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TITLE: Elastomeric Auxetic Urogential Meshes: Exploring Alternatives to Knitted Polypropylene

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14. ABSTRACT

To date, we have completed the finite element analysis (FEA) of various auxetic geometries and have selected the geometry that we deem is the most appropriate for our novel mesh. A paper detailing the results from the FEA was submitted to the Journal of Biomechanical Engineering and is currently undergoing a second review, with a high probability of publication. We have manufactured the novel elastomeric auxetic mesh from polydimethylsiloxane (PDMS). PDMS is a soft elastomer that has a material stiffness which is similar to vaginal tissue (PDMS material stiffness = 9.9 MPa, vaginal stiffness = 6-14 MPa). Using mechanical testing, we have verified that the pores of this novel mesh expand in response to mechanical loading and that this mesh has elastic-like property (i.e. the mesh returns to its original configuration when stretched). We have completed implantations on 10 Sham-operated animals (no mesh implanted) and 7 mesh implanted animals. We have also begun the testing and analysis of tissue and histological examination of the immune response to mesh in the rabbit vagina. Our preliminary mechanical testing results reveal that prolapse mesh negatively impacts the contractile function of vaginal smooth muscle (VSM) in a similar manner as it does the nonhuman primate. These results are significant and suggest that the rabbit is an appropriate model for assessing the impact of prolapse mesh on the rabbit VSM. Currently, we are exploring new options for another elastomer, polycarbonate urethane, from which to manufacture our novel device. In the next reporting period, we will continue manufacturing the elastomeric auxetic mesh and implanting it into animals until we have reached our study endpoints.

15. SUBJECT TERMS

prolapse, auxetic, pore, porosity, elastomer

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1. INTRODUCTION:

Roughly 300,000 surgeries are performed annually in the US to repair pelvic organ prolapse (POP) – a common condition in women in which the pelvic organs descend into the vaginal lumen. The changing demographics of the VA population with more women utilizing VA benefits and services combined with the increased risk of developing POP in women engaging in strenuous activity portends a rapidly escalating demand for repair of POP and related conditions among female veterans. Following POP surgical repairs utilizing a woman's own tissues, 40% will fail by 2 years, and 70% by 4 years prompting surgeons to seek materials to augment repairs, most commonly polypropylene mesh. While current literature supports the use of knitted, lightweight, wide pore polypropylene, the ideal mesh has not been defined and no mesh to date is without complications.

Currently, all meshes used in POP repairs are hernia meshes simply remarketed as 510K devices for a different indication. Hernia meshes are comprised of a stiff plastically deforming polymer (polypropylene), whose use prior to 2011 was motivated by the need for mesh products to be similar to their abdominal hernia counterparts so that a 510K device status could be retained. Thus, prolapse meshes were never developed specifically for the mechanical and physiologic needs of the vagina and polypropylene was never deemed the ideal polymer. In addition, for hernia repairs, large pores (>1mm) have been shown to be critical for successful host tissue integration. Yet, recent research has revealed that when implanted for POP repairs, unlike in the abdominal wall, polypropylene mesh pores are much more likely to collapse below the critical 1 mm threshold. Pore collapse leads to localized areas of increased mesh burden (density) and altered mesh mechanics (increased stiffness) increasing the risk of complications, particularly pain and mesh erosion/exposure.

In response to the Department of Defense Discovery Award in the Program Topic Area "Advanced Prosthetics", we propose to develop a novel synthetic prolapse mesh based on an auxetic pore geometry that 1) undergoes elastic as opposed to plastic deformation and 2) maintains or exceeds its initial pore sizes when loaded, with 3) a material stiffness that matches that of the vagina. An "auxetic" structure is one that has a negative Poisson's ratio. Thus, instead of the middle of the device collapsing in on itself when placed in tension, as in most current polypropylene meshes, the middle expands leading to increased pore sizes with mechanical loading. The meshes will be constructed using elastomeric polymers. Unlike polypropylene, these meshes will extend and contract as they are loaded and unloaded respectively; thereby behaving more **similar to native supportive tissues. We hypothesize that these novel elastomeric, auxetic prolapse meshes will evoke improved tissue integration and preserve vaginal function relative to current commonly used polypropylene prolapse meshes.**

2. KEYWORDS: prolapse, auxetic, pore, porosity, elastomer

3. ACCOMPLISHMENTS:

What were the major goals of the project?

