC biology under well defined, physiologic conditions. The specific aims build on our accomplishments to date and involve the use of our existing one-dimensional "slab" model of a reconstituted arterial wall in studies of vascular EC biology, including both steady and pulsatile laminar flow conditions. They also involve the further development and optimization of our model, including its extension to a tubular configuration. Although the proposal for this current project requested four years of funding, the award was only for two years. Thus, though the current effort builds on the following three specific aims, the focus in each aim is very selective. The three specific aims are:

1. To continue to evaluate our reconstituted, one-dimensional "slab" arterial wall model under both steady and pulsatile flow conditions, including studies using the model in its existing form as well as with future, evolutionary changes incorporated, with a focus on:

- **extracellular matrix**
- **signal transduction**
- **vasoactive substrance secretion**

2. To further develop and optimize the three-dimensional SMC collagen matrix structure including:

a. the determination of the composition required to achieve appropriate elastic characteristics for this three-dimensional SMC matrix structure.

b. the optimization of vascular SMC growth and density in the three-dimensional matrix structure;

c. the determination of the conditions necessary to maintain vascular SMC in a contractile phenotype

3. To extend our existing one-dimensional "slab" model of the arterial wall to a tubular configuration with its evaluation focusing on:

- **vascular cell morphology**
- **endothelial cell turnover**
- **vasoactive substance secretion**
- **platelet adhesivity**

There are two important underlying assumptions to this proposed effort. One is that, in the study of vascular biology in cell culture, the use of a static, no flow environment with cells plated on tissue culture plastic represents an abnormal, non-physiologic condition. Through the research under the present grant, a model arterial wall has been developed which can be used in studies of vascular biology, including those focused on hemodynamic effects, on the interaction of vascular cells both with each other and with components of the extracellular matrix, and on transport across the endothelium. The development of this reconstituted blood vessel wall needs to be continued and further evaluated; however, it represents a further engineering of the cell culture environment so as to make it more physiologic.

Perhaps even more important, however, the development of such a reconstituted blood vessel in culture and the studies proposed will provide important information which can help guide the development of tissue-engineered vascular prostheses. As will be discussed in the next section, on-going efforts include those focused on the development of a hybrid graft (e.g. a synthetic material pre-seeded with an inner lining of EC prior to implantation) and those where the goal is to reconstitute an entire living blood vessel in culture, including both EC and SMC. It is the latter approach which is being pursued at Georgia Tech; however, in both of these efforts

a major issue is the achievement of an endothelium with functional integrity. In the current two-year effort we plan to develop a tubular configuration of our current model and begin to evaluate this.

In the co-culture model we have developed, porcine aortic SMC are seeded with soluble rat tail collagen type I, and the collagen is allowed to polymerize in order to obtain a three-dimensional matrix with SMC. The cells bind to the collagen fibers and contract the gel to a final area which depends on many factors including the number of SMC seeded. Once the collagen lattice has fully contracted, porcine aortic EC are seeded at a very high cell density, with the cells rapidly becoming confluent. EC grown on collagen gels have been shown to secrete their own ECM, thus no proteins were layered underneath the EC.

The most important achievement to date has been the attainment of a quiescent EC monolayer. The culture of EC on top of gels of collagen type I resulted in a dramatic shutdown of cell growth. Previous reports have demonstrated a higher rate of cell turnover in EC cultured on adsorbed collagen compared to EC on plastic. This suggests that the growth inhibition observed in EC on collagen gels was induced by both the three-dimensional structure of the matrix and the organization of the collagen into fibers. These results emphasize the role of tension-dependent interactions between EC and the ECM which may be involved in the regulation of cell growth and differentiation as it was previously demonstrated with capillary EC. When EC and SMC were grown in co-culture, similar values of EC thymidine uptake were obtained, with there being only a slight further decrease in EC growth.

Porcine aortic EC cultured on plastic and exposed to shear stress exhibited a decreased growth rate. This decrease, which is on the order of 40%, is consistent with results obtained with BAEC and shows that flow has a significant effect on EC growth, independent of the origin of

the cells. Experiments using the bromodeoxyuridine incorporation technique suggest that 35 cpm/1000 cells corresponds to a population of approximately 1% dividing cells. Flow thus was able to decrease the number of dividing cells from 4% to 2.5% suggesting that, although important, it is not enough to bring the cells into a growth state similar to that found *in vivo*.

