OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED 2013-11-18

Application Identifier 6631626-01-5646153

5. APPLICANT INFORMATION **Organizational DUNS*:** 047006379

Legal Name*: President and Fellows of Harvard College
Department: Sponsored Programs Admin
Division: Harvard Medical School
Street1*: 25 Shattuck St
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02115-6027

Person to be contacted on matters involving this application
Prefix: First Name*: Rachel Middle Name: M. Last Name*: Cahoon Suffix:
Position/Title: Director, Sponsored Programs Administration
Street1*: Harvard Medical School
Street2: SPA, Gordon Hall 509
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02115-0000
Phone Number*: 6174320050 Fax Number: 617-432-2651 Email: rachel_cahoon@hms.harvard.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1042103580C5

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY* NIH/NIDDK

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Biliary pathophysiology and prevention by "Somah" during liver preservation

12. PROPOSED PROJECT
Start Date* 07/01/2014
Ending Date* 06/30/2016

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MA-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Arun Middle Name: Last Name*: Chaudhury Suffix:

Position/Title: Research Fellow
Organization Name*: President and Fellows of Harvard College
Department: Medicine
Division: Harvard Medical School
Street1*: Brigham and Women's Hospital
Street2: Surgery
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02115-0000
Phone Number*: 857-203-6044 Fax Number: 857-203-5592 Email*: arun_chaudhury@hms.harvard.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$353,925.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$353,925.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Ms. First Name*: Barbara Middle Name: J. Last Name*: Rankin Suffix:

Position/Title*: Senior Sponsored Programs Officer
Organization Name*: President and Fellows of Harvard College
Department: Sponsored Programs Admin
Division: Harvard Medical School
Street1*: Harvard Medical School
Street2: Gordon Hall, Sponsored Programs Administration
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02115-0000
Phone Number*: 6174322662 Fax Number: 6174322651 Email*: barbara_rankin@hms.harvard.edu

Signature of Authorized Representative*
Rankin, Barbara J.

Date Signed*
11/18/2013

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Harvard Medical School
Duns Number:	0470063790000
Street1*:	VA Boston Healthcare System
Street2:	1400 VFW Parkway
City*:	West Roxbury
County:	
State*:	MA: Massachusetts
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	02132-4927
Project/Performance Site Congressional District*:	MA-008

File Name

Additional Location(s)

Funding Opportunity Number: PA-12-139. Received Date: 2013-11-18T16:02:09.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number A3431-01

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ● No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Chaudhury_Abstract.pdf
8. Project Narrative*	Chaudhury_Project_Narrative.pdf
9. Bibliography & References Cited	Chaudhury_R21_References_F.pdf
10.Facilities & Other Resources	Chaudhury_R21_Facilities_Resources.pdf
11.Equipment	

Funding Opportunity Number: PA-12-139. Received Date: 2013-11-18T16:02:09.000-05:00

Abstract

Liver transplantation is the only definite therapy for both acute and chronic liver failure. However, graft dysfunction occurs due to non-physiological states during storage. A major feature of post-transplant complication is related to biliary dysfunction, including intraluminal biliary casts and non-anastomotic biliary stricture. Traditionally, livers are stored in University of Wisconsin (UW) solution. An improvised organ storage solution, "Somah", with rationally designed components to maintain perfusion and high energy phosphates have been recently demonstrated to provide superior status of storage of solid organs like heart in vitro. Preliminary data demonstrated biliary ductular reactive changes and mucosal injury in porcine DCD (donation after cardiac death) livers cold-stored in UW solution as early as 6 hrs. However, these histopathological changes could not be visualized in livers cold-stored in Somah for even 72 hrs. We hypothesize that the ability of Somah to restore intracellular ATP levels in vitro is the basis for preservation of cholangiocyte integrity during preservation. The current proposal aims to examine the pathophysiological basis for biliary dysfunction of livers stored in vitro and explore rational basis for biologically preconditioning storage solutions to prevent cholangiocyte cellular injury. In biliary epithelial cells, the solute carrier protein, SLC17A9-positive vesicles, store and secrete ATP in bile. ATP in bile stimulates chloride and water secretion and decreases bile viscosity and facilitates bile flow. We hypothesize that when biliary ATP is deficient, bile stasis may damage to mucosa of cholangiocytes. In vitro storage of livers in ischemic conditions has also been reported to damage actin within cholangiocytes. We plan to examine whether cytoskeletal injury disrupts SLC17A9 vesicular trafficking. We hypothesize that restoration of intracellular high energy phosphates by the novel organ storage solution, “Somah”, prevents biliary damage by supplying the substrate ATP for effective actomyosin action (myosin II is an ATPase) and vesicular content, i.e., ATP, of SLC17A9 micropinosomes (SLC17A9 is a VNUT, a vesicular nucleotide transporter). The specific aims aimed to test our hypotheses are: (a) Aim 1# To examine whether extracellular ATP secretion is impaired and SLC17A9 containing vesicles is decreased in biliary epithelium during in vitro liver storage and (b) Specific Aim 2# To examine whether non-muscle myosin II (NMMII) isoforms are diminished in biliary epithelium during in vitro liver storage Anticipated outcome is that livers stored in Somah will have intact functional SLC17A9 positive vesicles that can traffic to cholangiocyte membrane post-stimulation for release of contents.

Project Narrative

Liver transplantation is a life-saving surgery for numerous conditions in which the liver, the body's main organ for detoxification, fails to function. However, a major obstacle is availability of high quality organs, as there is lack of rationally designed storage solutions which may provide physiologic protection in vitro. Here we show the efficacy of a novel solution Somah ("elixir of life", in Sanskrit), in protection of liver structure and function for 3 days ex vivo, which will facilitate storage for a long period of time and organ transfer between geographically separated sites.

Facilities and other resources

1. Dedicated lab space for the work will be provided by Dr. Hemant Thatte.
2. Laboratory and office space including computers are available for conducting the experiments and analyses.
3. In addition, dedicated animal facilities are available for maintaining animals proposed for the experiments.
4. For optical imaging analyses, confocal microscopy suites in core research facility at VA Boston HealthCare System (West Roxbury) campus will be used. Zeiss and Nikon confocal microscopes are available. The team have greater than decade long experience in cutting edge microscopy and have incorporated microscopy for novel translational investigations.
5. Vibratomes and cryostat machines are available for obtaining liver sections.
6. Spectrophotometers and western blotting apparati are available.
7. Functional Evaluation of Livers after extended storage:

Pilot experiments have been performed and are described below.

Preparation of Blood for Ex Vivo Studies.

Systemically heparinized blood was collected intraoperatively, leukodepleted, and stored at 4°C. Prior to the commencement of experiments, the hematocrit was adjusted to 10% using Somah solution (now *perfusate*). The perfusate was assessed during the course of the experiment for gases, ionic composition and pH, and if required, glucose, K^+ and Ca^{2+} levels, and pH were adjusted to the physiological levels, using glucose, KCl, $CaCl_2$ and $NaHCO_3$ solutions, respectively.

Ex Vivo Perfusion Using "Somah Device"

Livers were stored in Somah for 72 hours at 4 ^{o}C. Upon storage, hepatic artery and portal vein were identified on the livers and canulated. The livers were kept in a polypropylene perfusion chamber attached to our custom built Somah Device that we use for *ex vivo* reanimation of hearts (Lowalekar et al 2013). An oxygenator, heat exchanger, CDI and Data acquisition device, with custom written software (Comdel Inc, Wahpeton, ND), are incorporated into the system for real-time monitoring of changes in perfusate pH, temperature, pO_2, pCO_2, K^+ and HCO^{3-}, pressure and flow rates. The Somah Device reservoir was filled with 2L perfusate. The livers were gently flushed through the portal vein with 2L of cold Somah, and then connected to the Somah Device via the hepatic artery and the portal vein. The reservoir outlet was diverted into two circuits; in first circuit, the perfusate drained by gravity into the portal vein. In the second circuit, perfusate was diverted through a pump to the hepatic artery via the oxygenator and heart exchanger. Temperature of the perfusate was raised to 37^{o}C over a 20 min period, and the perfusate was circulated through the liver over a 2-hour period. Perfusate pressure in the portal vein was adjusted to between 8-10 mmHg by changing the height of the reservoir. Perfusate pressures were maintained between 80-100 mmHg through the hepatic artery by adjusting the pump. The liver perfusate drained into the chamber through the hepatic vein and

other tributaries, and was returned to the reservoir by another pump. Perfusate draining from the hepatic vein was temporally sampled for albumin, liver enzymes and other metabolites as mentioned below.

8. Source of chemicals, antibodies and other useful reagents

    - CoStorSol (University of Wisconsin Solution; UWS): Preservation Solutions Inc, (Elkhorn, WI)
    - Somah solution provided by Somahlution, LLC (Jupiter, Fl)
    - Other chemicals (quinacrine etc) shall be obtained from Sigma-Aldrich (St. Louis, MO)
    - VetScan iStat, VetScan VS2, Comprehensive Diagnostic Profile cartridges for measuring blood gases, electrolytes, lactate, glucose, AST, ALT and CK enzymes: Abaxis Inc (Union City, CA)
    - Adenosine 5′-triphosphate (ATP) Bioluminescent Assay Kit: Perkin Elmer
    - Western Blotting supplies: BioRad
    - SLC17A9 antibody and blocking peptide: MBL (standardized in lab; also available from Sigma and SCBT)
    - Non-muscle myosin II (A,B,C) antibodies: Cell Signaling
    - Proximity Ligation Assay Kit: Duolink (Olink Bioscience)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Arun Middle Name Last Name*: Chaudhury Suffix:

Position/Title*: Research Fellow
Organization Name*: President and Fellows of Harvard College
Department: Medicine
Division: Harvard Medical School
Street1*: Brigham and Women's Hospital
Street2: Surgery
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02115-0000

Phone Number*: 857-203-6044 Fax Number: 857-203-5592 E-Mail*: arun_chaudhury@hms.harvard.edu

Credential, e.g., agency login: ACHAUDHURY

Project Role*: PD/PI Other Project Role Category:

Degree Type: M.D. Degree Year: 2004

	File Name
Attach Biographical Sketch*:	Chaudhury_Biosketch_Nov2013_C.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11534121
Funding Opportunity Number: PA-12-139 . Received Date: 2013-11-18T16:02:09.000-05:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Hemant Middle Name S. Last Name*: Thatte Suffix:

Position/Title*:	Associate Professor of Surgery
Organization Name*:	President and Fellows of Harvard College
Department:	VA West Rox
Division:	Harvard Medical School
Street1*:	VA Boston Healthcare System
Street2:	Department of Surgery (MC112)
City*:	West Roxbury
County:	
State*:	MA: Massachusetts
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	02132-0000

Phone Number*: 6173237700 Fax Number: 6173635592 E-Mail*: hemant_thatte@hms.harvard.edu
E.6202

Credential, e.g., agency login: hthatte

Project Role*: Other (Specify) Other Project Role Category: Co-Investigator

Degree Type: Ph.D. Degree Year: 1985

	File Name
Attach Biographical Sketch*:	Thatte_Biosketch_Nov2013_F.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Haiyan Middle Name Last Name*: Cao Suffix:

Position/Title*:	Instructor in Surgery
Organization Name*:	President and Fellows of Harvard College
Department:	VA West Rox
Division:	Harvard Medical School
Street1*:	Harvard Medical School
Street2:	VA Boston Healthcare System
City*:	West Roxbury
County:	
State*:	MA: Massachusetts
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	02132-4927

Phone Number*: 857-203-6254 Fax Number: 857-203-5592 E-Mail*: haiyan_cao@hms.harvard.edu

Credential, e.g., agency login: CAOHY526

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: M.D. Degree Year: 1991

	File Name
Attach Biographical Sketch*:	Cao_Biosketch_Nov2013_C_FINAL.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Xiu-Gui Middle Name Last Name*: Lu Suffix:

Position/Title*:	Instructor in Surgery
Organization Name*:	President and Fellows of Harvard College
Department:	VA West Rox
Division:	Harvard Medical School
Street1*:	Boston VA Med Ctr-W Roxbury
Street2:	Orea Bldg - R2B114
City*:	West Roxbury
County:	
State*:	MA: Massachusetts
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	02132-0000

Phone Number*: 6173237700 Fax Number: 6173558336 E-Mail*: xiu-gui_lu@hms.harvard.edu

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: M.D. Degree Year: 1970

	File Name
Attach Biographical Sketch*:	Lu_Biosketch_Nov2013.pdf
Attach Current & Pending Support:	

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Chaudhury, Arun	Instructor in Surgery
eRA COMMONS USER NAME (credential, e.g., agency login) ACHAUDHURY	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Medical College Kolkata, India	MBBS	09/00	Medicine
All India Institute of Medical Sciences, New Delhi, India	MD	06/04	Anatomy
Monell Chemical Senses Center, Philadelphia, PA	Postdoctoral	02/07	Nutritional Neurosciences
Harvard Medical School, Boston, MA	Postdoctoral	06/13	Neurogastroenterolgy

A. Personal Statement

My investigative interests are focused on pathophysiology of gastrointestinal orders and include keen translational endeavors to address refractory problems in gastroenterology and hepatology. In this application, I extend my prior experience of neuronal cell biology and neurotransmission to investigate stochasticity of cholangiocyte function during preservation in vitro prior to hepatic transplantation. I have always incorporated or introduced cutting-edge, yet simple and elegant methodologies to investigate the most pressing problems involving nutrition and digestive health and disease.

B. Positions and Honors

Positions and Employment

1999-2000 Internship, Dept. of Medicine, Surgery, OBGYN, Medical College Hospitals, Kolkata
2000-2004 Junior & Senior Resident, Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi
2005-2007 Postdoctoral Fellow in Nutritional Neurosciences, Monell Chemical Senses Center, University of Pennsylvania, Philadelphia, PA
2007-2013 Research Fellow in Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School and Center for Swallowing & Motility Disorders, VA Medical Center, Boston
2007-present Member, The Harvard Clinical and Translational Science Center (Harvard CATALYST)
2013-present Instructor, Department of Surgery, Brigham and Women's Hospital

Honors

1994 Ranked 5th in Medical Entrance Exam (West Bengal Joint Entrance Examination)
1998 Govt. of West Bengal Merit Award for Medical Class Topper (full tuition waived)
1999 Certificate of Honors, Ophthalmology, RIO, MCH, India
2003 Commonwealth Science Council Travel Award to MBL, Woods Hole, MA
2003 Department of Science and Technology (DST, Govt. of India) Travel Award to MBL, Woods Hole, MA
2003 International Brain Research Organization (IBRO) (Asia-Pacific) Travel Fellowship for Analytical and Quantitative Light Microscopy (AQLM) Course at Marine Biological Lab (MBL), Woods Hole, MA
2003 IBRO Fellowship for FENS (Federation of European Neurosciences) Summer School on Peripheral Nervous System, Ofir

2003	CSIR (Council for Scientific & Industrial Research, Govt. of India) Fellowship for Enteric Nervous System (ENS) Conference, Banff
2003	European Union Travel Fellowship, Baltic Summer School on Neurodegenerative Diseases, Kiel
2004	16th IFAA (International Federation of Association of Anatomists) Young Investigator Award, Kyoto
2004	WERC/IBRO (West European Regional Council/International Brain Research Organization) PhD fellowship (one out of only 3 candidates chosen from a global pool of applicants)
2006	American Motility Society (AMS) Young Investigator Award
2010	Extraordinary Scientific Investigator, Department of State, Govt. of USA
2011	Best Scientific Poster, 16th American Motility Society Meeting, St. Louis, MO
2013	US Permanent Residency, Extraordinary Investigator Category

C. Selected Peer-reviewed Publication and Patent Citations (Selected from 53 peer-reviewed publications)

Most relevant to the current application

1. Chauhan R, Roy TS, Chaudhury A, Shariff A. Variant human pancreas: aberrant uncinate process or an extended mesenteric process. *Pancreas*. 2003 Oct;27(3):267-9. PMID: 14508134
2. Chaudhury A, Shariff A, Srinivas M, Sabherwal U. Changes in nitrergic innervation of defunctionalized rat colon after diversion colostomy. *Neurogastroenterol Motil.* 2004 Aug;16(4):475-87. PMID: 15306003
3. Horn CC, Ciucci M, Chaudhury A. Brain Fos expression during 48 h after cisplatin treatment: neural pathways for acute and delayed visceral sickness. *Auton Neurosci*. 2007 Mar 30;132(1-2):44-51. PMID 17092780; PMC1865123.
4. Rao YM, Chaudhury A, Goyal RK. Active and inactive pools of nNOS in the nerve terminals in mouse gut: implications for nitrergic neurotransmission. *Am J Physiol Gastrointest Liver Physiol*. 2008 Mar;294(3):G627-34. PMID: 18096606; PMC2497337.
5. Goyal RK, Chaudhury A. Physiology of normal esophageal motility. *J Clin Gastroenterol*. 2008 May-Jun;42(5):610-9. PMID: 18364578; PMC2728598.
6. Chaudhury A, Rao YM, Goyal RK. PIN/LC8 is associated with cytosolic but not membrane-bound nNOS in the nitrergic varicosities of mice gut: implications for nitrergic neurotransmission. *Am J Physiol Gastrointest Liver Physiol*. 2008 Sep;295(3):G442-51. PMID: 18635601; PMC2536782.
7. Chaudhury A, He XD, Goyal RK. Role of PSD95 in membrane association and catalytic activity of nNOSalpha in nitrergic varicosities in mice gut. *Am J Physiol Gastrointest Liver Physiol*. 2009 Oct;297(4):G806-13. Erratum in: *Am J Physiol Gastrointest Liver Physiol*. 2010 Oct;299(4):G100-2. PMID: 19679819; PMC2763812.
8. Goyal RK, Chaudhury A. Mounting evidence against the role of ICC in neurotransmission to smooth muscle in the gut. *Am J Physiol Gastrointest Liver Physiol*. 2010 Jan;298(1):G10-3. PMID:19892937; PMC2806097.
9. Goyal RK, Chaudhury A. Pathogenesis of achalasia: lessons from mutant mice. *Gastroenterology*. 2010 Oct;139(4):1086-90. PMID: 20800108.
10. Chaudhury A, He XD, Goyal RK. Myosin Va plays a key role in nitrergic neurotransmission by transporting nNOSα to enteric varicosity membrane. *Am J Physiol Gastrointest Liver Physiol*. 2011 Sep;301 (3):G498-507. PMID: 21680773; PMC3174543.
11. Chaudhury A, He, XD, Goyal RK. Role of myosin Va in purinergic vesicular neurotransmission in the gut. *Am J Physiol Gastrointest Liver Physiol*. 2012 Mar;302 (6):G598-607. PMID: 22207579; PMC3311306.
12. Chaudhury A, Goyal RK. Myosin activators in gaseous neurotransmission. Patent application pending, USPTO/Harvard University, November 2012.
13. Goyal RK, Sullivan MR, Chaudhury A. Progress in understanding of inhibitory purinergic neuromuscular transmission in the gut. *Neurogastroenterology Motility*, 2013 Mar;25(3):203-7. PMID: 23414428.
14. Goyal RK, Chaudhury A. Structure activity relationship of synaptic and junctional neurotransmission. *Autonomic Neurosciences: Basic and Clinical*, 2013 Jun;176(1-2):11-31. PMID: 23535140; PMC3677731.
15. Chaudhury A. Evidence for Dual Pathway for Nitrergic Neuromuscular Transmission in Doubt: Evidence Favors Lack of Role of ICC. Gastroenterology. 2013 Nov;145(5):1160-1. PMID: 24070723.

D. Research Support

Ongoing Research Support

SomahLution (Industry) Thatte HS (PI) 09/01/2011-08/31/2014
Title: Evaluation of Somah Technology in Preservation of Abdominal Organs. Define the role of Somah Technology in its ability to preserve structure and function in livers and kidneys from beating and non-beating heart donors.
Role: Instructor

5R01 DK062867-08 Goyal (PI) 04/01/2004-03/31/2014
Nitrergic Neuro-Smooth Muscle Transmission in the Gut
The overall purpose of the proposed studies is to extend the previous studies of regulation of catalytically active nNOS in the nitrergic varicosities and determine how nNOS is transported to the membrane, attached there and regulated to produce NO. This information will help identify abnormalities in the steps that may impair nitrergic neurotransmission. These studies will help define pathophysiology of impaired nitrergic neurotransmission that have no anatomical evidence of loss or damage to nitrergic nerves.
Role: Research Fellow (through 6/30/2013)

Completed Research Support

None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Thatte, Hemant Sadashiv	Associate Professor of Surgery (Cardiothoracic)
eRA COMMONS USER NAME (credential, e.g., agency login) hthatte	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
MPKV University, Pune, India	B.Sc.	06/79	Agriculture, Genetics
University of Mysore, Mysore, India	M.Sc.	06/81	Fd. Tech., Biochemistry
University of Minnesota, Minneapolis, MN	Ph.D.	06/85	Fd. Sci., Biochemistry
Stanford University School of Medicine, Stanford, CA	Postdoctoral	06/88	Medicine
Harvard Medical School, Boston, MA	Postdoctoral	06/92	Biol Chem Molec Pharm
Harvard Medical School, Boston, MA	Postdoctoral	06/92	Medicine

A. Personal Statement

My research over greater than a decade has been translational cardiac surgery. It involved development of novel solutions for protection of the heart and surgical conduits during surgery (Gala), and recently, invention of Somah Technology for preservation and resuscitation of heart from beating and non-beating heart donors for transplant. I am a cell biologist and biochemist with strong training in biophysics and have published studies on cellular regulation of eNOS in endothelial cells; cellular effects of electric fields; anisotropy and mobility of plasma membrane components; preservation on structure and function of human surgical bypass conduits and organs, using cell biology, biochemistry, biophysics and cellular imaging techniques. I have years of experience in the designing and execution of experiments, novel or otherwise as well as supervision of personnel. Preliminary evidence shows the utility of Somah in cytoprotection of porcine DCD livers stored at 4 degrees C for 72 hrs. This may be revolutionary in expansion off high quality donor pool, a much needed necessity in the field of hepatic transplantation.

B. Positions and Honors

Positions and Employment

1992-1996 Research Associate, Biological Chemistry Molecular Pharmacology, Harvard Medical School, Boston, MA
1996-1999 Instructor, BCMP and Medicine, Harvard Medical School and Brigham and Women's Hospital
1999- Health Research Scientist, Department of Veteran's Affairs Medical Center, West Roxbury, MA
1999- Staff Scientist, Department of Surgery, Brigham and Women's Hospital, Boston, MA
2000-2006 Instructor in Surgery, Harvard Medical School
2001- Director, Cardiac Surgery Research, VA Boston Healthcare System, Boston, MA
2006-2012 Assistant Professor of Surgery, Harvard Medical School, Boston, MA
2012- Associate Professor of Surgery, Harvard Medical School, Boston, MA

Other Experience and Professional Memberships

1999-2008 Director, Multi-photon Imaging Core, Boston VA HealthCare System, West Roxbury, MA

Honors

1978-1979 National Merit Scholar
1979-1981 Merit Scholarship, M.Sc. Program, University of Mysore

1979 B.Sc., *summa cum laude*, University Gold Medals, M.P.K.V. University
1989-1991 Research Fellowship, Cooley's Anemia Foundation, New York, NY
1996-1998 National Research Service Award, NIH, Bethesda, MD.
1996 U.S. Patent # 5669396:Treatment of vaso-occlusive crisis in sickle cell disease by inhibition of adhesive interactions between sickle lymphocytes and sickle red blood cells.
2002 Lapides Award for Neurourology research- 1st place. "Localization of Hypocritin in the urinary bladder and its physiological relevance"; (with co-authors).
2003 Lapides Award for Neurourology research- 3rd place. The presence and function of Gap junctions in rat urinary bladder"; (with co-authors).
2003- Mention in Marquis International Who's Who in Healthcare and Medicine
2003 Patent # 6,569,615. Composition and Methods for Tissue Preservation
2004 *US Patent pending*. Preconditioning and physiological distension of human vessels preserved in GALA (The Tejasa Perfusion System).
2006 *US Patent 7,575,856*. Method and Composition of Resuscitating, Maintaining and Transporting of Cadaveric Human Heart.
2006 *Patent pending*: Methods for in vitro long-term preservation of human bypass conduits.
2006 *Patent pending*: Application of GALA as cardioplegia, cardioprotective and resuscitation agent.
2007 *Patent pending:* Lazarus Organ Preservation Solution: Enhancing transplant organ viability and storage time; Enabling potential organ harvesting from non-beating heart donors
2007 Award of distinction. Massachusetts Chapter of the American College Surgeons. Troponin I After Cardiac Surgery and its Implications on Myocardial Protection.
2008 *Patent pending:* Methods and Development of a Novel Cardioplegia Solution
2008 *Patent pending*: Methods and Development of a Novel Volume Replacement, Pump Prime and Resuscitation Solution

C. Selected Peer-reviewed Publications (Selected from 53 peer-reviewed publications)

Most relevant to the current application

1. Goetz RM, Thatte HS, Prabhakar P, Cho MR, Michel T, Golan DE. Estradiol induces the calcium-dependent translocation of endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. USA.* 1999; 96:2788-2793. PMID: 10077589; PMC15847.
2. Igarashi J, Thatte HS, Prabhakar P, Golan DE, Michel T. Calcium-independent activation of endothelial nitric oxide synthase by ceramide. *Proc. Natl. Acad. Sci. USA.* 1999; 96:12583-12588. PMID: 10535965; PMC22998.
3. Thatte HS, Biswas KS, Najjar SF, Rhee JH, Birjiniuk V, Crittenden MD, Michel T, Khuri SF. Multi-photon microscopy in the evaluation of human saphenous vein. J Surg Res, 95:37-43, 2001. PMID: 11120633
4. Thatte HS, Biswas KS, Najjar SF, Rhee JH, Birjiniuk V, Crittenden MD, Michel T, Khuri SF. Multi-photon microscopy in the development of a new solution, GALA, for vessel preservation. Ann Thorac Surg 2003; 75:1145-1152. PMID: 12683553
5. Thatte HS, Rhee JH, Zagarins S, Treanor PR, Birjiniuk V, Crittenden MD Khuri SF. Acidosis induced apoptosis in human and porcine heart. Ann Thorac Sur 2004; 77:1376-1383. PMID: 15063270
6. Thatte HS, Dygert JH, Kumbhani DJ, Najjar SF, Treanor PR, Khuri SF. Intracoronary shunt induced endothelial cell damage in porcine heart. J Surg Res 2006, 131:168-174. PMID: 16412472
7. Kumbhani DJ, Healey NA , Thatte HS, Nawas S, Birjinuik V, Crittenden MD, Treanor PR, Khuri SF. Diabetic patients undergoing cardiac surgery are at greater risk for developing intraoperative myocardial acidosis. J Thorac Cardiovasc Surg, 2007;133:1566-1572. PMID: 17532958
8. Rousou L, Taylor K, Lu XG, Crittenden M, Haime M, Khuri SF, Thatte HS. Saphenous Vein Conduits harvested by Endoscopic Technique Exhibit Structural and Functional Damage. Ann Thorac Surg 2009; 87:62-70. PMID: 19101270
9. Hussaini BE, Treanor P, Healey N, Tilahun D, Srey R, Pochay V, Lu XU, Khuri SF, Thatte HS. Evaluation Of Blood Components Exposed To Coated Arterial Filters In Extracorporeal Circuits. Perfussion 2009; 24:317-323. PMID: 19965951
10. Thatte HS, Rousou L, Hussaini BE, Lu XG, Treanor PR, Khuri SF. Development and Evaluation of a Novel solution, Somah, for the Procurement and Preservation of Beating and Non-beating Donor Hearts for Transplantation. Circulation 2009; 120:1704-1713. PMID: 19822811

11. Hussaini BE, Treanor P, Healey N, Tilahun D, Srey R, Pochay V, Lu XU, Khuri SF, Thatte HS. Evaluation Of Blood Components Exposed To Coated Arterial Filters In Extracorporeal Circuits. Perfusion 2009; 24:317-323. PMID: 19965951
12. Ikuta T, Thatte HS, Tang JX, Mukerji I, Knee K, Bridges KR, Head CA. Nitric oxide reduces sickle hemoglobin polymerization: Potential role of nitric oxide-induced charge alteration in depolymerization. Arch Biochem Biophys, 2011; 510-53-61. PMID: 20421111
13. Hussaini BE, Treanor P, Healey N, Lu XG, Khuri SF, Thatte HS. Multifactorial comparison of modified and conventional perfusion strategies in a porcine model of Cardiopulmonary Bypass. J Surg Res 2011; 168:7-15.
14. Hussaini BE, Lu XG, Wolfe JA, Thatte HS. Evaluation of VirtuoSaph endoscopic vein harvesting technique on structural and functional viability of saphenous vein endothelium. J Cardiothorac Surg 2011; 6:82. PMCID: PMC3125322
15. Lowalekar SK, Lu XG, Thatte HS. Further Evaluation of Somah: Long-Term Preservation, Temperature Effect and Prevention of Ischemia-Reperfusion Injury in Rat Hearts Harvested After Cardiocirculatory Death. Transpl Proceed 2013;45:3192-3197. PMID: 24182783

D. Research Support

Ongoing Research Support

DoD/ONR N00014-09-1-0028 Thatte HS (PI) 10/01/09-09/30/12
Title: Molecular mechanisms of 94 GHz microwave radiation mediated cellular response. The effects of microwaves at the cellular level are not known. However, we do know that microwave radiation increases the body temperature and cause lesions in the cornea. Other than the well-defined (heating) effect of microwaves on water molecules, such radiation must induce a cellular response, at the non-lethal level, even if it is mediated by microwave-induced oscillation of water molecules. Therefore the goal is to demonstrate the coupling of microwaves and elucidation thereof, of a cellular response, in cultured stem cell differentiated neurons and/or epithelial cells, using unexposed cells as controls for comparison.
Role: PI

VA Merit Review Thatte HS (PI) 10/01/2010-09/30/14
Title: Functional Evaluation of Somah Technology on Heart Transplant. Define the role of Somah Technology in preservation and resuscitation of heart from beating and non-beating heart donors for transplant.

Role: PI

SomahLution (Industry) Thatte HS (PI) 09/01/2011-08/31/2014
Title: Evaluation of Somah Technology in Preservation of Abdominal Organs. Define the role of Somah Technology in its ability to preserve structure and function in livers and kidneys from beating and non-beating heart donors.
Role: PI

Completed Research Support

Terumo (industry) Thatte HS (PI) 01/01/10-06/30/10 Title: Evaluation of VirtuoSaph Endoscopic Saphenous Vein Harvesting System.
We evaluated the ability of this technique to preserve structural and functional viability of human saphenous vein during EVH using imaging and biochemical techniques.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Cao, Haiyan	Instructor in Surgery
eRA COMMONS USER NAME (credential, e.g., agency login) CAOHY526	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Shanghai Medical University, Shanghai, China	MD	07/91	Medicine
Peking University Health Science Center, P.R. China	Ph.D.	07/03	Child Neurology

A. Personal Statement

I have developed a novel method for antibody-mediated targeted gene transfer, a general strategy to target gene transfer to specific classes or subclasses of neurons. I developed a chimeric gC--Staphylococcus A ZZ domain protein, which binds to antibodies, and performed helper virus-free HSV-1 (Herpes Simplex Virus 1) vector packaging. I expect to extend myself into translational medicine. The field of organ transplantation amalgamates well with my intentions because currently there is a great shortage of organs for transplant and numerous attempts are being made to broaden the donor criteria by including marginal donors, some of which have a history of infections such as hepatitis, HSV, etc. I expect to device ground-breaking ways to allow the use of marginal donors for organ transplantation by attenuating the infection within the organs prior to transplantation. One of the ways to achieve this would be by extending the time of *ex vivo* preservation of organs while maintaining their vitality. This will provide the window of opportunity to process the organs *ex vivo* and devise ways to reduce the possibility of the transfer of donor's infection to the recipient.

B. Positions and Honors

Positions and Employment

07/1991-07/1994 Resident, Pediatrics, Shanxi Children's Hospital, Taiyuan, Shanxi, China
07/1994-07/1997 Resident, ICU, Shanxi Children's Hospital, Taiyuan, Shanxi, China
07/1997-08/1998 Chief Resident, Pediatrics, Shanxi Children's Hospital, Taiyuan, China
07/2003-12/2004 Attending Doctor, Child Neurology, Shanxi Children's Hospital, Taiyuan, Shanxi, China
12/2004-09/2011 Research Fellow, Neurology, Harvard Medical School/VA Boston Healthcare System, Boston, MA
12/2011-Present Instructor, Department of Surgery, Harvard Medical School, VA Boston Healthcare System, Boston, MA

Honors and Prizes

1990 Outstanding Student Award, Shanxi Medical University, Taiyuan, Shanxi, China
1991 Outstanding Graduate Award, Shanxi Medical University, Taiyuan, Shanxi, China
1994 Outstanding Resident Award, Shanxi Children's Hospital, Taiyuan, Shanxi, China
1996 Outstanding Resident Award, Shanxi Children's Hospital, Taiyuan, Shanxi, China
1997 Outstanding Resident Award, Shanxi Children's Hospital, Taiyuan, Shanxi, China

Professional Societies

2005-Present Member, Society for Neuroscience

2005-2007 Member, American Association for the Advancement of Science, (AAAS)

C. Selected Peer-reviewed Publication and Patent Citations

Most relevant to the current application

1. **Cao, H.**, Zhang, G., and Geller, A.I. (2010) Antibody-mediated targeted gene transfer to NMDA NR1-containing neurons in rat neocortex by helper virus-free HSV-1 vector particles containing a chimeric HSV-1 glycoprotein C--Staphylococcus A protein. Brain Research 1351, 1-12. PMID: 20599821: PMC2929402.
2. Zhang, G., Zhao H., **Cao, H.**, and Alfred I. Geller (2012) Overexpression of either lysine-specific demethylase-1 or CLOCK, but not Co-Rest, improves long-term expression from a modified neurofilament promoter, in a helper virus-free HSV-1 vector system. Brain Res. In Press. Brain Res. 1436:157-67.
3. **Cao, H.**, Zhang, G., and Geller, A.I. (2011) Antibody-mediated targeted gene transfer of helper virus-free HSV-1 vectors to rat neocortical neurons that contain either NMDA receptor 2B or 2A subunits. Brain Res. 1415:127-35.
4. Zhang, G., Li, X., **Cao, H**., Zhao H., and Geller, A.I. (2011) The vesicular glutamate transporter-1 upstream promoter and first intron each support glutamatergic-specific expression in rat postrhinal cortex. Brain Res. 1377:1-12.
5. Zhang, G., **Cao, H**., Kong, L., O'Brien, J., Baughns, A., Jan, M., Zhao, H., Wang, X., Lu, X., Cook, R.G., and Geller, A.I. (2010) Identified circuit in rat postrhinal cortex encodes essential information for performing specific visual shape discriminations. Proc. Natl. Acad. Sci. USA, 107, 14478–14483.
6. Zhang, G., **Cao, H**., Li, X., Zhao, H., and Geller, A.I. (2010) Genetic labeling of both the axons of transduced, glutamatergic neurons in rat postrhinal cortex and their postsynaptic neurons in other neocortical areas by Herpes Simplex Virus vectors that coexpress an axon-targeted ß-galactosidase and wheat germ agglutinin from a vesicular glutamate transporter-1 promoter. Brain Research, 1361, 1-11
7. Zhang G, Liu M, **Cao H**, Kong L, Wang X, Cook RG, Geller AI (2009) Improved spatial learning in aged rats by genetic activation of protein kinase C in small groups of rat hippocampal neurons. Hippocampus 2009; 19, 413-423.
8. **Cao, H**., Kong, L., Wang, X., Liu, M., Zhang, G., and Geller, A.I. (2008) Enhanced nigrostriatal neuron-specific, long-term expression by using neural-specific promoters in combination with targeted gene transfer by modified helper virus-free HSV-1 vector particles. BMC Neuroscience 9, 37.
9. Bo, T., Jiang, Y.W., **Cao, H.Y**., Wang, J.M., and Wu, X.R. (2004) Long-term effects of seizures in neonatal rats on spatial learning ability and NMDA receptor expression in the brain. Developmental Brain Research 152, 137-142.
10. **Cao, H.Y**., Jiang, Y.W., Liu, Z.W., and Wu, X.R. (2003) The effect of recurrent epileptiform discharges induced by magnesium-free treatment on developing cortical neuron in vitro. Developmental Brain Research 142, 1-6.

D. Research Support

Ongoing Research Support

VA Merit Review **Thatte HS (PI)** **10/01/2010-09/30/14**
Title: Functional Evaluation of Somah Technology on Heart Transplant. Define the role of Somah Technology in preservation and resuscitation of heart from beating and non-beating heart donors for transplant.
Role: Instructor

Completed Research Support

None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Lu, Xiu-Gui	POSITION TITLE Instructor in Surgery
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Shanghai Medical University, Shanghai, China	MD	06/70	Medicine

A. Personal Statement

The scientific research is my career. I have studied several organs including liver, heart, bladder and brain for more than thirty years. My expertise is related to RNA, DNA and protein investigations dealing with the techniques in biochemistry, immunology and molecular biology. I am a cell biologist and have studied Smooth and skeletal muscle cells, epithelial cells and endothelial cells and tissue engineering. Since 2005 my research has been translational in cardiac surgery; I have participated in the invention of Somah Technology for preservation and resuscitation of heart from beating and non-beating heart donors for transplant. I am involved in different projects as a manager, run experiments day to day basis, analyze data and interpret results, and help write manuscripts.

B. Positions and Honors

Positions and Employment

1976-1986 Research Associate, Institute of Basic Medical Sciences, China
1986-1993 Research Assistant Professor, Institute of Basic Medical Sciences, China
1994-2005 Research Fellow Harvard Medical School, Boston, MA
2001- Research Health Scientist, VA Boston Healthcare System, MA
2006- Instructor in Surgery, Harvard Medical School, Boston, MA

Honors

Received these awards from the Ministry of Health of the P.R.C.

1984 Scientific and technological award for study of chemiluminscence ABEI,
1986 Scientific and technological award for study of Anti-HBc antibody
1991 Scientific and technological award for study of high speed ELISA detection anti- HBc kit.
2007 *Patent pending:* Lazarus Organ Preservation Solution: Enhancing transplant organ viability and storage time; Enabling potential organ harvesting from non-beating heart donors

C. Selected Peer-reviewed Publications

Most relevant to the current application

1. Rousou L, Taylor K, Lu XG, Crittenden M, Haime M, Khuri SF, Thatte HS. Saphenous Vein Conduits harvested by Endoscopic Technique Exhibit Structural and Functional Damage. Ann Thorac Surg 2009; 87:62-70. PMID: 19101270
2. Hussaini BE, Treanor P, Healey N, Tilahun D, Srey R, Pochay V, Lu XG, Khuri SF, Thatte HS. Evaluation Of Blood Components Exposed To Coated Arterial Filters In Extracorporeal Circuits. Perfussion 2009; 24:317-323. PMID: 19965951

3. Thatte HS, Rousou L, Hussaini BE, Lu XG, Treanor PR, Khuri SF. Development and Evaluation of a Novel solution, Somah, for the Procurement and Preservation of Beating and Non-beating Donor Hearts for Transplantation. Circulation 2009; 120:1704-1713. PMID: 19822811
4. Hussaini BE, Treanor P, Healey N, Tilahun D, Srey R, Pochay V, Lu XG, Khuri SF, Thatte HS. Evaluation Of Blood Components Exposed To Coated Arterial Filters In Extracorporeal Circuits. Perfusion 2009; 24:317-323. PMID: 19965951
5. Hussaini BE, Treanor P, Healey N, Lu XG, Khuri SF, Thatte HS. Multifactorial comparison of modified and conventional perfusion strategies in a porcine model of Cardiopulmonary Bypass. J Surg Res 2011; 168:7-15.
6. Hussaini BE, Lu XG, Wolfe JA, Thatte HS. Evaluation of VirtuoSaph endoscopic vein harvesting technique on structural and functional viability of saphenous vein endothelium. J Cardiothorac Surg 2011; 6:82. PMCID: PMC3125322
7. Lowalekar SK, Lu XG, Thatte HS. Further Evaluation of Somah: Long-Term Preservation, Temperature Effect and Prevention of Ischemia-Reperfusion Injury in Rat Hearts Harvested After Cardiocirculatory Death. Transpl Proceed 2013;45:3192-3197. PMID: 24182783

D. Research Support

Ongoing Research Support

VA Merit Review Thatte HS (PI) 10/01/2010-09/30/14

Title: Functional Evaluation of Somah Technology on Heart Transplant. Define the role of Somah Technology in preservation and resuscitation of heart from beating and non-beating heart donors for transplant.

Role: Instructor

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
First Name*: Arun
Middle Name:
Last Name*: Chaudhury
Suffix:

2. Human Subjects

Clinical Trial? ❍ No ❍ Yes
Agency-Defined Phase III Clinical Trial?* ❍ No ❍ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ❍ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

Funding Opportunity Number: PA-12-139. Received Date: 2013-11-18T16:02:09.000-05:00

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Modular Budget

OMB Number: 0925-0001

Budget Period: 1

Start Date: 07/01/2014 **End Date:** 06/30/2015

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	150,000.00
Consortium F&A	
Total Direct Costs*	**150,000.00**

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	MTDC Off Campus	28.70	150,000.00	43,050.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS , Michael Leonard , 212.264.2069

Indirect Cost Rate Agreement Date: 05/31/2013

Total Indirect Costs **43,050.00**

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) **193,050.00**

Funding Opportunity Number: PA-12-139. Received Date: 2013-11-18T16:02:09.000-05:00

PHS 398 Modular Budget

Budget Period: 2

Start Date: 07/01/2015 **End Date:** 06/30/2016

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	125,000.00
Consortium F&A	
Total Direct Costs*	**125,000.00**

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	MTDC Off Campus	28.70	125,000.00	35,875.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS , Michael Leonard , 212.264.2069

Indirect Cost Rate Agreement Date: 05/31/2013

Total Indirect Costs **35,875.00**

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) **160,875.00**

Funding Opportunity Number: PA-12-139. Received Date: 2013-11-18T16:02:09.000-05:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium F&A for Entire Project Period ($)	275,000.00
Section A, Total Consortium F&A for Entire Project Period ($)	
Section A, Total Direct Costs for Entire Project Period ($)	275,000.00
Section B, Total Indirect Costs for Entire Project Period ($)	78,925.00
Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period ($)	353,925.00

2. Budget Justifications

Personnel Justification	Chaudhury_R21_Personnel_justification.pdf
Consortium Justification	
Additional Narrative Justification	Chaudhury_R21_Additional_Narrative.pdf

Funding Opportunity Number: PA-12-139. Received Date: 2013-11-18T16:02:09.000-05:00

Personnel Justification

Personnel

Dr. Arun Chaudhury, M.D., Principal Investigator, (7.2 calendar months) will supervise the overall progress of the projects, conduct all experiments and endeavor to complete the proposed specific aims. Dr. Chaudhury is an expert neuroanatomist/cell biologist and has extensive training in animal microsurgery, nutritional neurosciences and extracellular single-fiber vagal recordings. Dr. Chaudhury has a Without Compensation appointment at VA Boston Healthcare System.

Dr. Hemant Thatte, M.D., Co-Investigator, (.60 calendar months) will supervise all aspects of the study and provide consultation as needed at any level. Dr. Thatte has a joint appointment with Harvard Medical School and the VA Boston Healthcare System. The effort requested represents Dr. Thatte's total effort dedicated to this project. Salary requested is the university's share of the investigator's effort.

Dr. Haiyan Cao, M.D., Other Significant Contributor will provide support as needed for the duration of the project. No compensation requested.

Dr. Xiu-Gui Lu, M.D., Other Significant Contributor will provide support as needed for the duration of the project. No compensation requested.

TBN, Research Technician (12 calendar months) will be mentored and instructed by Dr. Chaudhury on all aspects of the study.

Additional Budget Justification

The budget is restricted to no more than $275,000 in direct costs over a two year period. Because the budget is modular and must be requested in increments of $25,000, we chose to request $150,000 in year one and $125,000 in year two.

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Chaudhury_R21_Specific_Aims.pdf
3. Research Strategy*	RESEARCH_STRATEGY_AC_F.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	Chaudhury_R21_Vertebrate_Animals.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	

Background Liver transplantation is state-of-the-art therapy aimed at ameliorating both acute and chronic liver failure (Calne 2012; Starzl 2012). However, graft dysfunction remains a major adversity post-transplant. Primary non-function, as well as delayed dysfunction of liver graft is mainly caused by ischemia-induced injury (Li and Crawford 2004; Birrer et al 2007). Reports indicate nearly intact morphological appearance of parenchymal cells (hepatocytes) even after extended cold preservation (Kukan and Haddad 2001). Non-parenchymal cellular damage may be the major cause of preservation-related graft injury. A major feature of post-transplant complication is related to biliary dysfunction (Vajdova et al 2000; Hubscher 2009; Beldi et al 2008). Disorganized biliary ductules and biliary casts obtunding the ductular microlumina result in graft dysfunction (Voigtlander et al 2013; Li and Crawford 2004; Brunner et al 2013). Biochemically, extensive evidence has been provided regarding the role of high-energy phosphates (HEPs) in cytoprotection during in vitro storage of livers (Lund et al 1975; Vajdova et al 2002; Schlegel et al 2013). Traditionally, livers are stored in University of Wisconsin (UW) solution. An improvised organ storage solution, "Somah", with rationally designed components to maintain perfusion and high energy phosphates have been recently demonstrated to provide superior status of solid organ storage in vitro (Thatte et al 2009; Lowalekar et al 2013). Our preliminary data show that biliary ductular reactive changes, detected in livers stored in UW solution as early as 6 hrs, could not be visualized in livers cold-stored in Somah for even 72 hrs. **The current proposal aims to examine the pathophysiological basis for biliary dysfunction of livers stored in vitro and explore rational basis for biologically preconditioning storage solutions to prevent cholangiocyte cellular injury.**

It has been demonstrated that SLC17A9 positive vesicle-secreted ATP by the biliary epithelial cells is a major constituent of bile (Sathe et al 2011; Gatof et al 2004). ATP in bile plays a critical role in determining biliary composition and fluidity during bile drainage (Chari et al 1996; Woo et al 2010; Fausther et al 2012). Biliary ATP may enhance absorption of hydrophobic bile salts (Li and Crawford 2004) and stimulate chloride secretion that dilutes bile (Feranchak and Fitz 2002). When biliary ATP is deficient, persistent presence of hydrophobic bile salts may damage biliary epithelium due to detergent action. This results in mucosal ulceration, creation of biliary sludge and subsequent blocking of ductular lumen (Hoekstra et al 2006; Monbaliu et al 2009). We hypothesize that restoration of intracellular high energy phosphates by the novel organ storage solution, “Somah”, prevents biliary damage. In vitro storage of livers in ischemic conditions damages actin within cholangiocytes (Sergi et al 2012). We plan to examine whether cytoskeletal injury disrupts SLC17A9 vesicular trafficking.

Specific Aim 1 To examine whether extracellular ATP secretion is impaired and SLC17A9 containing vesicles is decreased in biliary epithelium during in vitro liver storage The reduction in ATP transporter SLC17A9 or its function may be the fundamental pathology that contribute to diminished extracellular release of ATP in the bile and the cyclical biochemical events that follow to cause biliary stasis and structural damage to bile ductules.

Specific Aim 2 To examine whether non-muscle myosin II (NMMII) isoforms are diminished in biliary epithelium during in vitro liver storage NMMII have been reported to be present in biliary epithelial cells (Puchtler et al 1975; O’Hara et al 2010). NMMII may transport subcortically located vesicles along actin tracks just prior to their release. Loss or reduction of function of NMMII may be responsible for inability of terminal secretion of SLC17A9-vesicles by cholangiocytes. Preconditioning storage media using recently available pharmacologic activators of myosin II like omecamtiv mecarbil (Leinwand and Moss 2011) may contribute to prevention of biliary epithelial damage during in vitro storage of livers. Anticipated outcome is that livers stored in Somah will have intact functional SLC17A9 positive vesicles that can traffic to cholangiocyte membrane post-stimulation for release of contents.

Significance This proposal aims to systematically identify pathophysiology of cellular changes of intrahepatic biliary ductules that occurs during in vitro storage of liver. It is being increasingly recognized that bile ductular damage is one of major reasons for delayed dysfunction after orthotopic liver transplantation. If the proposed aims are achieved, rationale biological modulation of storage solutions will usher in paradigm shift of harvesting and storing DCD (donation after cardiac death) livers, which in turn will address the critical issues of shortage of organ supply and provide high quality livers for transplantation. Our preliminary evidence support the proposed hypotheses.

Despite advances in transplant surgery, post-operative outcomes after solid organ transplantations have shown only marginal improvement (Cecka 2003). This is related to inclusion of older, marginal and non-beating heart organ sources in the donor pool, which are being increasingly adapted because of continued organ shortage (Maathuis et. al., 2004). Utilization of organs from marginal donors has imperatively resulted in decreased graft function and survival, when compared to organs obtained and used for transplant from younger donors in previous years (Alexander et. al., 1996). Maintaining the organ viability during extracorporeal storage prior to transplantation is an important prerequisite for successful post-transplant long-term outcomes, which is dependent on the temporal explant storage conditions.

Liver transplantation, despite the procedural morbidity, is a treatment of choice for end stage organ failure, which otherwise culminate in fatal outcomes (Calne 2012). Hence the clinical indications for transplantation have increased, while supply of quality organs continues as a challenge. Optimal storage conditions for DCD livers may revolutionize high quality organ supply. Requirement for improved organ preservation has been identified since the earliest days of liver transplantation for favorable long-term outcomes (Starzl 2012). In most transplant centers, livers are cold flushed and cold static stored in either extracellular or intracellular type storage solutions for preferably less than 12 hours prior to transplantation. In vitro damage is thought to occur because of initiation of multifactorial degenerative cascade because of progressive loss in high energy phosphates (HEP) due to hypoxia, which becomes self-limiting with the production of lactate and protons as a result of decoupling of aerobic from anaerobic metabolism (Taylor and Baicu 2010, Lund et al 1975).

Recently, it has been demonstrated that a newly formulated organ storage solution, “Somah”, counterbalanced many of the impairing factors encountered by the porcine and rat solid organs like hearts and kidneys during prolonged cold static storage and at higher temperatures (Thatte et al 2009; Lowalekar et al 2013; Lowalekar et al 2013). Somah provided optimum cell protection by maintenance of high-energy phosphate levels, and preserved structure and function of cardiac myocytes and endothelium in hearts and kidneys obtained from BHD and DCD donors during short-term hypothermic storage as well as long-term storage (Thatte et al 2009; Lowalekar et al 2013; Lowalekar et al 2013).

There are only scant reports that have systemically reported histopathologic findings of biliary injury during in vitro storage of livers (Karimian et al 2013, Yanaga et al 1990, Shin et al 2013, Brunner et al 2013). Biliary damage causes major morbidity post-transplantation (Kochhar et al 2013; Calne 1977). Bile ductules are known to have heterogeneous dimensions (Han et al 2012). Our preliminary studies using 14 female pig livers revealed that bile ducts and ductules of different diameters were disorganized when the DCD livers were stored in UWS, but not in Somah during hypothermic (4° C) storage for 72 hours. Livers stored in UW solution showed: (i) Blocked ductular lumina by epithelial sludge (ii) Mucosal ulcerations (iii) Ductular nuclear proliferations with cellular arrangements in multiple directions (iv) Mononuclear cellular infiltration at the base of these ductules, as well as in the portal area. These changes in the biliary structures have been earlier reported to be the cause of graft dysfunction post-transplant,

including contributing to non-anastomotic biliary stricture and biliary dysfunction (Karimian et al 2013).

Remarkably, our preliminary results show that Somah is cytoprotective for the cholangiocytes, and morphological changes seen in biliary ductules in livers stored in UWS were not seen when the livers are stored under identical conditions in Somah (**Figures 1 and 2**). We hypothesized that the provision of high energy phosphates may be responsible for cytoprotective action of Somah in comparison to UWS (**Figure 3**). Livers stored in Somah in the cold for 72 hrs did not show any evidence of biliary ductular injury (**Figure 4**). Furthermore, these livers synthesized albumin when reperfused for 2 hrs post-storage (**Figure 5**).

In order to investigate the pathophysiology underlying biliary ductular damage during liver preservation in vitro, we plan to examine the function of biliary epithelial cells to secrete ATP during in vitro storage.

Our hypotheses is based on the following precedent evidentiary: (a) Solute carrier protein, SLC17A9, a vesicular nucleotide transporter (VNUT) which acts as ATP transporter (Sawada et al 2008), is responsible for extracellular ATP secretion into bile by biliary epithelial cells (Sathe et al 2011; Villanger et al 1993) (b) cycling of SLC17A9 vesicles depends on cytoskeletal integrity (Sathe et al 2011; Veel et al 1990) (c) actomyosin, especially non-muscle myosin II, in the cell periphery is responsible for final steps of vesicular secretion (Erdmann et al 2013; Wang et al 2011; Bond et al 2011; Even-Ram et al 2007; Togo and Steinhardt 2004; Cai et al 2010) and (d) non-muscle myosin II isoforms are present in biliary epithelial cells (O'Hara et al 2010). As suggested earlier (Woo et al 2010), lack of ATP in bile may result in change in composition of bile, which becomes prone to cholestasis.

Microfilament actin has been demonstrated to be functionally deficient in cholangiocytes during in vitro storage (Sergi et al 2012). It is well-known that myosins interact with actin to coordinate vesicular release in subcortical cytoskeleton of epithelial cells. We hypothesize that in vitro storage conditions causes down regulation of non-muscle myosin II isoforms in biliary epithelia, which results in inability of SLC17A9-vesicle mediated extracellular ATP secretion by biliary epithelial cells. Lack of ATP secretion in bile may be the major change that elicits biliary epithelial damage, mucosal sloughing and luminal obstruction, the backpressure further stimulating reactive ductular proliferation and portal neutrophilic infiltration. Preconditioning of storage media by pharmacologic modulator of myosin II may be a critical component in prevention of biliary epithelial damage, which may further impact postgraft non-dysfunction.

Approach

Central rationale We hypothesize that restoration of intracellular high energy phosphates by the novel organ storage solution, “Somah”, prevents biliary damage by supplying the substrate ATP for effective actomyosin action (myosin II is an ATPase) and vesicular content, i.e., ATP, of SLC17A9 microvesicles (SLC17A9 is a VNUT, a vesicular nucleotide transporter).

Specific Aim 1 To examine whether extracellular ATP secretion is impaired and SLC17A9 containing vesicles is decreased in biliary epithelium during in vitro liver storage

Rationale ATP in bile is a potent secretagogue, stimulating biliary epithelial cell (BEC) secretion. ATP stimulated chloride and water secretion decreases bile viscosity and promotes biliary flow (Woo et al 2010; Marinelli 1999). In vitro storage diminishes cellular HEP stores. The reduction in ATP transporter SLC17A9 or its function may be the fundamental pathology that contribute to diminished extracellular release of ATP in the bile. Failure of ATP release may lead to bile inspissation and obstruction; it may also lead to mucosal damage by bile salts. Because

of ability of Somah to restore cellular HEP stores, we hypothesize that this contributes to extended storage of livers in vitro in Somah by maintaining function of SLC17A9 in BECs.

Proposed experiments *Materials (same for specific aims 1&2)* Porcine and rat livers stored in University of Wisconsin and Somah solutions will be used for experiments. Storage conditions in vitro will be varied; cold storage and subnormothermic storage at 21 degrees C for 6 hrs, 24 hrs and 72 hrs will be examined. Comparisons will be made with freshly harvested liver tissues.

To examine SCL17A9 vesicular exocytosis in biliary mucosa Liver sections will be visualized by live cell confocal microscopy to examine vesicular ATP release. Namely, vibratome sections, obtained from both periportal area as well as from deep within the liver, will be loaded with quinacrine, which is known to enrich in ATP containing vesicles (Sathe et al 2011). Quinacrine exocytosis in real time will be examined by stimulating sections with secretin, as secretin is known to enhance flow in biliary ductules (Marinelli et al 1999). Control experiments will be performed by preincubation with somatostatin which is known to inhibit secretin action (Tietz et al 1995). Exocytosis will be examined in biliary epithelial cells forming the ductules in portal areas. Quinacrine and SLC17A9 double labeling will help obtain evidence whether the quinacrine was concentrated in the ATP containing vesicles. Finally, bulk ATP release from sections will be examined by luciferin-luciferase assay and comparisons made between livers stored in UWS and Somah. Western blots and ELISA assays will be performed to examine whether there are changes in SLC17A9 expression during acute in vitro storage. Hepatic sections will be incubated in vitro using V-type H^+-ATPase inhibitor bafilomycin and anti-SLC17A9 antibody respectively. Sections will be examined at the end of 72 hrs whether in vitro pharmacologic inhibition of SLC17A9 produces biliary cast, luminal obstruction or mucosal sloughing or ductular wall reactive hyperplasia, i.e., simulates histopathologic changes of prolonged in vitro storage in UWS.

Possible outcomes Cholangiocyte SLC17A9 pump failure shall be demonstrable in livers stored ex vivo in UWS but may not occur in tissues stored in Somah.

Anticipated problems and alternatives The proposed experiments will examine efficiency of vesicular ATP release. No major problems are anticipated. The team are well accustomed to examine exocytosis mechanisms by optical means and biochemical assays (e.g., Chaudhury et al 2012; Chaudhury and Goyal 2012; Thatte et al 2009). SLC17A9 antibodies are well-characterized, commercially available including blocking peptides (Chaudhury et al 2012). ELISA kits for SLC17A9 are also commercially available. Team is well-trained in veterinary care of pigs and cutting edge expertise in large animal surgery and in vitro perfusion techniques (e.g., Thatte et al 2009).

Specific Aim 2 To examine whether non-muscle myosin II (NMMII) isoforms are diminished in biliary epithelium during in vitro liver storage

Background In vitro liver storage has been reported to cause damage to cellular actin (Sergi et al 2012). It has also been proposed that cytoskeletal elements may be involved in intracellular transport of SLC17A9 vesicles (Sathe et al 2011). We hypothesized that in vitro storage of liver may cause diminution in expression or function of non-muscle myosin II. NMMII interacts with actin in terminal subcortical zone and facilitates vesicle exocytosis (Bond et al 2011). NMMII has been reported to be present in BECs (O'Hara et al 2010). We plan to examine whether SLC17A9 and NMMII physically interact in the cholangiocytes, and whether this interaction is affected during prolonged in vitro storage. Finally we will examine whether newly reported myosin motor activators (Malik et al 2011; Chaudhury and Goyal 2012) can prevent biliary epithelial damage.

Proposed experiments

***To examine role of NMMII in SCL17A9 vesicular exocytosis* in biliary epithelial cells**
Staining for NMMII will be performed and its localization examined in the cholangiocytes. Proximity ligation assay (PLA), as described earlier (Chaudhury et al 2011; Chaudhury et al 2012), will be used to test NMMII-SLC17A9 interaction. Functional assay of NMMII will be performed to examine functionality. Secretin stimulated sections will be examined for phosphorylated regulatory light chains of myosin II, which are known to be associated with active myosin II (Bond et al 2011). Phospho-NMMII and SLC17A9 interactions will be examined by PLA. Liver sections will be incubated in vitro using blebbistatin and ML-9 that inhibit myosin II and myosin light chain kinase (the kinase responsible for activating myosin II) respectively. Liver sections will be examined at the end of 72 hrs whether in vitro pharmacologic blockade of myosin II produces biliary epithelial sloughing or provokes biliary proliferative reaction. Finally, the recently reported myosin II activator omecamtiv mecarbil will be added to the storage solutions (UWS and Somah) and sections examined at end of 72 hrs for histological evidence of biliary epithelial damage. We hypothesize that omecamtiv will be further protective for biliary epithelium, in addition to extracellularly supplemented high energy phosphates.

Possible outcomes Functionality of NMMII in SLC17A9 vesicular trafficking may be affected in livers stored in UWS, but not organs stored in Somah for extended periods of time ex vivo.

Anticipated problems and alternatives Omecamtiv is a cardiac myosin II activator, but all myosin II (non-muscle and muscle) possess greater than 70% molecular homology and based upon this similarity, this high risk experiment is proposed. If it does produce a difference in protecting biliary epithelium, it will produce a paradigm shift in standards of storage media for orthotopic liver transplantation.

Innovation and translational significance of the proposed investigation This is the first endeavor to systematically examine pathophysiology of acute bile duct injury during in vitro storage. It is likely that availability of high energy phosphate in Somah prevented bile duct injury. While ATP is synthesized in cytoplasm in membrane bound organelles, there are numerous mechanisms that facilitate release of ATP in the extracellular space (Lazarowski et al 2011). Increasingly it is being identified that much like within the nerve terminals (Chaudhury et al 2012; Goyal and Chaudhury 2013), vesicle mediated compartmentalization and secretion is an essential component of epithelial physiology (Townley et al 2012; Sathe et al 2011; Cho and Broder 1993; Cameron et al 1993; Buanes et al 1988; Jung et al 2004; Benedetti et al 1993). Recent evidence also provide intriguing insight of cytoskeletal elements and force generating protein within epithelial cells, simulating event of vesicular neurotransmission (Ku et al 1999). Given the paucity of donors, as well as high quality organs, these investigations focused on optimization of storage mechanisms shall be at the forefront of translational significance in the field of organ transplantation. Though some of the proposed experiments such as the utility of myosin activators in preventing bile duct damage may be interpreted as high risk, supportive preliminary evidence can usher in new standards of liver storage. This proposal shall provide groundwork for future exploration of feasibility of storage of human DCD livers.

Timeline

During Year 1, endeavors shall involve wet bench experiments with focus on accomplishing all proposed experiments in specific aims 1 and 2. Year 2 will be focused on analyzing data, performing additional experiments if needed and dissemination of new knowledge by conference presentation and research publications.

Preliminary Data

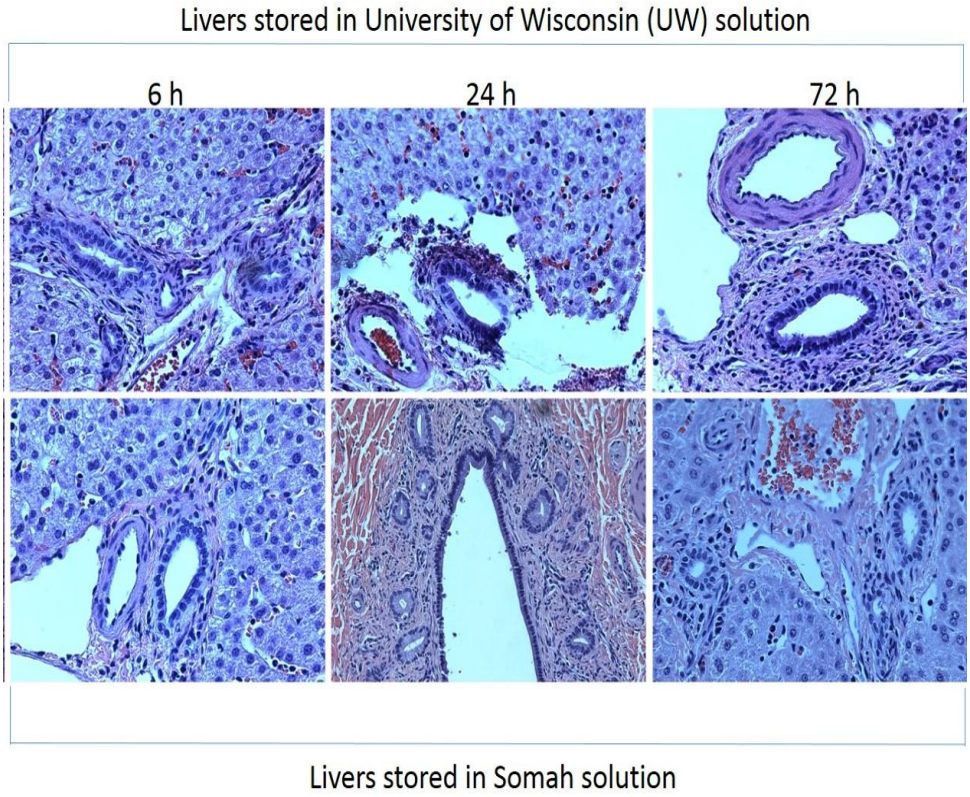

Figure 1 Porta of livers at 6, 24 and 72 hrs stored in University of Wisconsin (UW) and Somah solutions Note that bile ductules shows mucosal ulceration and disorganized, heaped, condensed nuclei in livers stored in UWS. These changes were seen as early as 6 hrs. In contrast, livers stored in Somah for 72 hrs showed normal appearing bile ductules in the portal region with clear, rounded uniform lumen. Also note that in the upper panels, some periportal hepatocytes show ballooning degeneration and apoptotic nuclei, which were not seen in livers stored in Somah.

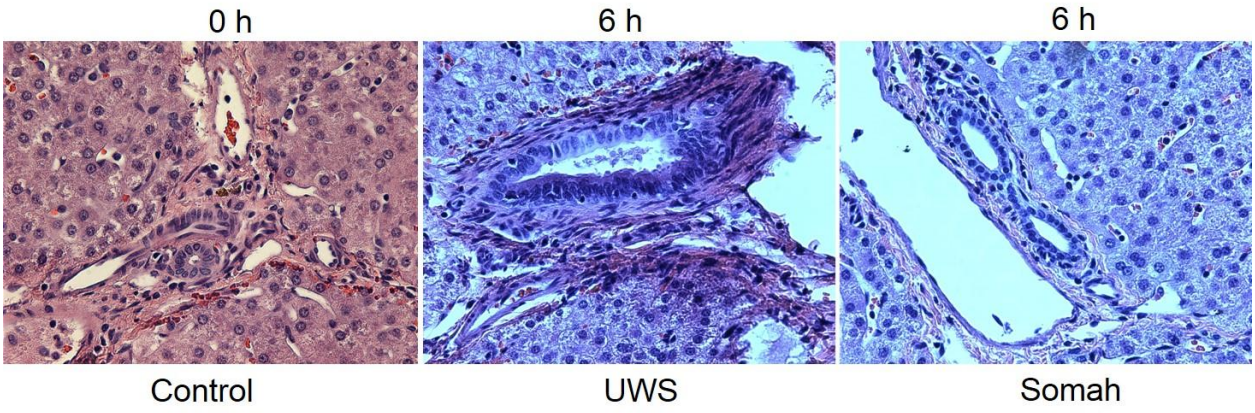

Figure 2 High power view (x400) of bile ductules obtained from livers cold-stored in UWS and Somah at 6 hrs Note the regularly arranged basal nuclei and clear lumen in a medium sized bile ductule seen at 0 hr (left panel). Note the metachromatic appearance of the ductular nuclei, including some condensed nuclei and reactive (proliferative) nuclear changes in the 3 o'clock position of liver stored in UWS. Note the sloughed material obstructing the ductular lumen and non-uniformly stained and ragged appearance of the mucosa. In contrast, lumina of bile ductules were regular appearing with intact mucosa in Somah stored livers. These changes were uniformly seen in ducts of different diameters.

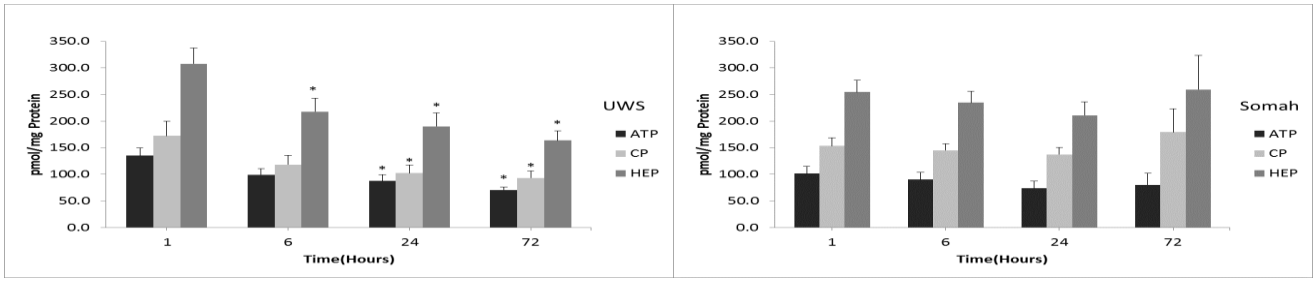

Figure 3 High energy phosphates (HEP) were conserved when livers were cold-stored in Somah, in comparison to UWS ATP and CP were measured using a bioluminescent assay kit (Sigma-Aldrich and GloMax-Multi+ Detection System, Promega).

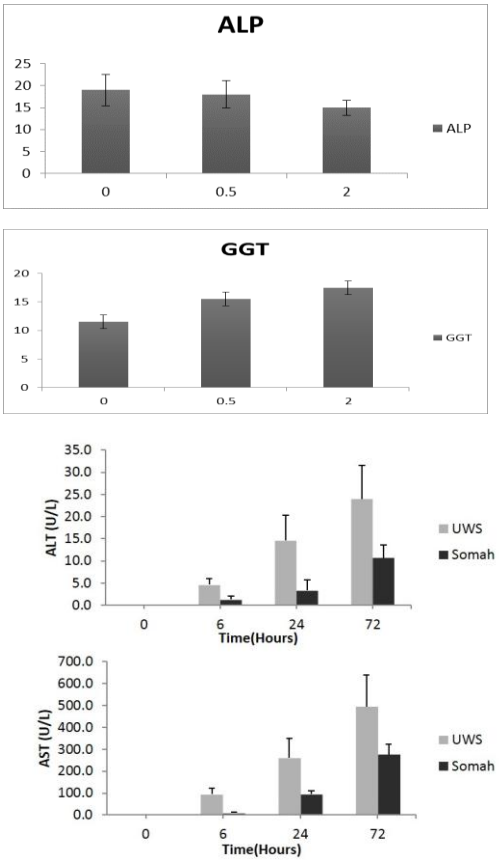

Figure 4 Biochemical evidence of lack of biliary epithelial injury during reperfusion of livers cold-stored in Somah Note the normal concentrations of alkaline phosphatase and γ–glutamyltransferase 2hrs after reperfusion of livers stored in Somah for 72 hrs at 4 degrees C. Porcine livers were reperfused using custom built perfusion device (Somah device) (Lowalekar et al 2013). ALT and AST levels were near normal, indicating lack of cellular injury when livers were stored in Somah solution for 72 hrs, in comparison to UWS. VetScan iStat and VetScan VS2 were utilized to determine biochemical parameters. DCD livers were obtained from 14 female swines (40-50 kg each), and biopsies obtained at 0 hr and livers stored either in UWS or Somah and examined at 6h, 24h and 72h.

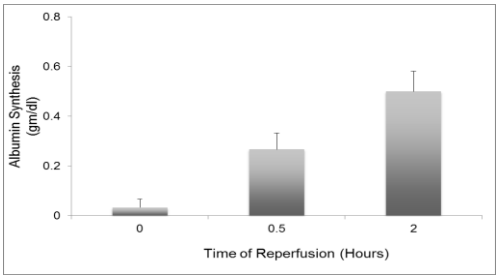

Figure 5 Reperfused livers cold-stored earlier in Somah for 72 hr synthesized albumin We plan to further examine hepatocyte synthetic ability by determining bilirubin composition, total protein and factor V synthesis after prolonged storage.

Proposed use of animals

Liver storage and procurement of samples: The study will be conducted in sixty female Yorkshire domestic pigs (*Sus scrofa*), each weighing 40-50 Kg, in accordance with a protocol approved by our Animal Studies Subcommittee (IACUC), VA Boston Healthcare System. The animals will be divided into two groups, comprising of thirty animals per group. UWS and Somah solutions will be compared for their ability for *ex vivo* preservation of the livers and the proposed specific aims will be examined regarding SLC17A9 pump function and intracellular trafficking. Whole livers will be dissected out 60±10 min after cardiac death, and then randomly stored in UWS (UW livers) or Somah solution (Somah livers) for 72 hours at 4^{o}C. Statistically significant differences among groups will be determined by ANOVA, followed by Scheffe's test for multiple comparisons. A p value<0.05 will be considered statistically significant.

Similar protocol will be followed using 60 male Wistar rats.

The rationale to perform these experiments in swines, despite their high costs of procurement, relates to similar anatomical and functional correlation with human livers. Per diem veterinary care of pigs is only moderate. Experiments with rat livers will help establish whether loss of high energy phosphates and biliary damage occurs across multiple species.

*Surgical Procedure***:** General anesthesia will be induced with intramuscular injections of Telazol 4-6 mg/kg and Xylazine 2 mg/kg. After intubation, animals will be maintained with IV propofol (10mg/kg/hour), remifentanyl (40-60 µg/hr) and Nimbex (cis-atracurium) 10-20 mg, and mechanically ventilated. The ear veins and the femoral artery and vein will be cannulated for intravenous access and blood pressure monitoring, respectively. External ECG leads will be placed, and the chest opened via a median sternotomy. The pericardium will be opened and elevated using 2-0 silk sutures to the skin. Systemic heparinization with 300 mg/kg will be achieved through the arterial line. Ten minutes after heparinization, a 9 fr, aortic vent cannula will be placed in the aortic root for infusion of cardioplegia. Aorta will be cross-clamped and hearts arrested as described earlier (Thatte et al 2009), and the heart-lung blocks will be extracted for other experiments. A median laparotomy will be performed, suprahepatic aorta cannulated and the abdominal organs flushed with 2L of ice cold UWS or Somah solution, at a pressure and flow rate of 100 mmHg and 300 ml/min respectively, till the perfusate return through the supra hepatic inferior vena cava (IVC) will be clear. The harvest will be concluded with a total hepatectomy after mobilizing the portal pedicle. The entire investigative team is well-versed in animal and human surgery and preliminary experiments have already been performed to assess feasibility. 5% attrition rate is expected due to operative fatal outcomes.

Veterinary care: Animals in this study will be under general care and supervision of VA veterinarian on staff.

Details of Procedures:

Physical Examination: A facility veterinarian will perform the physical examination, - includes recording of general condition, rectal body temperature, respiratory rate, heart rate, and lung auscultation, for each animal within 3 days prior to the experiment, after arrival of the animals to our facility.

Fasting: Animals will be fasted for 12 hours prior to tranquilization and induction of anesthesia. Food, including any dietary supplements, will be withheld. Water will be provided ad lib.

Body Weights: Body weights will be recorded on the day of surgery.

Analgesia and Anesthesia
The animals will be premedicated with Telazol® (4-6 mg/kg, [IM]) and Xylazine (2 mg/kg, IM). The animals will be anesthetized with isoflurane or sevoflurane, delivered through a facemask, to effect. The animals will be intubated, and maintained in anesthesia with inhalant anesthetic. Drugs for appropriate anesthetic management will be available for administration, if indicated. (Thiopental, 10 mg/Kg, IV injection). The drug, dose, and route of administration will be documented in the surgical records.

Pain Assessment and Control:
Assessment of pain or distress will be based on many different criteria. We will look for the following signs and symptoms when assessing the pain in the swine:

- Decreased activity
- Abnormal postures, muscle flaccidity or rigidity
- Poor grooming
- Decreased food or water consumption
- Decreased fecal or urine output
- Weight loss (generally 20-25% of baseline), failure to grow, or loss of body condition (cachexia)
- Dehydration
- Decrease or increase in body temperature
- Decrease or increase in pulse or respiratory rate
- Physical response to touch (withdrawal, lameness, abnormal aggression, vocalizing, abdominal splinting, increase in pulse or respiration)
- Teeth grinding
- Self-aggression
- Inflammation
- Photophobia
- Vomiting or diarrhea
- Objective criteria of organ failure demonstrated by hematological or blood chemistry values, imaging, biopsy, or gross dysfunction

Euthanasia
At the completion of the experiment, euthanasia will be performed in accordance with accepted American Veterinary Medical Association (AVMA) guidelines (1.2 meq/kg KCl IV or overdose of sodium pentobarbital IV until complete circulatory arrest). This is the standard protocol used at the VA for rapid euthanasia without causing distress, discomfort or anxiety to the animal, and is consistent with AVMA guidelines. The specific endpoint criteria to be used for identifying sick animals will include weight loss (20% of initial weight), inactivity, and inability to ambulate. If

euthanasia is required, the organ will be saved for evaluation. Decision to euthanize will be based on condition of the animal, and the team taking care of the animals, in consultation with the staff veterinarian.

Carcass disposal
Animals that have died or are euthanized, and are to be disposed of, will be placed in a sealed plastic bag and then deposited in the red bag within the red biohazard container located in the animal morgue coolers. Carcasses will be sectioned to a maximum of 50 pounds for each biohazard bag.

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