To construct an elastomeric auxetic mesh using two different polymers: 1) Polydimethylsiloxane (PDMS) - an FDA approved hydrophobic elastomer that has been implanted in hundreds of thousands of patients to date and 2) Polycarbonate Urethane (PCU) - a novel, highly durable hydrophilic elastomer with tunable mechanical properties that affords its use in load-bearing applications. In Aim 1: computational modeling was used to design and then to construct a novel device that affords sufficient anatomic support to the vagina and hence, the pelvic organs while maximizing device porosity. Each elastomer from which the device was constructed had a modulus equivalent to that of vaginal tissue. In Aim 2: we developed and validated the rabbit model for mesh implantation. In Aim 3: the host response to elastomeric auxetic mesh implantation versus that of the current commonly used prototype polypropylene prolapse mesh (Restorelle) is being evaluated.

What was accomplished under these goals?

Aim 1: Our mechanical testing protocol showed that the bowtie auxetic geometry is the most favorable geometry given that the pores of the bowtie model expanded and maintained auxetic behavior at increasing loads with the least amount of model deformation and limited elongation (~30%). Based on these results, we manufactured a novel elastomeric auxetic mesh using the bowtie pore geometry from polydimethylsiloxane (PDMS) and polycarbonate (PCU). The material stiffness of PDMS and PCU utilized were both on the same order of magnitude as vaginal tissue (PDMS material stiffness = 10 MPa, PCU material stiffness = 70 MPa, vaginal stiffness = 6-14 MPa (human) and 25-34 MPa (animal)).

Aim 2: We developed a novel method to implant mesh onto the internal rabbit vagina via a lumbar colpopexy. We have completed our sample size of 10 Restorelle implanted animals and 10 Sham animals (no mesh implanted). Comparable to what we found in NHPs, polypropylene mesh has a negative impact on vaginal smooth muscle function with a 43% decrease in contractility (Figure 1). The observed decrease in contractile function was also associated with a thinning of the vaginal smooth muscle layer (Figure 2). We also observed mesh buckling with Restorelle despite implanting the meshes in a flat configuration (Figure 2). In the areas where the mesh buckled, we observed mesh exposure, a common complication in women (Figure 2). This result suggest that not only is the rabbit a good model for testing the host response to mesh with tensioning and loading but is also useful for understanding mechanisms of mesh exposure.

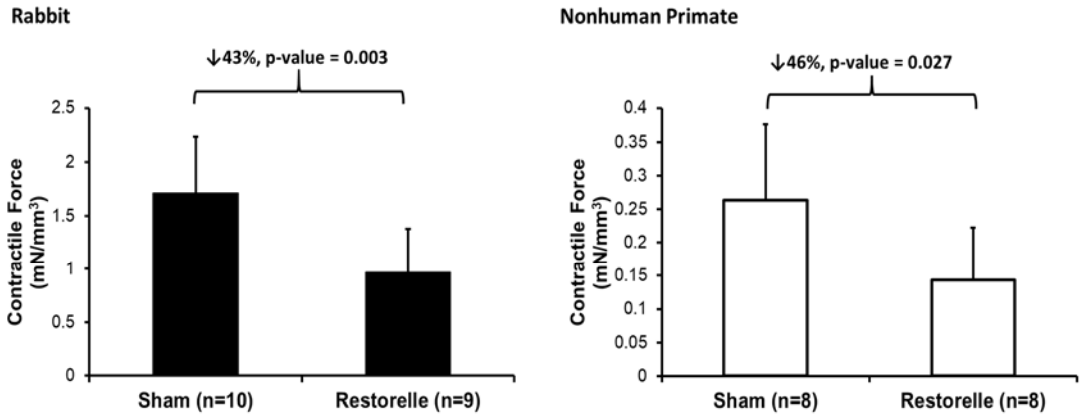

Figure 1: Contractile force of the rabbit (left) and nonhuman primate (right) vagina in response to 120 mM KCl. The contractile force of the rabbit vagina significantly decreased by 43% with the implantation of Restorelle via a lumbar colpopoexy and this result is similar to the 46% decrease in contractility with the implantation of Restorelle onto the nonhuman primate vagina via an abdominal sacral colpopexy.