When EC co-cultured with SMC were exposed to shear stress, no significant further decrease in EC growth rate, as compared to static co-culture, was observed. Thus, the measured EC growth rate for both cases represented a factor of five decrease as compared to PAEC on plastic. This corresponded to the presence of approximately 1% dividing cells and is a number very similar to that found in the endothelium of blood vessels, indicating that ECM proteins in a fibrous form may be a prerequisite for the conservation of a differentiated, quiescent state of the endothelium. With such low growth rates, any effect of flow on the growth rate of co-cultured EC obviously would be difficult to observe.

Much of our effort in the last year has gone into trying to better understand the characteristics of our SMC-seeded collagen gel. From a biological point of view, it will be important to understand the relationship of the mechanical organization of the three-dimensional collagen matrix with the resident SMC structure and function. Smooth muscle cells are being studied in three types of model systems: (a) floating matrices where tension is isotropic, (b) bound matrices where tension is anisotropic, and (c) stress-relaxed matrices where tension is rapidly dissipated by mechanical release. The effects of these mechanical forces on SMC orientation, phenotypic morphology, cytoskeletal architecture and actin isoform intracellular localization are being examined by immunofluorescence and confocal laser scanning light microscopy. Also, scanning, transmission, intermediate voltage and immunogold electron microscopy are being used to visualize the interrelationships among ECM and SMC. The mechanisms by which SMC remodel ECM is studied by examining secretion of matrix

components and, particularly in stress-relaxed matrices, the release of intrcellular proteins such as actin into the matrix. Proteins released into the matrix are examined by SDS-PAGE and immunoblotting. This remodeling and associated ECM-SMC interactions are important not only in embryogenesis and in maintenance of vasomotor tone in the vascular system, but also in pathological conditions such as atherosclerosis in which SMC modulate from a contractile to a synthetic phenotype with concomitant increased matrix synthesis.

From a mechanical point of view, the material properties of the SMC-seeded collagen gel are being studied using uniaxial tensile and stress relaxation tests. The uniaxial tensile testing is performed by displacing the specimen and storing the force and particle location information. This process is continued until the gel breaks. In the analyses, the new thickness and width are calculated. The force exerted by the gel and the new cross-sectional area are then used to calculate cauchy stress and green strain. The stress relaxation test is performed by applying a step change in strain to the specimen. The new particle location and the time varying force are then stored. This information is then used to find cauchy stress versus time. In the preliminary studies conducted to determine the mechanical properties of the collagen-smooth muscle cell lattice, it has been found that the material exhibits very nonlinear, plastic-like material responses. The critical breaking stress of the gel is largely dependent on seeding density and the variation of the stress versus strain curve depend on strain rate. For example, gels seeded with an innoculum of 1 million cells had a critical breaking stress of 23,054 $\pm$ 1620 N/m^2 and 7346 $\pm$ 1124 N/m^2 for the stain velocities of 2.3 mm/sec and 0.13 mm/sec, respectively. Likewise, for gels seeded at 2 million cells, the critical breaking stresses were 32,974 $\pm$ 5,277 N/m^2 and 13,497 $\pm$ 2469 N/m^2 for the fast and slow displacement rates, respectively. There is also a fluid-like response in the sense that in the stress relaxation studies, the lattice significantly relaxes after a step change in strain. When stress relaxation testing is performed on gels with the seeding densities of 1 and 2 million cells, the peak stresses

seen are 12249 ± 2520 N/m^2 and 5507 ± 778 N/m^2, respectively.

There is much work yet to be done on the optimization of collagen gels. One of the critical aspects yet to be investigated is the addition of an elastic material. Also the appropriate seeding density which most closely correlates to *in vivo* cell densities must be achieved in order to more closely mimic the material properties of blood vessels. Currently, emphasis is being placed on the development of techniques for the construction of tubular constructs. The initial techniques employed, although not necessarily optimal, have been successful. This effort continues, and it is for such tubular constructs that we are exploring options for incorporating elasticity into the material.

