15:30:58 OCA PAD INITIATION - PROJECT HEADER INFORMATION 07/20/90

Active

Project #: E-25-M44 Cost share #: Rev #: 0
Center # : 10/24-6-R6966-0A0 Center shr #: OCA file #:
Work type : RES
Contract#: LETTER DTD 4/24/90 Mod #: Document : CONT
Prime #: 5 RO1 HL41175-03 Contract entity: GTRC

Subprojects ? : N
Main project #:

Project unit: MECH ENGR Unit code: 02.010.126
Project director(s):
NEREM R M MECH ENGR (404)894-2768

Sponsor/division names: UNIVERSITY OF TEXAS / SAN ANTONIO, TX
Sponsor/division codes: 400 / 045

Award period: 900501 to 910430 (performance) 910731 (reports)

Sponsor amount	New this change	Total to date
Contract value	84,657.00	84,657.00
Funded	84,657.00	84,657.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: VASCULAR HEALING--CELL BIOLOGY & RHEOLOGIC FACTORS

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U
Defense priority rating : N/A
Equipment title vests with: Sponsor

ONR resident rep. is ACO (Y/N): N
NIH supplemental sheet
GIT X

Administrative comments -
INITIATION OF PROJECT. CONTINUATION OF E-25-1180.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 08/09/91

Project No. E-25-M44_____ Center No. 10/24-6-R6966-0A0_

Project Director NEREM R M_____ School/Lab MECH ENGR_____

Sponsor UNIVERSITY OF TEXAS/SAN ANTONIO, TX_____

Contract/Grant No. LETTER DTD 4/24/90_____ Contract Entity GTRC

Prime Contract No. 5 R01 HL41175-03_____

Title VASCULAR HEALING--CELL BIOLOGY & RHEOLOGIC FACTORS_____

Effective Completion Date 910430 (Performance) 910731 (Reports)

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

Comments_____
_____

Subproject Under Main Project No. _____

Continues Project No. E-25-M80_____

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

NOTE: Final Patent Questionnaire sent to PDPI.

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER 1 RO1 HL 41175-04	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Schwartz, Colin J.	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION The University of Texas Health Science Center	5/1/91	4/30/92

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Vascular Healing--Cell Biology and Rheologic Factors

(SEE INSTRUCTIONS)

1. Brief Summary of Plans:

Based on encouraging and continuing progress this year (-03), no major changes in our overall research plan are anticipated. The changes to our original research plan, which are detailed below, reflect recent rapid developments in our knowledge concerning cytokines and cell adhesion molecules influencing endothelial cell-to-cell, cell-to-substrate, as well as platelet and monocyte adherence to endothelial cells. Additionally, progress in our laboratories at UTHSCSA and GIT, especially related to shear stress influences on endothelial cell-to-substrate focal adhesion points and endothelial secretory product(s) influencing monocyte recruitment and adhesion, will allow us to focus more precisely our efforts to optimize both development of a pre-endothelialized vascular graft and also probe cellular mechanisms regulating the biology of vascular endothelial cells residing in different flow environments.

Initial objectives established for Specific Aim #1 directed at defining endothelial cell growth on porous graft materials under steady or pulsatile flow conditions have been achieved. Some studies will continue to explore molecular mechanisms governing the shear dependent decrease in cell proliferation observed. The knowledge gained from these studies has allowed us to initiate *in vitro* testing of a confluent pre-endothelialized vascular graft. Additionally this year, we plan to continue a collaboration initiated in the current year with Dr. Dan Urry at The University of Alabama at Birmingham to examine the influence of elevated laminar shear stress on the proliferation and retention of BAEC cultured on synthetic elastomeric polypeptides.

Since most of the major objectives of Specific Aim #2 designed to determine influence of different shear stress on graft endothelial monolayer integrity have been completed, plans for this year will focus on cellular and molecular mechanisms regulating the expression and distribution of cell-to-substrate focal adhesion sites. Additional studies will continue to define the cell-to-cell adhesion molecules responding to the flow environment.

The objectives of Specific Aim #3 to examine the influence of shear stress preconditioning of BAEC on platelet and monocyte adherence have been achieved. Studies planned for this next year (-04) are designed to 1) examine monocyte and platelet adherence under flow conditions and 2) define specific cellular molecules including cytokines and cell adhesion molecules potentially mediating shear stress associated responses. The only deviations from the original design will be to examine the expression of several of these possible mediators; specifically, MCP-1, ICAM-1, and VCAM-1 by measuring mRNA levels in shear stress preconditioned BAEC using northern blot analyses. Plans in Specific Aim #4 to develop and test a preconditioned, endothelialized vascular prosthesis have been initiated and are designed to continue on schedule in year -04. At the current rate of progress, *in vivo* testing should be initiated within the first 6 months of year -04. As is evident in the progress in year -03, continued success in this program involves and depends upon an integrated and coordinated effort among the investigators at both UTHSCSA and GIT.