Aim 3: The objective of this aim is 1) to evaluate the host response to an elastomeric auxetic mesh and 2) to compare this response to a commercially available polypropylene mesh, Restorelle. To accomplish this objective, we implanted the elastomeric auxetic meshes manufactured from PDMS (n=10) and PCU (n=10) (developed in Aim 1) as well as Restorelle (n=10) (implanted in Aim 2) onto the rabbit vagina for 12-weeks via a lumbar colpopexy. After 12 weeks, the mesh-vagina complexes are in the process of being excised and the active mechanics (i.e. function of the vaginal smooth muscle), histomorphology (i.e. smooth muscle morphology and thickness, collagen structure), immunohistochemical (i.e. macrophage labeling), and biochemical (i.e. total collagen content and GAG content)

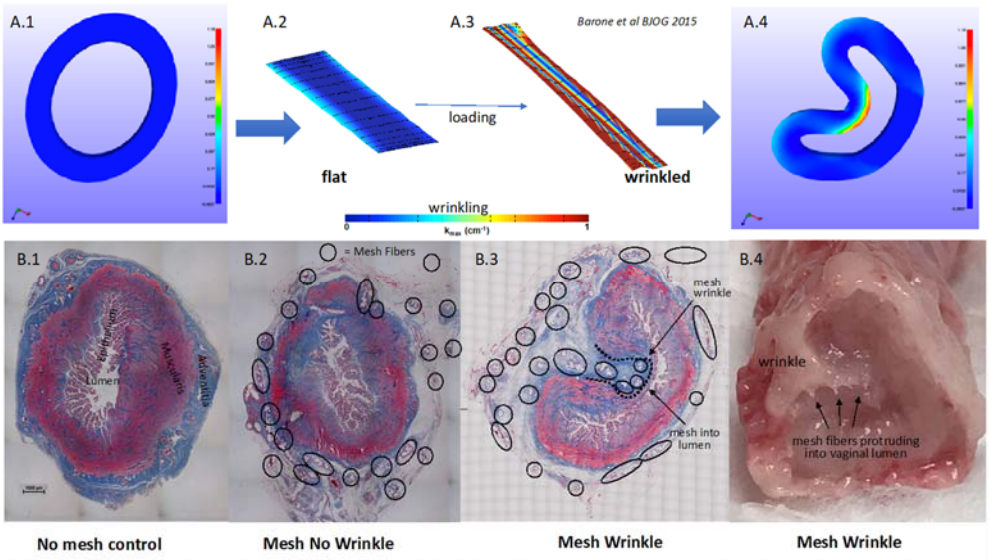

Figure 2. Mesh wrinkling and impact on the vagina after implantation by sacrocolpopexy in the rabbit. A.1 is a modeled cross-section of the vaginal muscularis. A.2-3 show a 3D image of mesh and corresponding surface curvature K_{max} with loading. Tensioning/loading mesh can cause wrinkling which pushes in on the vagina as shown in the shape (A.4) with corresponding cross-section and gross anatomy (below, B.3-4). Computational analysis (upper panel) deforming the geometry on the left (A.1) into the geometry on the right (A.4)reveals a strain distribution that is consistent with what would be predicted from a simple beam analysis. In the cross section image of a mesh wrinkle (B.3), locations where curvature and tissue strain are the highest corresponds to where the vaginal vaginal wall (especially smooth muscle) has thinned to the point where mesh fibers (circled in black) enter into the lumen of the vagina (mesh exposure). The is in contrast to the middle image (B) where mesh was implanted flat without wrinkles leading to general smooth muscle thinning relative to control, but no regions where mesh fibers are close to the lumen of the vagina. Thus, these data show that mesh wrinkling should be avoided, which one of

endpoints will be assessed. To date, 37 out of the 40 rabbits have reached the 12-week endpoint. The remaining 3 rabbits will be sacrificed in mid-November. For the active mechanics of this study, we performed a vaginal contractility assay in which the mesh-vagina complexes or vagina alone in the case of Sham were exposed to 3 stimuli in order to assess the contractile function of the vaginal smooth muscle: 120 mM KCl (assesses muscle-mediated contractions), electrical field stimulation - 20 V for a duration of 5 secs and the frequency was increased from 1-64 Hz (assesses nerve-mediated contractions), and 10^{-7} to 10^{-4} M phenylephrine (an α_1-adreno-receptor agonist), applied non-cumulatively (assesses receptor-mediated contractions). Preliminary results (Table 1) demonstrate that the contractile force in response to 120 mM KCl for the PCU mesh was significantly less than that of Sham (p-value = 0.005). There was no significant difference in response to electrical field stimulation, p-value = 0.322. Compared to Sham, Restorelle, and PDMS, the receptor-mediated contractions for PCU were significantly less, p-value = 0.004, 0.034, and 0.020, respectively.