Finally, two other areas of activity are as follows. First, we are interested in the shear stress regulation of the expression of the calcium/calmodulin-sensitive nitric oxide (NO) synthase protein and its mRNA. Eventually, we plan to conduct studies using our co-culture model; however, the present studies were performed in bovine aortic endothelial cells (BAEC) to determine if this induction of NO synthase is accompanied by an enhanced NO production and to investigate mechanisms responsible for regulation of NO synthase expression by shear stress. Shear stresses of 15 dynes/cm^2 for 3 hours resulted in a two-fold induction of NO synthase mRNA content in BAEC as quantified by Northern analysis. A variety of cell signaling studies have been conducted. Particularly relevant to the shear stress regulation of NO and NOS is the use in these studies of the potassium channel antagonist tetraethylammonium chloride (3 mM), which prevented the increase in NO synthase mRNA in response to shear. Also, nocodazole (5 µg/ml), which blocks microtubule polymerization, abolished the shear-induced increase in mRNA of NO synthase in both BAEC and in human aortic endothelial cells, while having no effect, or slightly increasing NO synthase mRNA expression in non-sheared cells. From these results and taken together with other studies, we conclude that shear-induced NO synthase mRNA

transcription is activated via potassium channel opening and subsequently involves microtubular polymerization.

A second area of activity involves the use of confocal microscope imaging in our studies of cell signaling. Again, our long term goal is to conduct such studies using our co-culture model and for both steady and pulsatile flow conditions; however, our initial efforts have used only an endothelial monolayer exposed to steady flow. Endothelial cells are grown on collagen-coated microscope slides and loaded with Fluo-3, a fluorescent calcium indicator. The cell-covered slides are mounted within a parallel plate flow chamber specifically designed for confocal microscopy. Intracellular calcium responses to flow in cell culture medium, with and without a chemical agonist (ATP), are examined. The intracellular distribution of calcium over a monolayer of endothelial cells is assessed using image analysis of fluorescence intensity. Following initiation of a low level flow (5 dyn cm^{-2}) in flow medium containing an agonist, there is a sustained elevation of calcium in most cells. In contrast, when no agonists are present in the flow medium there is a rapid, transient calcium flux in some cells and a slight elevation of calcium over the monolayer after the onset of flow. Further development of this flow system should facilitate study using our co-culture model of the artery wall. This would facilitate study of biological signaling mechanisms and may also be useful for investigation of aspects of shear stress implicated in the regulation of vascular biology and the development of atherosclerosis.

Finally, at the time our previous grant, BCS-9111761, was ending and our new, current grant was starting, a big turnover in the personnel in my laboratory occurred. This was due to four students finishing their Ph.D.s Those working on our current grant thus represent a new team. New graduate students are Eric Francke and Dror Seliktar, with Karen Schnetzer continuing. Also, Dr. Lu Hilenski has joined the laboratory as a research faculty

member working in the area of tissue engineering and Ms. Jane Thomas is our new research technician.

Although there is much still to do, we believe that we are making progress. This is evidenced by our recent publications and presentations. Those which have occurred since the submission of the proposal for our current grant are listed below.

Publications arising from NSF Grants BCS-9111761 and BES-9412010 during the period January 1, 1994 - June 30, 1995.

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.

Ziegler, T. and Nerem, R.M., "Tissue Engineering a Blood Vessel: Regulation of Vascular Biology by Mechanical Stresses," J. Cell. Biochemistry, Vol. 56, No. 2, pp. 204-209, 1994.

Nerem, R.M. and Sambanis, A., "Tissue Engineering: From Biology to Biological Substitute," Tissue Engineering, Vol. 1, No. 1, pp. 3-13, 1995.

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.

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.

Girard, P.R. and Nerem, R.M., "Shear Stress Modulates Endothelial Cell Morphology and F-Actin Organization Through the Regulation of Focal Adhesion-Associated Proteins," J. Cell. Physiology, Vol. 163, pp. 179-193, 1995.

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 (in press).

Thoumine, O., Ziegler, T., Girard, P.R., and Nerem, R.M., "Elongation of Confluent Endothelial Cells in Culture: The Importance of Fields of Force in the Associated Alterations of their Cytoskeletal Structure," Experimental Cell Research, (in press).

