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1 Introduction

In the life of a combat soldier, traumatic cataract in ocular lenses may result from blast loading, whereby (i) the lens capsule (Fig.1) is perforated by intraocular foreign bodies (IOFBs [Walter, 1962, Mader et al., 1993, Parver et al., 1993, Wong et al., 1997, Mader et al., 2006, Weichel and Colyer, 2008]) which in turn damage the lens fiber cells, (ii) the lens is loaded fluid dynamically by the surrounding aqueous and vitreous humors [Banitt et al., 2009] (see Fig.1), and/or (iii) the lens internal substance (crystallins lens fiber cells) is stressed by the passing shock wave. Traumatic cataract can result in a partially or fully clouded lens, complete dislocation of the lens (floating between aqueous and vitreous humors, see Fig.1), or zonule rupture such that partial or full vision loss may occur. The mechanisms of traumatic cataract formation that may require cataract surgery (implantation of an intraocular lens (IOL)) are not well understood in comparison to the mature and ever-improving surgical technology and procedures. The **hypothesis of the research** is that an ultrastructurally-based computational finite element model of the ocular lens subjected to blast loading can assist in better understanding how traumatic cataract is formed in the combat soldier, and in turn improve our understanding of traumatic cataract in civilians whose eyes are subjected to impact loading. The **scope of the research** is to develop a multi-scale, ultrastructurally-based, computational model of the ocular lens subjected to blast loading, in conjunction with imaging methods to identify lens capsule and internal substance structure and mechanical experiments for calibrating material model parameters.

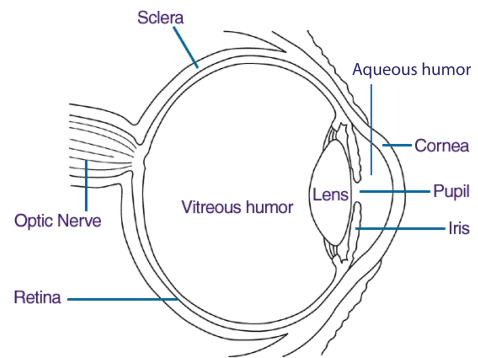


Figure 1. Eye cross-section.
www.nei.nih.gov.

2 Body

The research Tasks focussed on over the past year have been the Cryo-EM/ET imaging of lens capsule type IV collagen (Task 6), nanoindentation of lens capsules (Task 5), and multiscale computational modeling (Task 1-3). A new PhD student has been recruited to the project, Ms. Julia Taussig, who is focussing on Tasks 5 and 6.

A research collaboration with Dr. Harvey Burd at Oxford University has been established that began during PI Regueiro's sabbatical there in Autumn 2014. Dr. Burd visited CU-Boulder in April 2015 to continue research interactions. Such collaboration has resulted in a published journal article (attached, and see Reportable Outcomes), as well as an interdisciplinary research proposal to the NIH (see Reportable Outcomes).

A multiscale computational model of the lens capsule, within a combined Eulerian-Lagrangian approach to large deformational loading of the lens, and solid-fluid interaction, is still being pursued in collaboration with Assoc. Prof. Franck Vernerey and his graduated PhD student, Dr. Louis Foucard, with assistance from PI Regueiro's PhD student, Mr. Boning Zhang.

We are currently working within a No Cost Extension (NCE), which will end 30 September 2016.

Next, research on the Tasks over the past year are summarized.

Task 5. smooth and sharp nanoindentation testing on porcine lenses to approximate lens capsule multiscale elasticity and strength parameters, and mechanical parameters of internal lens substance material: 5a - Design and fabricate a fixture to clamp lens capsules for nanoindentation onto samples immersed in aqueous solution. 5b - Test use of new fixture in nanoindentation machine. 5c - Conduct smooth nanoindents on porcine lens capsules.

Ms. Taussig has been conducting nanoindentation tests on well-defined materials such as polycarbonate to learn how to properly nanoindent materials with the newly-obtained Integrated Nanoindenter-Raman Spectroscopy Instrument in Dr. Virginia Ferguson's lab, and to analyze the results. She is also nanoindenting soft/compliant materials and thin materials (e.g., thin polydimethylsiloxane samples with varying crosslinking ratios) and analyzing the results in preparation to properly nanoindent ocular lens capsules which are also soft and thin. She is learning about different analysis methods such as a viscoelastic analysis for compliant materials and poroelastic analysis for permeable materials. Ms. Taussig is also trying to determine how to properly attain Raman spectra of ocular lens capsules using the Integrated Nanoindenter-Raman Spectroscopy Instrument. A question to be answered is if Raman spectra change with location on the ocular lens capsule (e.g., anterior versus posterior orientation). She tried to obtain spectra but attained inconclusive results. She is conducting a literature search to determine how to attain more conclusive results.

Task 6. imaging of lens fiber cell geometry using confocal laser scanning microscopy (CSLM), and type IV collagen ultrastructure in lens capsule using cyro-electron tomography: 6b - On as-received porcine lens capsules, image type IV collagen ultrastructure in lens capsule using cyro-electron tomography.

We met with the two lab directors, Dr. Thomas Giddings and Dr. Andreas Hoenger, and research associate Ms. Cynthia Page to re-start the research with Ms. Taussig being trained in the imaging techniques. Ms. Taussig conducted a Dimethylmethylene Blue Assay (DMMB) and Cryo-EM imaging study to determine whether she could remove glycosaminoglycans (GAGs) from ocular lens capsules in order to isolate the type IV collagen meshwork. The intent was to remove GAGs from the ocular lens capsule to increase ease of visualization of the type IV collagen network.

DMMB Assay: Ms. Taussig soaked lens capsules in trypsin and put the samples on a shaker for varying amounts of time: 0 hr, 1 hr, 16 hr, and 24 hr (note: also called "trypsinization") to digest and remove the GAGs. She used a plate reader to find the samples' absorbances of wavelengths of light known to be used to find concentrations of GAGs in samples (she conducted a DMMB assay used by collaborators in Dr. Renschler's laboratory at CU-Boulder). She made solutions with varying concentrations of chondroitin sulfate (a GAG molecule) to make a standard curve. She used Matlab (The MathWorks, Inc.) to find a linear fit for the standard curve, and used the linear fit equation to find concentrations from absorbance values for the ocular lens capsule triplicate samples for each time point (0 hr, 1 hr, 16 hr, and 24 hr). She found that as time of trypsinization increases, more GAGs are removed (less GAGs remain in the tissue). After 16 hours, the rate of GAG-removal significantly decreases. It appears that little additional GAG-removal occurred between 16 hours and 25 hours of trypsinization. Ms. Taussig plans to repeat the previously-mentioned experiment with more tissue samples to increase significance of results about GAG removal via trypsinization. She is also planning on finding an assay to determine how much type IV collagen is removed during trypsinization. If trypsinization removes a significant amount of

collagen from ocular lens tissue, other enzymes will need to be explored for GAG removal to improve imaging of the type IV collagen structure.

CryoET Imaging: Ms. Taussig is currently working on CryoET of ocular lens capsules with Ms. Page. She is trying to image ocular lens capsules that have not been trypsinized, ocular lens capsules that have been trypsinized for one hour, and ocular lens capsules that have been trypsinized for 24 hours.

2c: Formulation and finite element implementation of multiscale perforating finite strain biphasic mixture (solid and fluid) solid-shell continuum model of lens capsule in Tahoe, to model cutting/ perforation of lens capsule based on implementation in subtasks 1c and 2b. 3b: Using result of subtask 3a, formulate and implement multiscale hierarchical, anisotropic, lens fiber cell equivalent soft viscoelastic constitutive model of the internal lens substance.

For the computational modeling, we are continuing to work on a large deformation hybrid Lagrangian-Eulerian simulation of the lens puncture tests in order to eventually model penetration by Intra-Ocular Foreign Bodies (IOFBs), but also shock propagation and solid-fluid interaction between the lens and vitreous and aqueous humors. This is in collaboration with Assoc. Prof. Franck Vernerey, and his graduated PhD student Dr. Louis Foucard, along with PI Regueiro's PhD student, Mr. Boning Zhang. The goal is to have one paper in review during the second year of the NCE.

Axisymmetric, multiscale, hyperelastic, membrane finite element model of the lens capsule at finite strain, with extension to poromechanics

For my sabbatical in Autumn 2014, I spent it in the Engineering Science Department at Oxford University, hosted by Dr. Harvey Burd. Dr. Burd is an expert in researching the mechanics of the ocular lens, and developing finite element models that can be used to simulate experimental test conditions, as well as physiologically-accurate loading conditions during accommodation. During my stay at Oxford, I worked with Dr. Burd on revising an earlier axisymmetric, multiscale, hyperelastic, membrane finite element model of the lens capsule at finite strain that he developed, helping to clarify the formulation of the global consistent tangent accounting for the multiscale network model of type IV collagen. Having a properly-formulated consistent tangent will lead to improved numerical simulation performance, making computations faster and possibly obtaining converged solutions that would not otherwise have converged. Our collaborative efforts resulted in a published journal paper (attached): Burd, H.J., Regueiro, R.A. (2015) "Finite element implementation of a multiscale model of the human lens capsule," *Biomech. Model. Mechanobiology* 14(6):1363-1378. Dr. Burd visited my research group in April 2015. We plan to continue our research collaboration, extending the model we worked on together to include poromechanics of the lens capsule. This research falls within the scope of the USAMRMC project. Also, our collaboration has led to an interdisciplinary research proposal submitted to the NIH (see Reportable Outcomes).

3 Key Research Accomplishments

- A tightly-coupled, properly-linearized, multiscale axisymmetric hyperelastic membrane finite element model of the lens capsule was developed, with parameters calibrated against experimental data for human lens capsule tissue, and used to predict other human ocular lens mechanics data.

4 Reportable Outcomes

1. Burd, H.J., Regueiro, R.A. (2015) “Finite element implementation of a multiscale model of the human lens capsule,” *Biomech. Model. Mechanobiology* 14(6):1363-1378.
2. Presented poster on *Large deformation indentation of porcine ocular lenses: experiments and computational modeling* at the Association for Research in Vision and Ophthalmology (ARVO) conference in Denver, CO, May 3, 2015.
3. Proposal in review: NIH PAR-15-085, “Relating biochemistry to ultrastructure and multi-scale mechanics in predictively simulating tissue mechanical behavior during aging: ocular lens accommodation as example,” 9/1/16 - 8/31/21, \$3,456,868 (PI Regueiro, Co-I Ferguson, Co-I Hoenger, CU-Boulder; Co-I Reilly, UTSA; Co-I Glickman, UTHSCSA; Co-I Burd, Co-I Wilson, University of Oxford, UK)

5 Conclusion

The research progress over the last year has focussed on revitalizing imaging of type IV collagen structure using cryo-electron microscopy and tomography (cryo-EM and ET) with a recently-recruited PhD student, Ms. Julia Taussig. Ms. Taussig is also revitalizing the nanoindentation research on ocular lens capsules using a newly-obtained wet-stage nanoindenter at CU-Boulder.

The collaborations with Dr. Vernerey of CU-Boulder and Dr. Burd of the University of Oxford continue to provide meaningful steps toward developing a multiscale computational model of the ocular lens being able to simulate large deformations under traumatic loading.

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