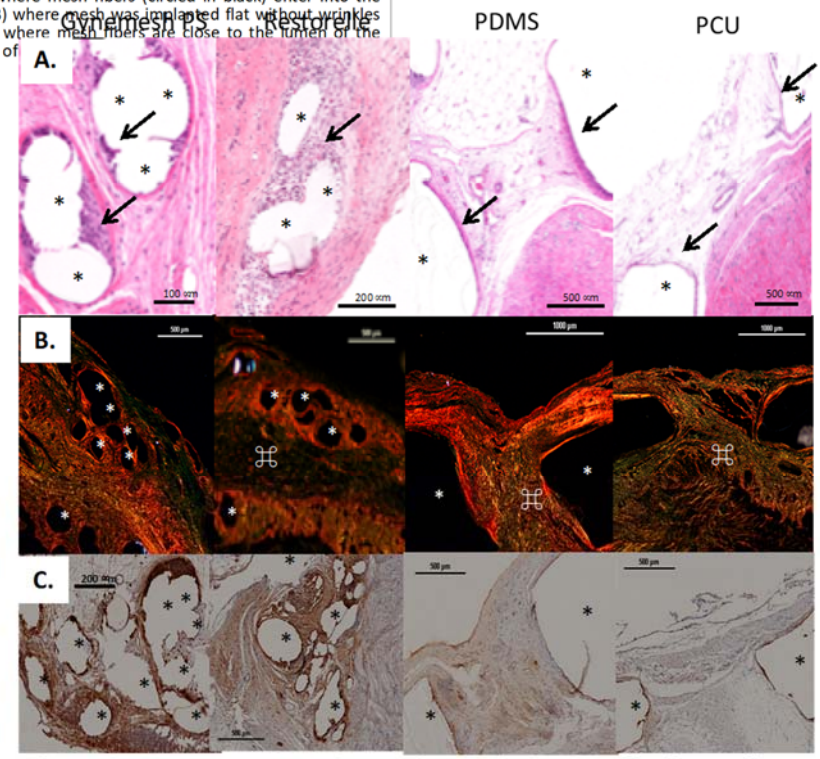

Figure 3. Host response at 3mos after implantation of polypropylene meshes Gynemesh and Restorelle vs EAMs - PDMS and PCU. A. Limited foreign body response (arrow) to the fibers (*) is seen in EAMs as compared to polypropylene meshes. B. Picrosirius red staining confirms presence of thick red collagen fibers (capsule) around Gynemesh PS with no normal tissue between pores (⌘)vs good tissue ingrowth in Restorelle and EAMs with green thin fibers and minimal fibrous capsule observed for PCU. C. Labeling with panmacrophage marker (RAM 11) shows markedly reduced macrophage response (brown stain) for the EAMs as compared to polypropylene.

Table 1: Maximum contractile force in response to 120 mM KCl, Electrical Field Stimulation, and Phenylephrine

	120 mM KCl (mN/mm^3)	Electrical Field Stimulation (g/g)	Phenylephrine (g/g)
Sham (n=10)	1.71 ± 0.52	0.45 (0.69)	1.50 (0.36)
Restorelle (n=9)	1.13 ± 0.50	0.51 (0.42)	1.26 (0.67)
PDMS (n=8)	1.42 ± 0.40	0.64 (0.21)	1.14 (0.80)
PCU (n=6)	0.85 ± 0.30	0.41 (0.26)	0.16 (0.62)
Overall p-value	0.005[a]	0.322[b]	0.011[b]
Sham vs Restorelle	0.05	N/A	N/A
Sham vs PDMS	0.668	N/A	N/A
Sham vs PCU	0.005	N/A	0.004
Restorelle vs PDMS	0.717	N/A	N/A
Restorelle vs PCU	0.809	N/A	0.034
PDMS vs PCU	0.149	N/A	0.020

Data represented as mean ± standard deviation and median (interquartile range).
[a]Overall p-value obtained using One-way ANOVA followed by Gabriel's pairwise test.
[b]Overall p-value obtained using Kruskal-Wallis followed by Mann-Whitney test with a Bonferroni correction with significance set to p-value < 0.0167.