James, N., Nerem, R.M., and Harrison, D.G., "Effects of Shear on Endothelial Cell Calcium in the Presence and Absence of ATP," The FASEB Journal, (in press).

Sato, M., Ohshima, N., and Nerem, R.M., "Viscoelastic Properties of Cultured Porcine Aortic Endothelial Cells Exposed to Shear Stress," Journal of Biomechanics, (in press).

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, (in press).

Helmlinger, G., Berk, B.C., and Nerem, R.M., "Flow-Induced Calcium Responses in Endothelial Cells are Synergistically Modulated by Serum," J. Cell. Engr., (in press).

Presentations at conferences during the period January 1, 1994 - June 30, 1995 on research conducted on NSF Grants BCS-9111761 and BES-9412010.

Nerem, R.M., "Cellular and Tissue Engineering," Okayama International Medical Forum, Okayama, Japan, February 2-4, 1994.

Nerem, R.M., "Vascular Endothelial Responses to Flow," Keystone Symposia on Tissue Engineering, Taos, NM, February 20-26, 1994.

Ziegler, T. and Nerem, R.M., "Co-Culture of Endothelial Cells with Smooth Muscle Cells in a Matrix of Collagen: Effect of Flow on Cell Morphology," Keystone Symposium on Tissue Engineering, Taoi, NM, February 20-26, 1994.

Nerem, R.M., "New and Potential Health Care Applications: Cellular and Tissue Engineering," DeLange Conference on Biotechnology, Science, Engineering, and Ethical Challenges for the 21st Century, Rice University, Houston, TX, February 28-March 2, 1994.

Nerem, R.M., "Cellular and Tissue Engineering," Thirteenth Southern Biomedical Engineering Conference, Rockville, MD, April 16-17, 1994.

Nerem, R.M., "Cell Culture Studies of Flow Effects on Vascular Biology," FASEB Summer Conference on the Endothelium and Cardiovascular Control, Copper Mountain, CO, June 26-July 1, 1994.

Nerem, R.M., Helmlinger, G., Thoumine, O., and Wiesner, T.F., "Regulation of Vascular Endothelial Biology by Flow," Symposium on Cell Mechanics and Cellular Engineering, Second World Congress of Biomechanics, Amsterdam, Netherlands, July 10-15, 1994.

Thoumine, O., Girard, P.R., and Nerem, R.M., "Oscillatory Shear Stress Modulates Cell-Matrix Attachment Proteins in Cultured Endothelial Cells," Second World Congress of Biomechanics, Amsterdam, Netherlands, July 10-15, 1994.

Nerem, R.M., "Tissue Engineering: An Introduction," Second World Congress of Biomechanics, Amsterdam, Netherlands, July 10-15, 1994.

Ziegler, T. and Nerem, R.M., "Influence of Collagen Matrix, Smooth Muscle Cells and Shear Stress on the Morphology and Cytoskeleton of Endothelial Cells," Second World Congress of Biomechanics, Amsterdam, Netherlands, July 10-1994.

Helmlinger, G., Berk, B.C., and Nerem, R.M., "The Modulation of Intracellular Free Calcium in Endothelial Cells Subjected to Various Fluid-Imposed Shear Stress and Media Conditions," Second World Congress of Biomechanics, Amsterdam, Netherlands, July 10-15, 1994.

Helmlinger, G., Berk, B.C., and Nerem, R.M., "The Intracellular Calcium Response of Single Endothelial Cells Subjected to Fluid-Imposed Steady and Pulsatile Shear Stress," 3rd International Symposium on Biofluid Mechanics, Munich, Germany, July 16-19, 1994.

Nerem, R.M., "Effects of Fluid Stresses on Cell Function," Workshop on Biomaterials: Cellular Responses to Implanted Materials," La Jolla, CA, September 12-14, 1994.

Nerem, R.M., "Endothelial Cell Calcium Dynamics in Response to Flow," American Physiological Society Conference on Mechanotransduction, Sarasota, FL, October 5-8, 1994.