2. Current Studies:

Specific Aim #1: Since the primary objectives of this aim were completed in the first two years, and are currently providing the basic endothelialized material for the other 3 specific aims, studies have been expanded to examine some of the molecular mechanisms mediating the pronounced decrease in BAEC and PAEC proliferation observed in response to elevated shear stress. Specifically, studies performed in a collaborative effort between the laboratories of Dr. R. M. Nerem of GIT and Dr. B.C. Burk at Emory University indicate that BAEC cultures preconditioned for 24 h to elevated steady state laminar or pulsatile shear stress exhibited a decreased precentage of cells in the S phase of the cell cycle accompanied by an increase in the percentage of cells in the G_0/G_1 phase as compared to no flow control cells using flow cytometry. Secondly, this same collaborative group demonstrated that BAEC preconditioned to elevated shear stress exhibited a seven-fold decreased response in cellular mRNA levels for c-myc protein relative to control BAEC when the cells were challenged with a pharmacological dose of α-thrombin. Finally, video observations at GIT of BAEC dividing under elevated shear stress reveal that these cells do not "round up" prior to or upon division but rather divide along the short axis of the elongated cells and simply extend in the direction of flow after division. These observations suggest that these cells do not break their cell-to-substrate attachments upon division and, thus, may have important implications relative to the maintenance of vascular endothelial integrity in high flow and shear stress environments.

Specific Aim #2: Studies within this Aim have proceeded on schedule based on previous studies in year -02 indicating that endothelial cells cultured on solid polyester film can withstand shear stress up to 200 dynes/cm^2 for indefinite periods without denuding, while cells cultured on porous (1 μ) polyester material can withstand shear stress of 300 dynes/cm^2 without any significant cell loss. As planned in the current year, these studies have been repeated at GIT using different flow environments including pulsatile and reversing flow. Similar to steady state laminar flow studies, BAEC cultured on solid polyester film remained attached in the presence of a variety of high shear pulsatile laminar flow environments over prolonged shear exposure periods. Those cells exposed to pulsatile flow did exhibit a more rapid rate of elongation and alignment with flow direction relative to cells exposed to the same mean shear stress. Also, BAEC exposed to prolonged reversing laminar flow with no mean flow (e.g., +30 dynes/cm^2 to -30 dynes/cm^2) also remained attached to the polyester substrate, though no cell shape change or alignment was observed. In contrast, BAEC exposed to a reversing laminar flow with a net mean flow (e.g., +30 dynes/cm^2 to -10 dynes/cm^2) initially elonged and aligned with flow and, then, after a variable lag time invariably detached from the solid polyester substrate. Studies are currently being performed to examine this response with cells cultured on the porous polyester substrate.

Secondly, Dr. Girard has recently completed studies, which will soon be submitted for publication, demonstrating the distribution of vinculin associated with focal cell contacts to the extracellular matrix in BAEC under different flow environments using fluorescent immunolabeling techniques. These studies indicate that BAEC preconditioned to 24 h elevated steady state shear stress (30 dynes/cm^2) exhibit a prominant localization of vinculin at the "upstream" end of most EC concomitant with changes in cell shape and alignment. In contast, endothelial cells under no flow conditions exhibit a predominant localization of vinculin in focal contracts distributed near the periphery of each cell. This approach should yield important insights as to the cellular mechanisms regulating the cell attachment responses to the different flow environments described above.

Finally, this year, Dr. Mowery, at UTHSCSA, has been conducting studies designed to identify cell surface proteins potentially involved in cell-to-cell adhesion interactions. As part of these studies, a 45 Kd protein has been isolated which appears to be expressed in response to flow conditions. Also studies to measure mRNA levels for the cellular adhesion molecule, ICAM-1, are being undertaken using northern blot analysis in BAEC preconditioned to elevated shear relative to static cultured BAEC.

Specific Aim #3: Understanding platelet-endothelial and monocyte endothelial interactions is critical not only to development of a small diameter vascular graft which can maintain patency *in vivo* but also to investigate the basic cellular processes mediating thrombosis and atherogenesis. Studies in year -03 within Specific Aim #3 have been concentrated on determining the cellular mechanisms regulating the previously documented decreased platelet and monocyte adherence to BAEC preconditioned for 24 h elevated shear stress. Experiments performed within the current year by Dr. Cayatte at UTHSCSA have demonstrated that the decrease in platelet and monocyte adherence observed in cells preconditioned to elevated shear stress (30 dynes/cm^2) relative to BAEC preconditioned to low shear stress (< 1 dyne/cm^2) is a time dependent process requiring a minimum of 4 h for expression. The results of these studies form the basis of a manuscript currently in preparation. Further, the observed difference in adherence of blood monocytes to BAEC preconditioned under different shear stress levels appears to be at least partially dependent upon a secretory product. Specifically, a molecule(s) appears to be secreted into serum free circulating culture medium by either BAEC or PAEC exposed to LS (< 1 dyne/cm^2) conditions for at least 4 h which enhances monocyte adherence to test endothelial cultures in a dose dependent manner. This as yet unidentified molecule(s) is present in either very low or undetectable levels in either HS or NS treated BAEC. Current efforts are directed toward isolating and identifying this agent. Concomitant studies to examine the expression of known cell adhesion molecules mediating monocyte (VCAM - 1) or platelet (GP1b) adherence are being conducted using both immunolabeling and mRNA northern blot analyses. Also, efforts are currently underway to assay IL-1 and MCP-1 levels in media of shear stress preconditioned cells by radioimmunoassay.

Specific Aim #4: Studies were initiated within this Aim in the current year to develop and test *in vitro* a small diameter, pre-endothelialized, and shear stress pre-conditioned vascular prosthesis. In cooperation with Spectrum Medical Industries, a 5 mm internal diameter cylindrical tube constructed from the 1 μ porous polyester mesh used in our previous studies has been developed. Also during this year, the Cell Culture Laboratory at UTHSCSA has developed a technique to successfully seed and culture endothelial cells in this potential prosthesis. Current studies are directed toward initiating the flow studies *in vitro* using this pre-endothelialized prosthesis and evaluating cell retention after prolonged pulsatile shear stress conditions as described in our initial design.

3. Human Subjects: No change

4. Vertebrate Animals: No change

5. Publications:

1. Cushing SD, Berliner JA, Valente AJ, Territo MC, Mahamad N, Parhami F, Gerrity R, Schwartz CJ, and Fogelman AM. Minimally Modified Low Density Lipoprotein Induces Monocyte Chemotactic Protein 1 in Human Endothelial Cells and Smooth Muscle Cells. Proc Natl Acad Sci, 87:5134-5138, 1990.

2. Sprague, EA, Nerem, RM, and Schwartz, CJ. Cellular Recognition and Transduction of Fluid Mechanical Shear Stress Signals. In: Liepsch, D. ed. Proceedings of 2nd International Symposium on Biofluid Mechanics and Biorheology. In press, 1990.

3. Sprague, EA, Cayatte, AJ, Levesque, MJ, Rozek, MM, Schwartz, CJ, and Nerem, RM. Shear Stress Related Decreases in Cell Proliferation and Platelet and Monocyte Adherence to Bovine Aortic Endothelial Cells Seeded on Solid or Porous Polyester Substrates. Accepted, ASME J. Biomech Engr, 1990.

4. Schwartz CJ. Current Concepts on the Role of Cholesterol in the Pathogenesis of Atherosclerosis. Highlights of a Conference: *Prevention and Regression of Atherosclerosis: New Research, New Concepts*. Published by Medical Information Services, Inc., pp 1-2, 1990.

5. Nerem RM. Influence of Flow on Vascular Cell Function. J of Japan Atherosclerosis Society, 18:631-633, 1990.

6. Holenstein R and Nerem RM. Parametric Analysis of Flow in the Intramyocardial Circulation. Annals of Biomedical Engineering, 18:347-365, 1990.

7. 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.

8. Sprague EA, Cayatte AJ, Nerem RM, and Schwartz CJ. Time-dependent Shear Stress Modullation of Platelet and Monocyte Adherence to Cultured Bovine Aortic Endothelial Cells. In: *Cardiovascular Science and Technology: Basic & Applied: II*. J. Norman, ed. Oxymoron Press, Louisville, Kentucky, pp. 125-128, 1990.

9. Geiger RV, Berk BC, Alexander RW and Nerem RM. Flow-induced CA^{2+} Chanbes in Bovine Aortic Endothelial Cells: Spatial and Temporal Analysis in Single Cells. In: *Cardiovascular Science and Technology: Basic & Applied: II*. J. Norman, ed. Oxymoron Press, Louisville, Kentucky, pp. 110-112, 1990.

10. Helmlinger G, Geiger RV, Schreck S, and Nerem RM. Pulsatile Flow Effects on Cultured Vascular Endothelial Cells. In: *Cardiovascular Science and Technology: Basic & Applied: II*. J. Norman, ed. Oxymoron Press, Louisville, Kentucky, pp. 116-118, 1990.

11. Nerem RM and Girard PR. Hemodynamic influences on the vascular endothelial biology. Toxicol Pathol 18: Part 1 (in press), 1990.

12. Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Nerem RM. The Pathogenesis of Atherosclerosis: An Overview. Clin Cardiol 14:1-16, 1991.

13 Prasad ARS, Nerem RM, Schwartz CJ, and Sprague EA. Stimulation of Phosphoinositide Hydrolysis in Cultured Endothelial Cells Exposed to Elevated Shear Stress. Submitted for publication, 1991.

14. Kelley JL, Kerbacher JJ, Gilchrist EP, Rozek MM, Sprague EA and Schwartz CJ. Purification of the 'Scavenger' Receptor for Chemically Modified Lipoproteins from Rabbit Carrageenan Granuloma Macrophages. In Preparation, 1991.

15. Valente AJ, Rozek MM, Graves DT and Schwartz CJ. Identification of Receptors for Smooth Muscle Cell-Derived Chemotactic Factor on Peripheral Blood Monocytes. In preparation, 1991.

16. Cayatte AJ, Nerem RM, Schwartz CJ, and Sprague EA. Inhibition of Platelet and Monocyte Recruitment to Cultured Endothelial Cells Preconditioned to Elevated Shear Stress. In preparation, 1991.

Abstracts:

1. Sprague EA, Cayatte AJ, Rozek MM, Nerem RM, and Schwartz CJ. Modulation of Platelet and Monocyte Adherence in Response to Elevated Shear Stress in Cultured Bovine Aortic Endothelial Cells. Presented at the First World Congress of Biomechanics, UCSD, La Jolla, CA, August 30-September 4, 1990.

2. Kerbacher JJ, Kelley JL, and Schwartz CJ. Influence of Cytoskeletal-disrupting Agents on the Metabolism of Acetyl-LDL by Cultured Vascular Endothelial Cells. Arteriosclerosis, 10:822a, 1990.

3. Rozek MM, Valente AJ, Cayatte AJ, Sprague EA, and Schwartz CJ. The Influence of Smooth Muscle Cell-derived Monocyte Chemotactic Protein (MCP-1) on Monocyte Adherence to Cultured Vascular Endothelial Cells. Arteriosclerosis, 10:758a, 1990.

4. Cayatte AJ, Sprague EA, Rozek MM, Nerem RM, and Schwartz CJ. Reduced Platelet and Monocyte Adherence to Endothelium Preconditioned by Laminar Steady-state Hemodynamic Shear Stress. Arteriosclerosis, 10:855a, 1990.

5. Kerbacher JJ, Gilchrist EP, Henzel WJ, Schwartz CJ, Kelley JL. The Intermediate Filament Vimentin Binds Chemically Modified LDL. J Cell Biol 111:177a, 1990.

6. Rozek MM, Valente AJ, Cayatte AJ, Sprague EA, and Schwartz CJ. The Influence of Smooth Muscle Cell-Derived Monocyte Chemotactic Protein (MCP-10 on Monocyte Adherence to Cultured Vascular Endothelial Cells. Circulation, 82:363, 1990.

7. Sprague EA, and Prasad ARS. Differential Regulation to Receptor-mediated Endocytosis of LDL and Modified LDL by Agents Influencing the Phosphoinositide Signal Transduction Pathway. Circulation, 82:2891, 1990.

8. Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Kerbacher JJ, Cayatte AJ, and Nerem, RM. Mechanisms in Atherogenesis and Occlusive Arterial Disease. Submitted to the International Symposium on Angiogenesis in St. Gallen, Switzerland, March 13-15, 1991

Books and Book Chapters:

1. **Schwartz CJ, Sprague EA, Valente AJ, Kelley JL, Edwards EH and Suenram CA. Inflammatory Components of the Human Atherosclerotic Plaque. In: S Glagov, WP Newman, III and SA Schaffer, eds. *Pathobiology of the Human Atherosclerotic Plaque*. Springer-Verlag New York, NY, pp. 107-120, 1990**