What opportunities for training and professional development has the project provided?

1. **Katrina Knight: NIH TL1 Clinical & Translational Science Post doctoral Fellow** in the Department of Medicine at the University of Pittsburgh. Project title: Development of a novel urogynecologic mesh to overcome current limitations of polypropylene. Dr. Knight through working with this grant has enhanced her surgical skills, aided with the development of the rabbit model for this study, and is currently focused on completing the final experimental endpoints of this study, which she will disseminate in 1 to 3 manuscripts. Over the past year, Dr. Knight has presented the work from this study in multiple settings including the annual meeting of the American Urogynecologic Society (AUGS) which has increased her visibility and enhanced her presentations skills.

2. **Aimon Iftikhar:** Current Ph.D. Candidate in Bioengineering at University of Pittsburgh; NIH TL1 Clinical & Translational Science Predoctoral Fellow Thesis Title: Development of a Clinically Relevant Rabbit Surgical Model of Pelvic Reconstruction to Evaluate the Immune Response to Novel Surgical Materials. She is currently testing a cytokine local delivery system and working on developing the rabbit model for use in this study and also presented at AUGS.

How were the results disseminated to communities of interest?

1. Annual Scientific Meetings:
    - American Urogynecologic Society
    - Society for Pelvic Research
    - Invited Speaker: Society of Women in Urology (SWIU) 6th Annual Meeting "The science behind the mesh: Fact vs fiction"; Fort Lauderdale, FL — January 2017
    - Duke University Department of Obstetrics and Gynecology Grand Rounds, "Urogynecologic Mesh Complications: what is the science telling us?" — June 2017
    - Invited Member of POP & Incontinence Workgroup: "National Women's Health Technologies Coordinated — September 2017

Registry Network (CRN) Think-Tank", collaboration between the FDA, the National Institute of Health (NIH)/National Library of Medicine (NLM), the Office of the National Coordinator for Health Information Technology (ONC), the American Congress of Obstetricians and Gynecologists (ACOG), American Urogynecologic Society (AUGS), clinicians, industry and other stakeholders, Silver Spring, MD

- Invited Speaker: The Royal Society of Science Meeting, London, England "Towards rebuilding vaginal support utilizing an extracellular matrix bioscaffold" — October 2017
- Invited Speaker: Endowed David Nichols, MD lectureship, Grand Rounds, Womens & Infants Hospital, Providence, RI, "The science behind urogynecologic meshes: learning from current products to improve future materials" — November 2017
- Invited Speaker: Reproductive Sciences Seminars, University of Colorado, Aurora, CO, "The Science Behind Urogynecologic Meshes: Learning from Current Products to Improve Future Materials" — March 2018

2. Local Meetings: Magee-Womens Research Institute Works-in-Progress Seminar Series
   - **Knight KM.** Development of an Elastomeric Auxetic Mesh for Prolapse Repair: An Alternative to Polypropylene Mesh. Magee-Womens Research Institute Works-in-Progress Seminar Series, Pittsburgh, PA, November 2017

What do you plan to do during the next reporting period to accomplish the goals?
During the next and final reporting period, we will complete Aim 3 which will involve biomechanical, biochemical, and histomorphological analyses of the tissue explants. We will also submit the findings of our work for publication. We will continue to explore the mesh wrinkle as a source of mesh related complications. We will resubmit our R01 further refining the properties of an elastomeric mesh manufactured from PCU. We will file a provisional patent on this device.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
The development of this product has significantly impacted the Urogynecologic and Pelvic Reconstructive Surgery Community as surgeons are very interested in novel materials that overcome the limitations of polypropylene mesh.

What was the impact on other disciplines?
Once the device is tested in large animals, we predict that it will be highly applicable to other disciplines that utilize mesh including general (abdominal and inguinal hernia repair) and thoracic surgery (diaphragmatic hernia repair).