Wiesner, T.F., Helmlinger, G., Berk, B.C., and Nerem, R.M., "A Mathematical Model of Calcium Signaling in Endothelial Cells Responding to Flow," 1994 Annual Fall Meeting of the Biomedical Engineering Society, Arizona State University, Tempe, AZ, October 14-16, 1994.

Greer, L.S., Vito, R.P., and Nerem, R.M., "Material Property Testing of a Collagen-Smooth Muscle Cell Latice for the Construction of a Bioartificial Vascular Graft," ASME International Congress and Exposition, Chicago, IL, November 6-11, 1994.

Ziegler, T., Alexander, R.W., and Nerem, R.M., "Effect of Shear Stress on the Morphology and Growth of Endothelial Cells Co-Cultured with Smooth Muscle Cells," ASME International Congress and Exposition, Chicago, IL, November 6-11, 1994.

James, N.L., Harrison, D.G., and Nerem, R.M., "Confocal Microscopy of Endothelial Cell Calcium Responses to Flow," American Heart Association 67th Scientific Sessions, Dallas, TX, November 14-17.

Nerem, R.M., "Tissue Engineering: From Biology to Biological Substitutes," NIST/NSF/NIH/FDA Workshop on Tissue Engineering: From Basic Science to Products, Gaithersburg, MD, November 22, 1994.

Nerem, R.M., "Tissue Engineering a Blood Vessel," 4th China-Japan-U.S.A.-Singapore Conference on Biomechanics, Taiyuan, China, May 21-27, 1995.

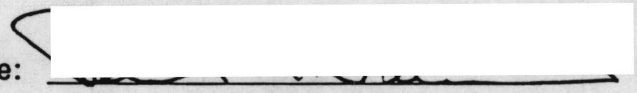
2. Statement of Funds Estimated to Remain Unobligated

Currently $2,218.83 remains unobligated. This will be spent prior to the end of August.

7. Updated Information on Animal Care and Use, Institutional Biohazard Committee, and Human Subject Certification

None

I certify that to the best of my knowledge (1) the statements herein (excluding scientific hypotheses and scientific opinions) are true and complete, and (2) the text and graphics in this report as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I understand that the willful provision of false information or concealing a material fact in this report or any other communication submitted to NSF is a criminal offense (U.S. Code, Title 18, Section 1001).

P.I. Signature: 

(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING

SECOND YEAR

PROPOSAL BUDGET

FOR NSF USE ONLY

ORGANIZATION	PROPOSAL NO.	DURATION (MONTHS) Proposed	Granted
Georgia Tech Research Corporation			
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR Robert M. Nerem	AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.6. show number in brackets)	NSF Funded Person-mos. CAL.	ACAD	SUMR	Funds Requested By Proposer	Funds Granted By NSF (If Different)
1. Robert M. Nerem ($13,099/mo)	1.0			$ 13,099	$
2. R. Wayne Alexander, Co-P.I.					
3. David G. Harrison, Co-I.					
4. Peggy R. Girard, Co-I.					
5. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
6. (6) TOTAL SENIOR PERSONNEL (1-5)				13,099	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( ) POST DOCTORAL ASSOCIATES					
2. (1) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)					
3. (3) GRADUATE STUDENTS				45,000	
4. ( ) UNDERGRADUATE STUDENTS					
5. (1) SECRETARIAL CLERICAL					
6. ( ) OTHER					
TOTAL SALARIES AND WAGES (A+B)				58,099	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) (25.1%)				3,235	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				61,334	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $1,000:)					
TOTAL PERMANENT EQUIPMENT					
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS) (trips for 2 scientific mtgs)				1,050	
2. FOREIGN					
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $					
2. TRAVEL					
3. SUBSISTENCE					
4. OTHER					
( ) TOTAL PARTICIPANT COSTS					
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				15,500	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				1,125	
3. CONSULTANT SERVICES					
4. COMPUTER (ADPE) SERVICES					
5. SUBCONTRACTS to Emory University School of Medicine				63,000	
6. OTHER					
TOTAL OTHER DIRECT COSTS				79,625	
H. TOTAL DIRECT COSTS (A THROUGH G)				142,009	
I. INDIRECT COSTS (SPECIFY RATE AND BASE) 40.0% of MTDC					
TOTAL INDIRECT COSTS				31,604	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)					
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPM 252 AND 253)					
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$173,613	$

PI/PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION		
INST. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked	Date of Rate Sheet	Initials-DGC

NSF Form 1030 (8/90) *Supersedes All Previous Editions*

*SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPM 233)

E-85-T03 #2

OMB Number 345-0058

NATIONAL SCIENCE FOUNDATION
4201 Wilson Blvd.,
Arlington, VA 22230

PI/PD Name and Address

Robert M. Nerem
Center for Space Research, C0600
U of Texas Austin
Austin TX 78712-9998

NATIONAL SCIENCE FOUNDATION
FINAL PROJECT REPORT

PART I - PROJECT IDENTIFICATION INFORMATION

1. Program Official/Org.	Fred G. Heineken - BES
2. Program Name	BIOENG & RSCH TO AID PERSONS/W DISAB PRO
3. Award Dates (MM/YY)	From: 09/94 To: 08/96
4. Institution and Address	GA Tech Res Corp - GIT Administration Building Atlanta GA 30332
5. Award Number	9412010
6. Project Title	Tissue Engineering a Blood Vessel

** You are encouraged to submit your Final Project Report electronically through the NSF FastLane home page (www.fastlane.nsf.gov). **

This Packet Contains
NSF Form 98A
And 1 Return Envelope

PART IV -- FINAL PROJECT REPORT -- SUMMARY DATA ON PROJECT PERSONNEL

(To be submitted to cognizant Program Officer upon completion of project)

The data requested below are important for the development of a statistical profile on the personnel supported by Federal grants. The information on this part is solicited in resonse to Public Law 99-383 and 42 USC 1885C. All information provided will be treated as confidential and will be safeguarded in accordance with the provisions of the Privacy Act of 1974. You should submit a single copy of this part with each final project report. However, submission of the requested information is not mandatory and is not a precondition of future award(s). Check the "Decline to Provide Information" box below if you do not wish to provide the nformation.

Please enter the numbers of individuals supported under this grant.
Do not enter information for individuals working less than 40 hours in any calendar year.

	Senior Staff		Post-Doctorals		Graduate Students		Under-Graduates		Other Participants[1]	
	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.
A. Total, U.S. Citizens	1	1			2	1				
B. Total, Permanent Residents										
U.S. Citizens or Permanent Residents[2]:										
American Indian or Alaskan Native										
Asian.										
Black, Not of Hispanic Origin.										
Hispanic										
Pacific Islander										
White, Not of Hispanic Origin										
C. Total, Other Non-U.S. Citizens										
Specify Country 1.										
2.										
3.										
D. Total, All participants (A + B + C)	1	1			2	1				
Disabled[3]										

☐ Decline to Provide Information: Check box if you do not wish to provide this information (you are still required to return this page along with Parts I-III).

[1] Category includes, for example, college and precollege teachers, conference and workshop participants.

[2] Use the category that best describes the ethnic/racial status fo all U.S. Citizens and Non-citizens with Permanent Residency. *(If more than one category applies, use the one category that most closely reflects the person's recognition in the community.)*

[3] A person having a physical or mental impairment that substantially limits one or more major life activities; who has a record of such impairment; or who is regarded as having such impairment. *(Disabled individuals also should be counted under the appropriate ethnic/racial group unless they are classified as "Other Non-U.S. Citizens.")*

AMERICAN INDIAN OR ALASKAN NATIVE: A person having origins in any of the original peoples of North America and who maintains cultural identification through tribal affiliation or community recognition.

ASIAN: A person having origins in any of the original peoples of East Asia, Southeast Asia or the Indian subcontinent. This area includes, for example, China, India, Indonesia, Japan, Korea and Vietnam.

BLACK, NOT OF HISPANIC ORIGIN: A person having origins in any of the black racial groups of Africa.

HISPANIC: A person of Mexican, Puerto Rican, Cuban, Central or South American or other Spanish culture or origin, regardless of race.

PACIFIC ISLANDER: A person having origins in any of the original peoples of Hawaii; the U.S. Pacific territories of Guam, American Samoa, and the Northern Marinas; the U.S. Trust Territory of Palau; the islands of Micronesia and Melanesia; or the Philippines.

WHITE, NOT OF HISPANIC ORIGIN: A person having origins in any of the original peoples of Europe, North Africa, or the Middle East.

PROJECT SUMMARY

The research on this grant has been focused on the development of a blood vessel substitute which employs a co-culture of endothelial cells (EC) and smooth muscle cells (SMC). The major contributions from this work are as follows: (i)the establishment of an EC-SMC co-culture model of the blood vessel wall and the initial evaluation of this model, including under conditions of flow; (ii) the determination of the influence of flow on the ability of EC to synthesize and secrete biologically active molecules; (iii) the study of how the mechanical environment of EC effects the extracellular matrix; (iv) the systematic investigation of pulsatile flow effects and through this the determination of how EC respond to different types of flow environments, with the influence of purely oscillatory flows being particularly intriguing; (v) the determination of the influence of mechanical factors on the proliferative activities of vascular cells, including the process of cell division itself; (vi) the development of a uniaxial cyclic stretch device and initial studies on the influence of cyclic stretch on the biology of vascular SMC; and (vii) the determination of the influence of flow and the associated shear stress on intracellular calcium, an important second messenger, through both experimental and modeling studies.

It should be noted that three graduate students worked on this project. Two of these are doctoral students, with one of these being a female. There also is one graduate student who worked on an M.S. degree. During the last five years, six received their Ph.D. degree based on work conducted with support of this project and its predecessor. In addition, several undergraduate students worked on the project. In addition, the unique interdisciplinary environment associated with the conduct of this project's activities should be noted. Not only is this a cooperative effort between an engineering school and a medical school, but it is one which is strongly integrated. As part of this integrated effort there are students from Georgia Tech working in the laboratories of Emory University School of Medicine and researchers from Emory working in the laboratories of Georgia Tech.

Journal publications related to this NSF supported project over the past five years are provided in the attached list.

Publications on NSF Grant BES-9412010

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.

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.

Girard, P.R. and Nerem, R.M., "Shear Stress Modulates Endothelial Cell Morphology and F-Actin Organization Through the Regulation of Focal Adhesion-Associated Proteins," J. Cell. Physiology, Vol. 163, pp. 179-193, 1995.

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.

Thoumine, O., Ziegler, T., Girard, P.R., and Nerem, R.M., "Elongation of Confluent Endothelial Cells in Culture: The Importance of Fields of Force in the Associated Alterations of their Cytoskeletal Structure," Experimental Cell Research, Vol. 219, pp. 427-441, 1995.

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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.

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Uematsu, M., Ohara, Y., Navas, J.P., Nishida, K., Murphy, T.J., Alexander, R.W., Nerem, R.M., and Harrison, D.G., "Regulation of Endothelial Cell Nitric Oxide Synthase mRNA Expression by Shear Stress," Americal Journal of Physiology: Cell Physiology, Vol. 269, pp. C12371-C1378, 1996.

Sato, M., Ohshima, N., and Nerem, R.M., "Viscoelastic Properties of Cultured Porcine Aortic Endothelial Cells Exposed to Shear Stress," Journal of Biomechanics, Vol. 29, No. 4, pp. 461-467, 1996.

Wiesner, T.F., Berk, B.C., and Nerem, R.M., "A Mathematical Model of Cytosolic Calcium Dynamics in Human Umbilical Vein Endothelial Cells," American Journal of Physiology: Cell Physiology, Vol. 270, pp. C1556-C1569, 1996.

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.

Inoue, N., Ramassami, S., Fukai, T., Nerem, R.M., and Harrison, D.G., "Shear Stress Modulates Expression of Cu/Zn Superoxide Dismutase in Human Aortic Endothelial Cells," Circulation Research, Vol. 79, pp. 32-37, 1996.

Matsumoto, T., Delafontaine, P., Schnetzer, K.J., Tong, B.C., and Nerem, R.M., "Effect of Uniaxial, Cyclic Stretch on the Morphology of Monocyte/Macrophages in Culture," ASME Journal of Biomechanical Engineering, Vol. 118, No. 3, pp. 420-422, 1996.