**Statement of Funds**

No other sources of funding are pending for this or related projects.

**A. Summary**

An incisional hernia is one of the most common complications of laparotomies, occurring annually in 10 to 15% of patients and up to 30% in certain subgroups of patients (Kingsnorth, 2006, Raffetto et al., 2003). This causes billions of dollars in lost productivity for patients and increased expense for health care systems. Even after an incisional hernia repair, the possibility of recurrence is as high as 54% depending on the technique used to repair (den Hartog et al., 2008). In some patients, incisional hernias result in bowel incarceration, obstruction, and strangulation.

This study seeks to elucidate the effect of doxycycline on wound healing and incision hernia repair at cellular and tissue levels in a rat incision hernia model. The current proposal aims to study the effectiveness of combining the usage of a well-known biologic mesh, acellular dermal matrix (ADM), and doxycycline, a tetracycline derivative known to inhibit matrix metalloproteinase-2 (MMP-2) (Gulub et al., 1991, Uitto et al., 1994). A midline laparotomy will be performed on the rats to create a hernia defect as detailed in Tharappel et al., 2013. The rats will then be fitted with either of two different types of prosthetic membranes commonly used in hernia repair surgeries: a synthetic mesh made of monofilament polypropylene or an acellular dermal matrix (ADM)

Successful completion of this project has the potential to pave the way to a therapeutic intervention of incisional hernia formation and also shed light on the complex cellular signaling pathways related to herniation.

**B. Background and Significance**

Understanding the cellular biology of incisional hernia, a condition affecting up to 20% of 2.5 million laparotomies performed per year in the United States alone, will help to minimize patient morbidity and lower health care costs for hundreds of thousands of patients per year. The research discussed in this proposal attempts to gain a greater understanding of a common condition affecting patients undergoing abdominal surgery of all types. The approach of using an inhibitor to block the collagenase that degrades the main structural component of the ECM of the abdominal wall is novel and has a significant potential for reducing hernia recurrences. Prior studies have demonstrated that the usage of ADM in certain repair protocols for hernia repair substantially reduced hernia recurrence when compared to other type of repair methods (Espinosa-de-los-Monteros et al., 2007). In our proposal, we are combining the efficacy of the ADM with a known inhibitor to a collagenase that degrades the main component of the ECM. We hope this approach has the benefit of combining the beneficial aspects of two different protocols, namely usage of ADM and a collagenase inhibitor regimen.

Additionally, this study aims to provide new insight into the cellular/biochemical mechanisms involved in IH formation by investigating other collagenase inhibitors and cytokines at the hernia repair site. **Innovation.** We are unaware of the approach described in our proposal as having ever been tried in any other laboratory. However, this is a more complete approach to repairing a recurrent hernia in the sense that it addresses two issues related to IH simultaneously.

1. It helps preventing the degradation of the ECM by inhibiting the collagenase responsible for this degradation; in this case, MMP-2.
2. It utilizes a more biocompatible matrix that is more readily accepted and incorporated by the host tissue. A faster incorporation of the ADM graft by rapid revascularization of the graft material is
considered to be helpful for clearing any infection at the cellular level if present at the site (Ayubi et al., 2008).

**Previous experience with similar studies and preliminary data:** A preliminary study was successfully conducted and the data was published (Tharappel et al., 2013). However, the preliminary study involved usage of only a PP mesh to study the difference between doxycycline treated and controls in a rat model of hernia creation and repair. This study found that there was a remarkable decrease of MMP-2, (Fig 1) and a significant increase in COL-1:COL-3 ratio (Fig 2) at the repair site of the doxycycline-treated rats. The biochemical effects resulted in an increase in repaired fascial tensile strength, although it was not statistically significant. Since then, we have done a second study using a higher number of animals (n=8) and longer survival times (6 weeks and 12 weeks). For both time points there was a statistically significant increase in tensile strength of the doxycycline treated repaired mesh-fascia interface (Fig 3,) (unpublished data; manuscript in preparation).

![Graphs showing MMP-2 and collagen type 1 and 3 analysis](image)

**C. Hypotheses**

**Hypothesis 1:** Doxycycline treated rats will have a lower level of MMPs at the hernia site and in the serum when compared to the untreated animals.

**Hypothesis 2:** The specimen from the doxycycline treated ADM hernia repaired rats will show a higher tensile strength when compared to all other animal groups.

**Hypothesis 3:** In doxycycline treated ADM fitted rats, MMP-2 inhibition by doxycycline and more biocompatibility provided by the ADM implant will result in a higher collagen 1 to collagen 3 ratio and thus a structurally stronger hernia repair.

**D. Specific Aims, Research Design, Methods, and Analysis**

**Specific Aim 1.** We will study the effect of doxycycline in the in the treated mesh implanted and primary repaired rats on the expression levels of MMP-2 at the protein and RNA levels and other endogenous collagenase inhibitors TIMP1 to 4 at the RNA transcript level. We also plan to analyze the pro-inflammatory cytokines TNF alpha and IL-6 in the treated mesh implanted and primary repaired animals. As stated in hypothesis 1, Doxycycline treated rats will have a lower level of MMPs at the hernia site and in the serum when compared to the untreated animals. Doxycycline is a specific inhibitor to MMP-2 in animal tissue. It has been shown that after abdominal aortic aneurysms (AAA)
repair, there is a much higher rate of incisional hernias in patients (Musella et al., 2001). It has already been shown that in AAA, MMP-2 is abundantly expressed and with a short-term doxycycline treatment, scientists were able to reduce it threefold when compared to untreated patients (Thompson and Baxter, 1999). Studies have shown that treating smooth muscle cells with doxycycline significantly reduced the MMP-2 levels in culture media (Liu et al., 2006). The rationale for investigating other MMP inhibitors and cytokines is to find out whether the altered levels of MMP-2 has any effect on the signaling of these factors at the cellular level.

**Specific Aim 2.** We will compare the tensile strength of the repaired abdominal wall-membrane interface among doxycycline treated and control animals using tensiometric analysis. This procedure is performed after necropsy using a computer controlled tensiometric equipment specifically adapted to biological specimens (Instron Systems). As stated in hypothesis 2, the specimen from the doxycycline treated ADM hernia repaired rats will show a higher tensile strength when compared to all other animal groups. Rationale: Previous studies have shown that ADM membranes quickly and easily integrate into the host tissue when they are used in hernia repair. In the presence of an MMP-2 inhibitor doxycycline, the animal group with ADM membrane will have a structurally stronger abdominal wall with more type 1 collagen incorporation in the ECM of the abdominal wall tissue..

**Specific Aim 3.** We will analyze and compare the vascular and cellular growth into the graft fascia interface area of the doxycycline treated and untreated animals using H&E staining from four groups: 1) polypropylene mesh (no doxycycline), 2) polypropylene mesh (doxycycline treated), 3) acellular dermal matrix (doxycycline treated), and 4) acellular dermal matrix (no doxycycline) implanted animals. The tissue from the repair area will be collected and processed for Western blot assay for collagen 1 to collagen 3 ratio analysis. As stated in hypothesis 3, In doxycycline treated ADM fitted rats, MMP-2 inhibition by doxycycline and more biocompatibility provided by the ADM implant will result in a higher collagen 1 to collagen 3 ratio and thus a structurally stronger hernia repair.

**Research Design.** Thirty six (36) male adult Sprague Dawley rats weighing ~400 gms will be used in this study. The rats will be randomly divided into six groups of 6 animals for each group. For a statistically valid number of animals/time point group, (see statistical analysis below) we need a minimum of 3 animals. Accounting for the risks during two major survival surgeries and survival, we determined at least 6 rats will be needed for each group. There will be two groups with ADM implant, two groups with synthetic polypropylene mesh implant, and two groups with no implants. One set of 6 animals from each category group will be under a doxycycline regimen of 30mg/kg/day starting from one day prior to hernia repair to the end of the experiment. The control group will be receiving an oral gavage of water for the same time period specified for the treatment group and receiving the same surgical treatments.

**Methods.** The following procedure for hernia formation and repair is detailed in Tharappel et al., 2013.

1) Each rat will undergo an initial operation to create a ventral abdominal wall hernia. A midline laparotomy will be performed on the rats to create a defect in the midline abdominal wall by excising peritoneum, abdominal wall muscle, and fascia to the level of the subcutaneous fat. Subcutaneous tissues will be closed with an absorbable suture while skin will be closed with a monofilament nylon suture or wound clips and an antibiotic ointment will be placed on the surgical incision. The rats will be recovered and monitored until ambulant. Analgesics (buprenorphine) will be administered
postoperatively. NSAIDS will be avoided due to their suppression of inflammation and possible interference with the variables being tested.

Table 1. Experimental Groups

<table>
<thead>
<tr>
<th>Time Point, 8 weeks</th>
<th>ADM Graft rats with Doxycycline</th>
<th>ADM Graft rats with no antibiotics</th>
<th>Polypropylene mesh rats with doxycycline</th>
<th>Polypropylene mesh rats with no antibiotics</th>
<th>Primary repair with doxycycline</th>
<th>Primary repair with no antibiotics</th>
<th>Total rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>36</td>
</tr>
</tbody>
</table>

2) The rats will be monitored for complications, pain, hernia recurrence, etc. over a 4-week period to allow for reperitonealization of the newly created hernia defect.

3) On post-operative day 27 through until the end of experiment, Doxycycline (30mg/kg) will be administered via oral gavage to the experimental group of animals while the control group will receive normal saline.

4) On post-operative day 28, the animals will be returned to the operating room for definitive hernia repair. Hernia repair will involve polypropylene graft or ADM graft placement as an underlay with 1 cm of overlap onto the native abdominal wall beyond the hernia defect. The control group will undergo a ventral hernia repair with absorbable sutures. Administration of doxycycline will be continued (30mg/kg/day) as a once per day oral gavage dose following hernia repair and up to the time of euthanasia. The control group and the experimental group (doxycycline treated) animals will be survived for 8 weeks following the hernia repair.

5) Following this time period, serum MMP-2 will be measured as previously described. Additionally, tissue at the area of graft-fascia overlay will be collected and analyzed for MMP expression and activity via western blotting, while MMP inhibitors and cytokine levels will be analyzed by qRTPCR and western blotting.

**Tensiometric procedures.**

Tensiometric tests will be performed on all grafts using an Instron 3000 test equipment (Instron Corp., Canton, MA). This will include determining the distraction forces required to separate the graft-fascia interface. Mechanical testing of the repaired interface fascia was performed within 6 h of tissue harvest. The abdominal tissue harvested from the repair area will be cut into a strip of tissue 4 cm long and 1.5 cm wide with the mesh-repaired area in the middle. It is then loaded into the pneumatic grips of an Instron E3000 machine equipped with a 250 Newton load cell. Tissue samples preloaded with 1 N force were then extended at a rate of 5 mm/min [22] until complete failure. Load and displacement data recorded were used to determine the maximum force required for failure of repaired tissue. Data analysis was conducted using Instron’s Bluehill software package.

**Western blotting**

Western blotting will be performed using antibodies for MMP-2, MMP-3, and MMP-9 that were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) using a previously described protocol [Tharappel et al., 2013]. Briefly, lysis buffer (1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 0.1 mg/mL phenylmethylsulfonyl fluoride, 2 mg/mL aprotinin, 2 mg/mL leupeptin, 2 mg/mL pepstatin
A, and 1x phosphate buffered saline) will be added to frozen abdominal wall tissue (one part tissue to four parts lysis buffer) and homogenized using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH). Homogenates will be centrifuged at 10,000 xg for 20 min, and the supernatants will be again centrifuged at 100,000 xg for 1 h. Supernatants will be collected, aliquoted, and stored at 80°C. One aliquot will be used for a protein assay using the BCA protein assay protocol (Pierce Protein Biology Products, Rockford, IL). The samples will be denatured by boiling for 5 min with 2x gel loading buffer (17.3% glycerol, 1.25 M b- mercaptoethanol, 5.2% sodium dodecyl sulfate, 0.22MTris, pH 6.8, 1e2 mgbromophenol blue). Thirty mg of protein from each sample will be electrophoresed (4% stacking gel and 8.5% separating gel at 175 V for 1 h) and then electroblotted onto polyvinylidene difluoride membranes (Bio- Rad Laboratories, Inc., Hercules, CA) at 100 V for 1 h. Membranes will be then incubated for 1 h in a blocking buffer (5% fat-free dry instant powdered milk, 1mMTris-base, 15 mM sodium chloride, and 0.05% Tween-20) at room temperature with shaking. The primary and secondary antibodies will be diluted in blocking buffer. They were then incubated with the membrane while shaking for 1 h, starting with the primary antibody and followed by the secondary antibody at room temperature. The membranes were then washed with three changes of wash buffer (1 mM Tris-base, 15 mM sodium chloride, and 0.05% Tween-20) after the primary and secondary antibody incubations. A Super Signal chemiluminescent substrate kit from Pierce (Rockford, IL) was used to detect antibodies bound to the membrane, and the images were analyzed by ImageJ software (NIH). Tissue from the graft-fascia interface will be collected at the time of euthanasia and part of it will be embedded in paraffin for H&E examination; immunohistochemical studies and the remainder of the tissue will be snap frozen in liquid nitrogen and stored in -80°C for nucleic acid isolation and histochemical assays.

**Real-time PCR.**

This procedure will be done as detailed in Tharappel et al., 2007. RNA for the real-time PCR templates will be prepared using Trizol reagent (Invitrogen) using the manufacturer's protocol. The cDNA templates for the real-time PCR (RT-PCR) reactions will be prepared with iScript cDNA synthesis kit (Bio-Rad). Real-time PCR reactions will be prepared with iQ SYBR Green Super mix (Bio-Rad) and will be amplified in a Bio-Rad MyiQ single-color real-time PCR detection system following the manufacturer's protocol. Oligonucleotides for the experiments will be obtained from Integrated DNA Technologies (Coralville, IA); sequence information and RT-PCR conditions will be available upon request. Primers for the RT-PCR will be from different exons so that amplicons from cDNA and contaminating genomic DNA would be of different lengths.

**Statistical analyses and considerations for sample size**

Normal MMP levels in rats are about 0.4 +/- .04. Assuming the standard deviations in both groups will be equal at about 10% (.04/.4), then detecting a 25% reduction in MMP levels in an experimental group compared to a control group at 80% power (standard) will require 3 animals in each group. Given the possibility of technical failure or experimental failure, we will be using 6 animals per group (control and treatment group) per arm. This will account for technical problems or concerns with animals falling out of the study.

The results will be presented as mean SEM. Differences between groups will be determined with analysis of variance. P <.05 is considered significant. Results of qRT-PCR will be compared with the Student t test for equality of slopes. Correlation will be determined with linear regression and expressed as correlation coefficient.
E. Budget

### SAGES RESEARCH GRANT APPLICATION
#### BUDGET SHEET

- **Detailed budget for 12 month period from** 01/01/2014 **through** 12/31/2014.
- **Dollar amount requested (Omit cents)**: 30000
- **Total for the grant request may not exceed $30,000.**

### Position Title, Time/Effort, Salary, Fringe Benefits, Sub-Totals

<table>
<thead>
<tr>
<th>NAME</th>
<th>POSITION TITLE</th>
<th>TIME/EFFORT</th>
<th>SALARY</th>
<th>FRINGE BENEFITS</th>
<th>SUB-TOTALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. John Scott Roth</td>
<td>Principal Investigator*</td>
<td>4 1.6</td>
<td></td>
<td></td>
<td>14312</td>
</tr>
<tr>
<td>2. Job Tharappel</td>
<td>Staff</td>
<td>20 8</td>
<td>10176</td>
<td>4136</td>
<td>14312</td>
</tr>
<tr>
<td>3. David Puleo</td>
<td>Co-I</td>
<td>1 0.4</td>
<td>1380</td>
<td>388</td>
<td>1768</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Consultant Costs
- Pathologist consultant: 1000

### Equipment
- Items: Subtotal

### Supplies
- Animal costs, Biologic Mesh, Synthetic mesh, sutures, Anesthesia, lab reagents: 7480

### Travel**
- Travel to a scientific meeting for data presentation: 1000

### Patient Care Costs
- Animal care expenses: 3640

### Consortual/Contractual costs

### Other Expenses
- Pathology lab expenses: 800

### TOTAL DIRECT COSTS
- 30000

---

**Budget Justification**

**Scott Roth**, (PI, 4% effort, no salary) Dr. Roth will be responsible for the overall conduct of the study. He will oversee all study related activities.

**Job Tharappel**, (Co-I, 20% effort) Dr. Tharappel will execute study related activities and will be responsible for maintaining all study records and a study timeline. He will be responsible for designing and conducting the animal studies, lab experiments, monitoring animals post-surgery, recording observations of wound healing and hernia repair.

**Davis Puleo** (Co-I, 1% effort) – Dr. Puleo will collaborate on this project, giving advice and direction on technical issues related to bioengineering side of the project related activities.

**Consultant Costs:** The H&E and immune-stained slides will be evaluated by a trained pathologist.

**Supplies:** It includes animals ($1700) Biologic and synthetic mesh $ (4000) sutures, anesthesia charges, lab reagents including antibodies and biochemical ($1700)

**Travel:** For air ticket and registration/lodging expenses for traveling to a scientific meeting to present data from this project.

**Animal care:** There will be a total of 13 weeks animal care (8 weeks post implantation+ 4 weeks pre-implantation and 1 week acclimatization) $280 per week for 36 animals.

**Pathology lab:** Paraffin tissue section processing and slide making for histological and immunological staining.
F. References


Update Special Husbandry (Single Housing) as per CPR

To: J. Scott Roth

From: Abhijit Patwardhan, Ph.D., Chairperson (Chairperson)
Institutional Animal Care and Use Committee (IACUC)

Subject: Approval of Protocol Amendment
Protocol Number: 2009-0597 Year: 2
Amendment: 0003

Date: 02/19/2013

On 02/19/2013, the IACUC approved your request for modification to your protocol entitled:

"Doxycycline Inhibition of MMP-2 and MMP-9 in Incisional Hernia Repair; Effect of Matrix Metalloproteinase II Inhibitor Doxycycline, on Incisional Hernia Recurrence Rates in a ADM or Polypropylene Mesh Implanted Rat Incision Hernia Model."

If this IACUC project is extramurally funded and the modification involves a substantive change in your scope of work, the sponsoring agency should be notified.

If you need further assistance or have questions, please call Angie Croucher in the Office of Research Integrity at 859/257-2549.
Available Resources

The animal experiments will be performed in the Department of Laboratory and Animal Research Laboratory at the University of Kentucky. Operating room facilities, technicians, and veterinary support are available to assist with all facets of this proposal. Laboratory analyses will be performed in the Department of Surgery Research laboratory in the University of Kentucky Chandler Medical Center. Tensiometric testing will be performed at the Wenner Gren Biomedical Research Facility at University of Kentucky directed by David Puleo, PhD. The PI for this proposal has extensive experience in clinical hernia repair. The PI has significant research experience and has utilized this hernia model previously. The Co-I of this proposal has several years of experience in small animal surgeries and all relevant laboratory protocols that will be used in this study. Our research lab in the division of General Surgery has all the equipment needed for this study and we have an excellent core facility with all the latest state-of-the-art instruments for pathology, imaging, molecular biology, and proteomics/genomics.
BIOGRAPHICAL SKETCHES

<table>
<thead>
<tr>
<th>Name of Investigator</th>
<th>Title</th>
<th>Department Affiliation</th>
<th>Present Nationality (If Non-US Citizen, Please indicate status)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. Scott Roth, MD</td>
<td>Professor of Surgery</td>
<td>Surgery/Division of General Surgery</td>
<td>U.S.</td>
</tr>
</tbody>
</table>

TRAINING AND EDUCATION (Begin with Baccalaureate training include Post-Doctoral and clinical training – if applicable)

<table>
<thead>
<tr>
<th>INSTITUTION</th>
<th>LOCATION</th>
<th>DEGREE</th>
<th>YEAR CONFERRED</th>
</tr>
</thead>
<tbody>
<tr>
<td>College of William and Mary</td>
<td>Williamsburg, VA</td>
<td>BS, Biology</td>
<td>1989</td>
</tr>
<tr>
<td>Medical College of Virginia, Va.</td>
<td>Richmond, VA</td>
<td>MD</td>
<td>1993</td>
</tr>
<tr>
<td>Commonwealth University</td>
<td></td>
<td>Intern &amp; Resident</td>
<td>1993-1998</td>
</tr>
<tr>
<td>University of Kentucky</td>
<td>Lexington, KY</td>
<td>MIS Fellowship</td>
<td>1998-1999</td>
</tr>
</tbody>
</table>

HONORS

1989 Phi Sigma Biology Honor Society, The College of William and Mary
2002 The John Bernard, Vick MD Teaching Award; East Carolina University Department of Surgery
2005 The Anthony Imbembo, MD Resident Teaching Award; University of Maryland Dept. of Surgery
2010 Recognition of Excellence in Student and Resident Teaching, University of Kentucky Dept. of Surgery

MAJOR RESEARCH INTEREST

I am the Chief of Gastrointestinal Surgery and my clinical interests include minimally invasive surgery with a focus in the areas of hernias (hiatal, inguinal, and ventral), gastroesophageal reflux, and hernias. My research has been focused in the area of abdominal wall hernias and tissue engineering for hernia repair.

RELATIONSHIP TO PROPOSED PROJECT

The goal of this project is to understand the effect of doxycycline and the ADM graft on wound healing and incisional hernia repair at the cellular and tissue levels in a rat incisional hernia repair (IHR) model. I have the specific clinical training and expertise in abdominal surgery, laparoscopic repair, and minimally invasive surgery, as well as the research experience required for the proposed project. We have assembled a strong research team with the combined expertise and motivation required to be successful.
## OTHER RESEARCH SUPPORT

“Feasibility study of the use of Flex HD® surgical implant in the closure of abdominal wall defects with component separation in clean or contaminated cases”  
**Role:** Principal Investigator  
**Dates of funding:** November 2010 – present  
**Source:** Musculoskeletal Transplant Foundation

“A Single Arm, Multi-Center, Retrospective Study with Prospective Follow-Up of Complex Ventral Hernia Repair Utilizing the AlloMax® Surgical Graft”  
**Role:** Principal Investigator  
**Dates of funding:** May 2009- present  
**Source:** CR Bard, Inc.

“Feasibility study of the use of Permacol Surgical Implant in the closure of abdominal wall defects after removal of infected prosthetic mesh”  
**Role:** Principal Investigator  
**Dates of funding:** March 2009- present  
**Source:** Covidien

“Evaluation of Rebound Hernia Device versus standard Polypropylene Mesh for laparoscopic inguinal hernia repair”  
**Role:** Principal Investigator  
**October 2009- present**  
**Source:** Minnesota Medical Development, Incorporated.

---

## RESEARCH AND/OR PROFESSIONAL EXPERIENCE

(Start with present position and list ALL experience relevant to project, Include Publications)

### Positions and Employment

<table>
<thead>
<tr>
<th>Date</th>
<th>Position</th>
<th>Institution and Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/13-present</td>
<td>Professor of Surgery</td>
<td></td>
</tr>
<tr>
<td>10/08-01/13</td>
<td>Associate Professor of Surgery, University of Kentucky College of Medicine; Lexington, KY</td>
<td></td>
</tr>
<tr>
<td>11/09-present</td>
<td>Adjunct Faculty, Fischell School of Biomedical Engineering, University of Maryland College Park, MD</td>
<td></td>
</tr>
<tr>
<td>07/08-09/08</td>
<td>Associate Professor of Surgery, University of Maryland School of Medicine; Baltimore, MD</td>
<td></td>
</tr>
<tr>
<td>2004-2008</td>
<td>Asst. Professor of Surgery, University of Maryland School of Medicine; Baltimore, MD</td>
<td></td>
</tr>
<tr>
<td>2000-2004</td>
<td>Asst. Professor of Surgery, Brody School of Medicine at East Carolina Univ.; Greenville, NC</td>
<td></td>
</tr>
<tr>
<td>1998-1999</td>
<td>Clinical Instructor, Dept. of Surgery, University of Kentucky College of Medicine; Lexington, KY</td>
<td></td>
</tr>
</tbody>
</table>

### Publications: Most Relevant to Project

### BIOGRAPHICAL SKETCHES (Continued)

#### RESEARCH AND/OR PROFESSIONAL EXPERIENCE
(Start with present position and list ALL experience relevant to project. Include Publications)

<table>
<thead>
<tr>
<th>No.</th>
<th>Author(s)</th>
<th>Title</th>
<th>Journal</th>
<th>Year</th>
<th>Volume</th>
<th>Pages</th>
<th>PMIDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>Diaz DF, Roth JS</td>
<td>Laparoscopic paraesophageal hernia repair with Acellular Dermal Matrix cruroplasty</td>
<td>Journal of the Society of Laparoendoscopic Surgeons</td>
<td>2011</td>
<td>15(3)</td>
<td>355-60</td>
<td>11444753</td>
</tr>
<tr>
<td>8.</td>
<td>Roth JS</td>
<td>Minimally invasive approaches to pancreatic pseudocysts</td>
<td>Curr Surg</td>
<td>2003</td>
<td>60</td>
<td>591-592</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Giurgiut M, Bendure L, Davenport D, Roth JS</td>
<td>The Endoscopic Component Separation Technique for Hernia Repair Results in Reduced Morbidity Compared to the Open Component Separation Technique</td>
<td>Hernia</td>
<td>in press</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>Albright E, Davenport D, Roth JS</td>
<td>Preoperative Functional Health Status Impacts Outcomes Following Ventral Hernia Repair</td>
<td>The American Surgeon</td>
<td>2012</td>
<td>78</td>
<td>230-234</td>
<td>2236983</td>
</tr>
</tbody>
</table>
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
Job C. Tharappel

POSITION TITLE
Senior Research Associate
Division of General 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.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>MM/YY</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kerala University; Kerala, India</td>
<td>BS</td>
<td>1983</td>
<td>Biology</td>
</tr>
<tr>
<td>Kerala University, Kerala, India</td>
<td>MS</td>
<td>1985</td>
<td>Zoology</td>
</tr>
<tr>
<td>Nagpur University, Maharastra, India</td>
<td>PhD</td>
<td>1993</td>
<td>Neurobiology</td>
</tr>
</tbody>
</table>

A. Personal Statement.
The main goal of this research project is the development of a mechanism to improve wound healing and hernia repair outcomes in patients undergoing repair with a human acellular dermal matrix. Through the use of pharmacological agents, matrix metalloproteinases are inhibited, resulting in a reduction in collagen degradation. As a result of inhibition of collagenases, wound healing will be improved and result in better outcomes. Over the last several years I have had many opportunities to work on projects that involved studies in rats and mice, including surgical procedures. With more than 15 years of experience in many lab protocols involving biochemical, immuno-histochemical, and molecular biology. I am confident that I can be a very significant contributor to this project from start to finish.

B. Positions and Honors

Positions and Employment
1988-1993 Research Assistant, Nagpur University, India
1994-1997 Post Doctoral Fellow, Dept. of Toxicology, University of Kentucky
1998-2003 Research Associate, Graduate Center for Nutritional Sciences, University of Kentucky
2003-2008 Senior Research Associate, Graduate Center for Nutritional Sciences, University of Kentucky
2008-2011 Senior Research associate, Gluck Equine Research Center, University of Kentucky
2011-present Senior Research Associate, Division of General Surgery, University of Kentucky

Honors
1988 Junior Research Fellowship (UGC) Nagpur University, India
1990 Senior Research Fellowship (UGC) Nagpur University, India
1993 Recipient of the best research presentation award, Nagpur University, India
1993 Research paper illustration selected for the cover page of the journal Brain Behavior and Evolution
1994-97 Fellow, NIH Training Grant

C. Selected Peer-reviewed Publications


D. Research Support

N/A
**NAME**  
David A. Puleo

**POSITION TITLE**  
Professor and Director

**eRA COMMONS USER NAME (credential, e.g., agency login)**  
david.puleo

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

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>MM/YY</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rensselaer Polytechnic Institute, Troy, NY</td>
<td>B.S.</td>
<td>05/87</td>
<td>Biomedical Engineering</td>
</tr>
<tr>
<td>Rensselaer Polytechnic Institute, Troy, NY</td>
<td>Ph.D.</td>
<td>05/91</td>
<td>Biomedical Engineering</td>
</tr>
</tbody>
</table>
A. Personal Statement

The overall goal of the proposed project is to evaluate effectiveness of doxycycline for improved healing following repair of incisional hernias. A key aspect of this objective will be mechanical testing of the fascia-mesh interface. The current application will utilize my previous research experience with evaluating mechanical properties of various synthetic and biological materials. I have over 20 years of experience participating on, assembling, and administering collaborative teams of investigators from multiple disciplines to attack research problems in the areas of biomolecule-stimulated cell and tissue responses and of tissue-implant interactions. Recent projects have included engineers, scientists, dentists, and physicians from the Colleges of Arts & Sciences, Dentistry, Engineering, Medicine, and Pharmacy. In summary, I have a demonstrated record of successful, productive, and collaborative research activities directed at research problems that require synergistic approaches to systematically address significant biomedical problems, such as repair of incisional hernias.

B. Positions and Honors

Research and Professional Experience

1987 Laboratory Assistant, Department of Materials Engineering, Rensselaer Polytechnic Institute, Troy, New York
1988 Research Associate, Castle Point Veterans Administration Medical Center, Castle Point, New York
1989 Laboratory Assistant, Department of Ophthalmology, Albany Medical College, Albany, New York
1987-1991 Teaching Assistant, Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York
1991-1997 Assistant Professor, Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky
1997-2004 Associate Professor, Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky
2000-2004 Director of Graduate Studies, Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky
2003-present Adjunct Associate Professor, Center for Oral Health Research, College of Dentistry, University of Kentucky Medical Center, Lexington, Kentucky
2003-2005 Acting Director, Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky
2004-2013 Professor, Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky
2005-2013 Director, Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky
2010-present Professor, Department of Orthopaedic Surgery, College of Medicine, University of Kentucky Medical Center, Lexington, Kentucky
2013-present Professor, Department of Biomedical Engineering, University of Kentucky, Lexington, Kentucky
2013-present Chair, Department of Biomedical Engineering, University of Kentucky, Lexington, Kentucky

Awards and Other Professional Activities

Research Initiation Award, National Science Foundation (1992)
Bourses de stage de recherche scientifique, Programme québécois de bourses d'excellence, Ministère de l'Éducation, Gouvernement du Québec (1998)
Chair (elected), Proteins and Cells at Interfaces Special Interest Group, Society For Biomaterials (1999-2001)
Member, inaugural Musculoskeletal Tissue Engineering study section, NIH (2005-2007)
C. Selected Peer-reviewed Publications

Most relevant to the current application


Additional publications of importance to the field (in chronological order)


D. Research Support

Ongoing Research Support

“MRI-R2: Instrument Acquisition: Nanomechanical Systems for in situ Mechanical Characterization of Materials in Application Environments”
Agency: NSF (0959896)
Period: 5/1/10-9/30/13
The overall goal of this program was to acquire instruments for micro- and nano-characterization of mechanical behavior of materials in controlled environments and under external stimuli.

“REU Site: A Multidisciplinary Research Experience in Engineered Bioactive Interfaces and Devices”
Principal Investigators: K.W. Anderson and J.Z. Hilt
Agency: NSF (0851716)
Period: 5/1/12-4/30/15
The overall goal of this program is to establish training activities for undergraduate students in research areas related to bioactive materials and devices.

“Modulating Inflammation and Fibrosis to Control Scarring in Muscle Wounds”
Principal Investigator: D.A. Puleo
Agency: NIH/NIAMS (R01AR060964-01A1)
Period: 8/1/12-7/31/16
The overall goal of this program is to develop multilayered, erodible polymer systems that prevent fibrotic healing of muscle defects.

Completed Research Support

“Recapitulation of a growth plate in a rabbit model”
Principal Investigator: D.A. Puleo
Agency: Shriners Hospital for Children (Lexington, KY)
Period: 10/1/06-9/30/10
The overall goal of this project was to obtain pilot data with salt-leached scaffolds for treating damaged growth plate.

“IGERT: Building Leadership Through a Program on Engineered Bioactive Interfaces and Devices”
Principal Investigator: K.W. Anderson
Agency: NSF (DGE-0653710)
Period: 9/1/07-8/31/12
The overall goal of this program was to establish a series of educational activities that integrate research, training, leadership and professional development, international perspectives, and ethical issues to better train the students to become the scientific leaders of tomorrow.

“Enhanced Oral Tissue Repair via Self Assembled Polymer Multi-layer Barriers for the Delivery of Antioxidants “
Principal Investigator: T.D. Dziubla
Period: 9/17/09-8/31/12
The goal of this project was to develop a layer-by-layer polymeric barrier that binds to oral mucosa and subsequently serves as a delivery system for antioxidant agents.

“Devices for Treating Inflammatory Bone Loss in an Oral Environment"
Principal Investigator: D.A. Puleo
Agency: NIH/NIDCR (R01DE019645),
Period: 6/1/09-2/28/13
The goal of this project was to develop a system for delivering biomolecules to treat dental implants compromised by oral microorganisms.

“Tissue Repair and Regeneration following Orthopedic and Craniofacial Trauma”
Principal Investigator: D.A. Puleo
Agency: USAMRAA (08246004)
Period: 7/1/09-7/30/13
The goal of this project was to develop a ceramic bone filler that enables timely and complete healing of large, infected bone defects, such as those sustained during military combat.

“Versatile Biosensing Platform for Monitoring Bone Markers for Space Medicine”
Principal Investigator: D.A. Puleo (transferred from Leonidas Bachas)
Agency: NASA EPSCoR
Period: 8/1/09-6/30/13
The goal of this project was to develop biosensors for diagnosis of bone health status.

“Cell Enhanced Hybrid Hydrogel Scaffolds for Tissue Engineering (Hy2STEP) to Regenerate a Native Physis”
Principal Investigator: T.A. Milbrandt
Agency: Orthopaedic Research and Education Foundation
Period: 8/1/11-7/31/13
The goal of this project was investigate cell-seeding of biodegradable scaffolds
Participation in SAGES

The PI of this project, Dr. Scott J. Roth has been a member of SAGES since 1998. He is also a member of the SAGES Guidelines Committee, Hernia Task Force, Legislative Committee and recently was appointed to the CPT Editorial Board. This year he was awarded the SAGES Recognition of Excellence Award as well as the Brandies Award to attend the Leadership Program in Health Policy in Management.

At the recent SAGES meeting in April 2013, Dr. Roth had 3 papers presented and one video presentation.