What was the impact on technology transfer?
None to date although we anticipate filing patent rights over the next several months.

What was the impact on society beyond science and technology?
None to date although we anticipate impacting the lives of women with prolapse and incontinence including military women.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change: Alternate elastomer: Although we initially proposed to use PVA as one of the elastomers, when studied at a microscopic level, we found that it is microporous – a property that would increase susceptibility to harboring bacteria and becoming infected. We are continuing to explore PDMS.

Actual or anticipated problems or delays and actions or plans to resolve them – we are working with Ken Gall – a material scientist at Duke University. We are currently using the elastomer polycarbonate urethane which is a tougher material than PDMS and is nonporous. Regarding delays in completing the project, it took us longer than anticipated to complete the implantations than anticipated. However, to date the implantations are completed and we will be completing the final experimental endpoints listed in the original scope of the work on the tissue explants during the no cost extension time period.

Changes that had a significant impact on expenditures - none
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents - none
Significant changes in use or care of human subjects: N/A
Significant changes in use or care of vertebrate animals: none
Significant changes in use of biohazards and/or select agents: N/A

6. PRODUCTS

- **Publications, conference papers, and presentations**

Presentations:

- **Knight K.**, Abramowitch S., Moalli P. In vivo Evaluation of the Host Response to an Elastomeric Mesh: An Alternative to Polypropylene Mesh. AUGS 38th Annual Scientific Meeting, Providence, Rhode Island, October 3-7, 2017

- **Knight KM.**, King GE., Palcsey SL., Moalli PA., Abramowitch SD. Impact of Prolapse Mesh on Vaginal Smooth Muscle Function: A Comparison Between the Rabbit and Nonhuman Primate. Society for Pelvic Research, Reno, Nevada, December 2-3, 2017

- **Knight KM,** Moalli PA, Abramowitch SD. Development and Evaluation of an Elastomeric Mesh for Pelvic Organ Prolapse Repair: An Alternative to Knitted, Polypropylene Mesh. 8th World Congress of Biomechanics, Dublin, Ireland, July 8-12, 2018

- **Knight K,** Abramowitch S, Moalli P. *In vivo* Evaluation of the Host Response to an Elastomeric Mesh: An Alternative to Polypropylene Mesh. Magee-Womens Research 9-90 Summit, Pittsburgh, PA, October 9-10, 2018

- **Knight KM,** Artsen AM, King GE, Palcsey SL, Abramowitch SD, Moalli PA. The New Zealand White Rabbit: An Alternative Model for Studying the Impact of Polypropylene Mesh on Vaginal Smooth Muscle Morphology and Function. AUGS 39th Annual Scientific Meeting, Chicago, Illinois, October 9-13, 2018

- **Iftikhar, A**. Nolfi AL, Artsen AM, Moalli PA. A Clinically Relevant Rabbit Surgical Model of Pelvic Reconstruction to Evaluate the Immune Response to Mesh in the Abdomen and Vagina. AUGS 39th Annual Scientific Meeting, Chicago, Illinois, October 9-13, 2018

Website(s) or other Internet site(s): Our organization has updated the Magee-Womens Research Institute Website which details our research projects and publications.
https://mageewomens.org/investigator/pamela-moalli-md-phd/

- Technologies or techniques: none

- **Inventions, patent applications, and/or licenses:** The University of Pittsburgh has decided not to patent our technology; thus, we are in the process of having the technology released to us so that we may pursue a patent application with another entity.

- Other Products: none

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	Pamela Moalli, Principal Investigator
Nearest person month worked:	0.7
Contribution to Project:	She oversees the biochemical, histomorphology and immunofluorescence outcomes. She holds weekly lab meetings with all of the personnel and staff included in this grant, review all technical aspects of data procurement, data obtained from the experiments, data analysis, and manuscript preparation.
Name:	Rui Liang, Research Associate
Nearest person month worked:	0.6
Contribution to Project:	Dr. Liang will be in charge of overseeing day to day activities regarding the biochemical, histomorphology and immunofluoresence outcomes.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Pamela Moalli, Principal Investigator
-DOD: 0.7 Calendar Months for Initial No Cost Extension

What other organizations were involved as partners? Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS (If applicable)
- COLLABORATIVE AWARDS NA
- QUAD CHARTS NA

9. APPENDICES: