Elasticity of the Mouse Ocular Lens Capsule as Measured by Osmotic Swelling

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IMPORTANCE

- Animal basement membranes are networks of laminin, type IV collagen, and other proteins essential for physiological functions. Deficiencies or abnormalities in the proteins can lead to muscular dystrophy, kidney disease, hearing loss, and, in the extreme, embryonic demise.
- The mechanical properties of basement membranes define their physiological roles. In an effort to determine how the complex structure influences the functional properties of the basement membrane, the change in mechanical behavior, specifically the Young's Modulus of Elasticity, is being investigated on samples with defective or absent associative proteins as compared to those with intact matrix components.
- Mice have been developed with defects in genes for critical membrane components: a mouse with X-linked Alport syndrome is available for this study. A mutation in a gene encoding one of the six collagen IV monomers results in an unstable collagen matrix due to a loss of two heterotimers. These mice die prematurely of kidney failure.

APPROACH

- The lens capsule has a similar composition to other basement membranes, but it is uniquely thick and can be studied in an uninterrupted state, making it ideal for studying the mechanical properties.
- Previously developed methods for determining the mechanical properties of lens capsules in other animals (pigs, cows, and humans) require dissection of the lens capsule, which is not well suited for the mouse due to the small size of its lens.
- A method utilizing osmotic swelling was developed with the porcine lens capsule to measure the elasticity of the membrane. This technique requires minimal manipulation of the lens capsule.
- Differences in manipulative techniques have given rise to differences in the measurements of mechanical properties. This method may more reliably measure the elastic modulus of the lens capsule because it is remaining in its anatomical state.

METHODS

- Eyes were removed from mice, which were ten to fifteen weeks old, 1 to 12 hours post mortem; the mice were stored on ice in a cold room held at 4°C. The lenses were extracted from the eyes and cleaned while submerged in a solution isotonic to the lens cytoplasm (about 0.9% w/v). Osmotic swelling was achieved by placing the lens in a hypotonic solution (0.1%, 0.3%, or 0.5%); plates were covered to prevent evaporation.
- Images were taken by a camera mounted on a dissection microscope every minute for six hours. The initial radius was obtained from a picture taken while the lens was in the isotonic solution, and the calibration was established from a picture of a calibrated circle.
- The images were analyzed in MATLAB; the equatorial diameter was measured after the colored picture was converted to black and white. The change in radius was plotted against time, and a simulated model was used to determine the elastic modulus.

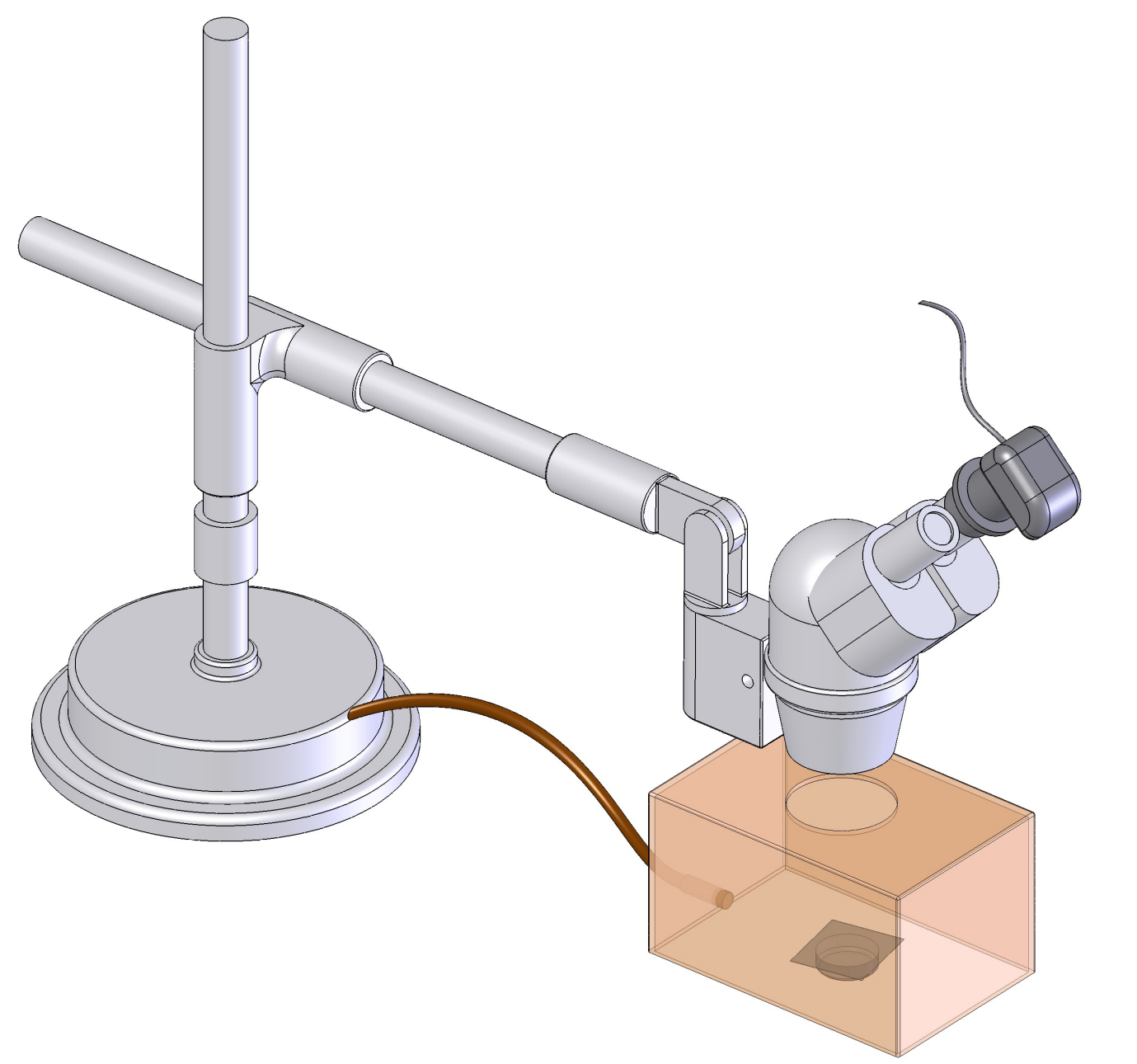


Figure 1: Apparatus set-up.

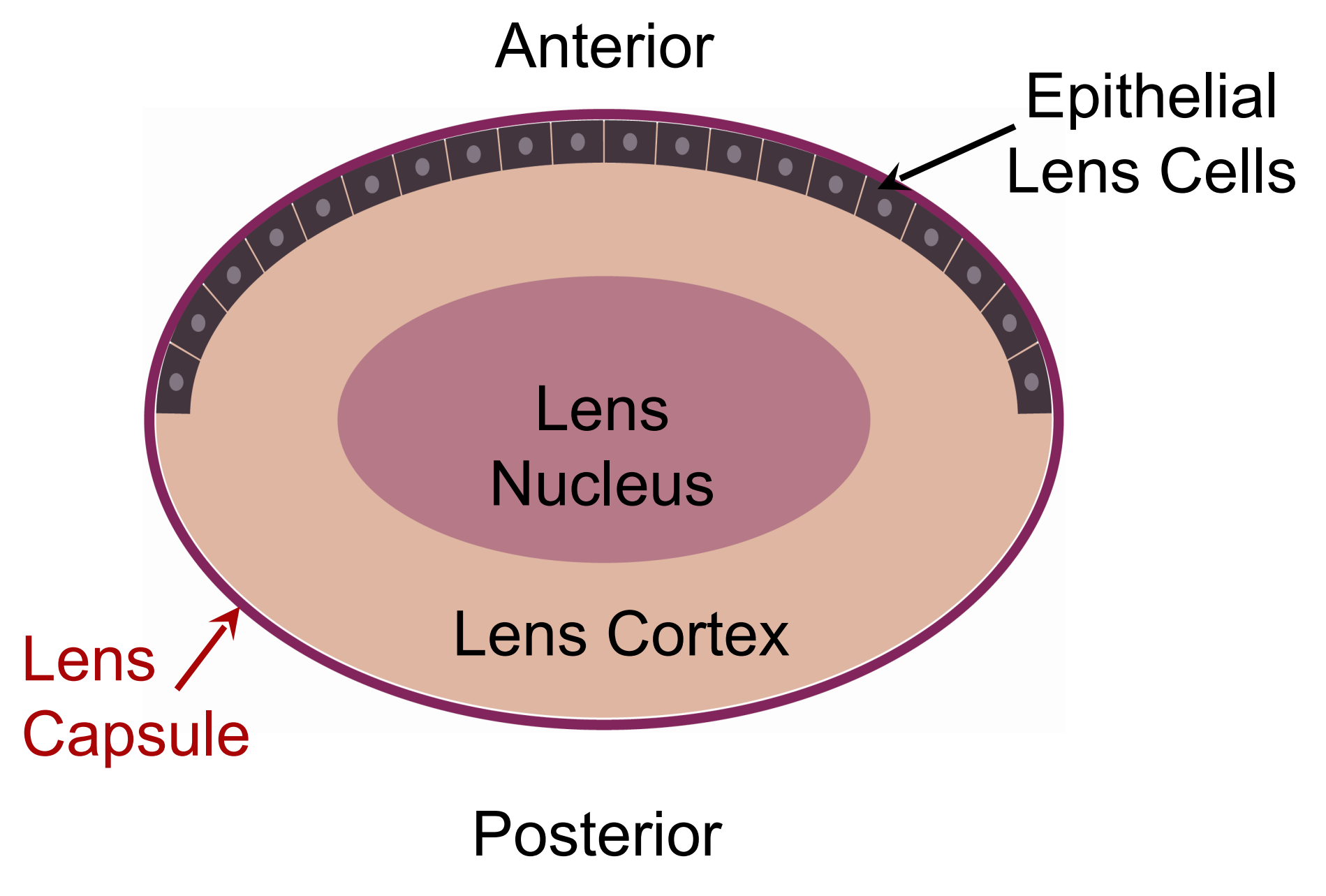


Figure 2: Anatomy of ocular lens.

L_P	Membrane Conductivity per unit Thickness (4.8×10^{-11} m•Pa^{-1}•s^{-1}) [1]
m	Bath Concentration
t	Time
r	Radius
υ	Poisson's Ratio (.47) [2]
h	Lens Capsule Thickness (equatorial: 9.4μm) [3]
R	Universal Gas Constant (8.314 m^3•Pa•K^{-1}•mol^{-1})
T	Ambient Temperature (293 K)

Table 1: Constants and experimental data in the model.

J_v	Solvent Mass Flux
K_T	Cumulative Conductivity
$\Delta\pi$	Osmotic Pressure
ΔP	Mechanical Pressure
r_C	Radius of Core
Zc*	Fixed Charge Density
E*	Young's Modulus of Elasticity
K_M*	Darcy's Conductivity of Mushy Zone

Table 2: Variables and optimized constants(*) in the model.

ACKNOWLEDGEMENTS:
Thank you to: Victor Barocas* for advising me throughout the experiment; Rouzbeh Amini* for developing the MATLAB simulation model and his help applying it to the mouse; Chun Wang* for providing the wild-type mice. *(Department of Biomedical Engineering)

RESOURCES:
[1]Fisher, R. F., 1987. The influence of age on some ocular basement membranes. Eye (London, England) Pt 2, 184-189.
[2]Fisher, R. F., 1969. Elastic constants of the human lens capsule. The Journal of Physiology 1, 1-19.
[3] Danysh, B. P., Czymmek, K. J., Olurin, P. T., Sivak, J. G., Duncan, M. K., 2008. Contributions of mouse genetic background and age on anterior lens capsule thickness. The Anatomical Record 291, 1619-1627.

MODEL

- Lens Expansion

$$J_v = \frac{1}{A}\frac{dV}{dt} = \frac{dr}{dt}$$

- Kedem-Katchalsky membrane transport

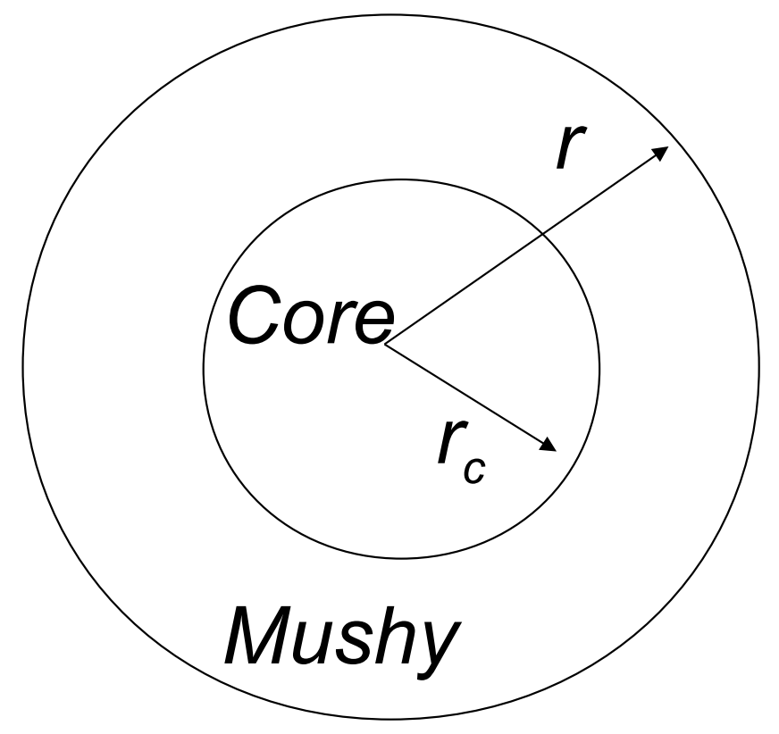
$$J_v = K_T(\Delta\pi - \Delta P)$$

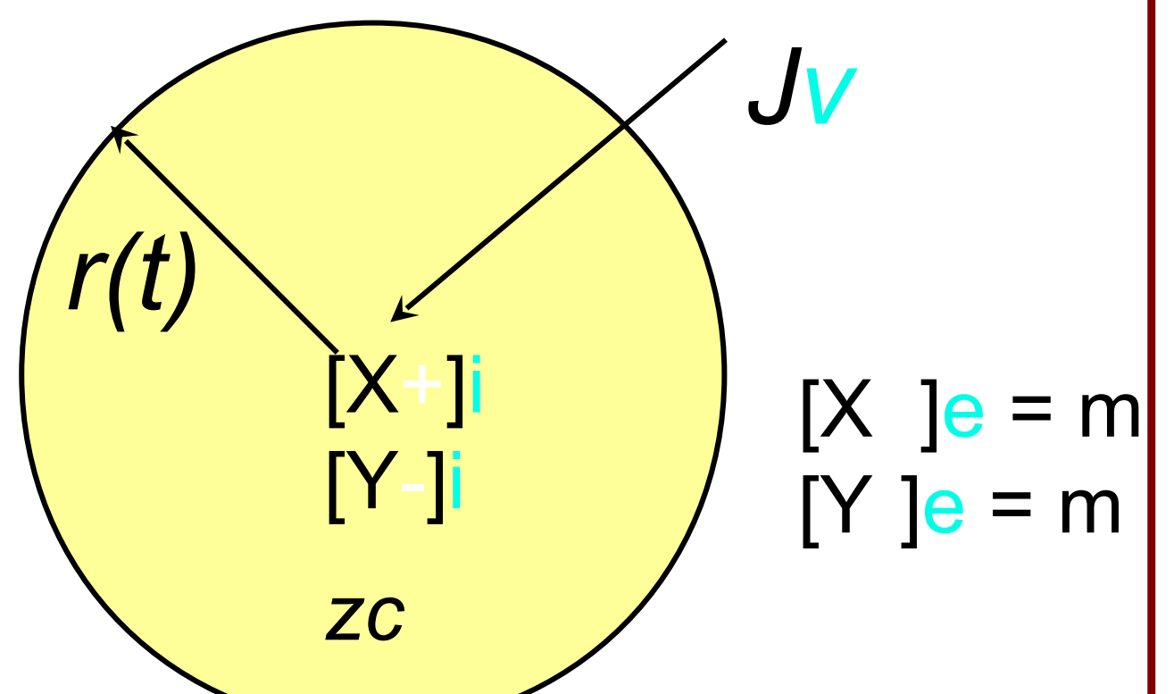
- Law of Laplace

$$\Delta P = \frac{2Eh}{r_0(1-\upsilon)}\left(1-\frac{r_0}{r}\right)$$

- Van't Hoff relation

$$\Delta\pi = 2RT\left(\sqrt{m^2+\left(\frac{r_0}{r}\right)^6\frac{(zc)^2}{4}}-m\right)$$





- From Geometry:

$$V_C = V - V_M = V_0 - V_{M0}$$

$$\frac{V_{M0}}{V_M} = \frac{r_0{}^3 - r_c{}^3}{r^3 - r_c{}^3}$$

- For simplification:

$$r_C = \sqrt[3]{\frac{r_0^3 - f(E,r,zc)r^3}{1-f(E,r,zc)}} \qquad f(E,r,zc) = \frac{2}{zc}\sqrt{\left[\frac{2Eh}{r_0(1-\upsilon)RT}\left(1-\frac{r_0}{r}\right)+m\right]^2-m^2}$$

- Final Governing Equation

$$\frac{dr}{dt} = 2RT\left(\frac{1}{L_p}+\frac{r-r_C}{K_M}\right)^{-1}\left[\left(\sqrt{m^2+\frac{(zc)^2}{4}}-m\right)-\Delta P\right]$$

- The MATLAB *ode45* routine was used to obtain the numerical, simulated solution. Values for the Young's Modulus (E), fixed charge density (zc) and Darcy's Conductivity (K_M) were chosen to minimize the sum of square error between the simulated and experimental radius (r) vs. time (t) data using the MATLAB *fminsearch* function.

RESULTS

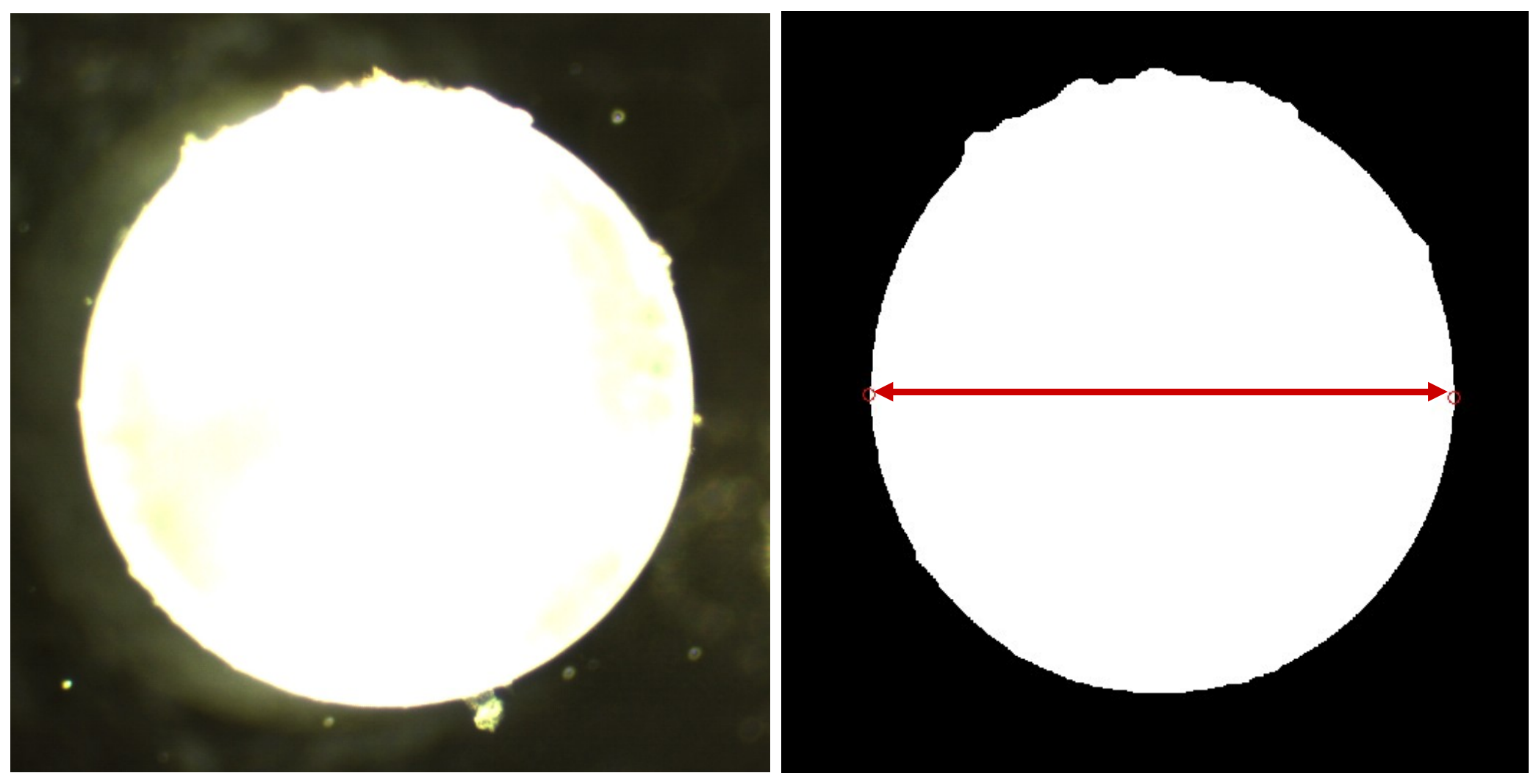
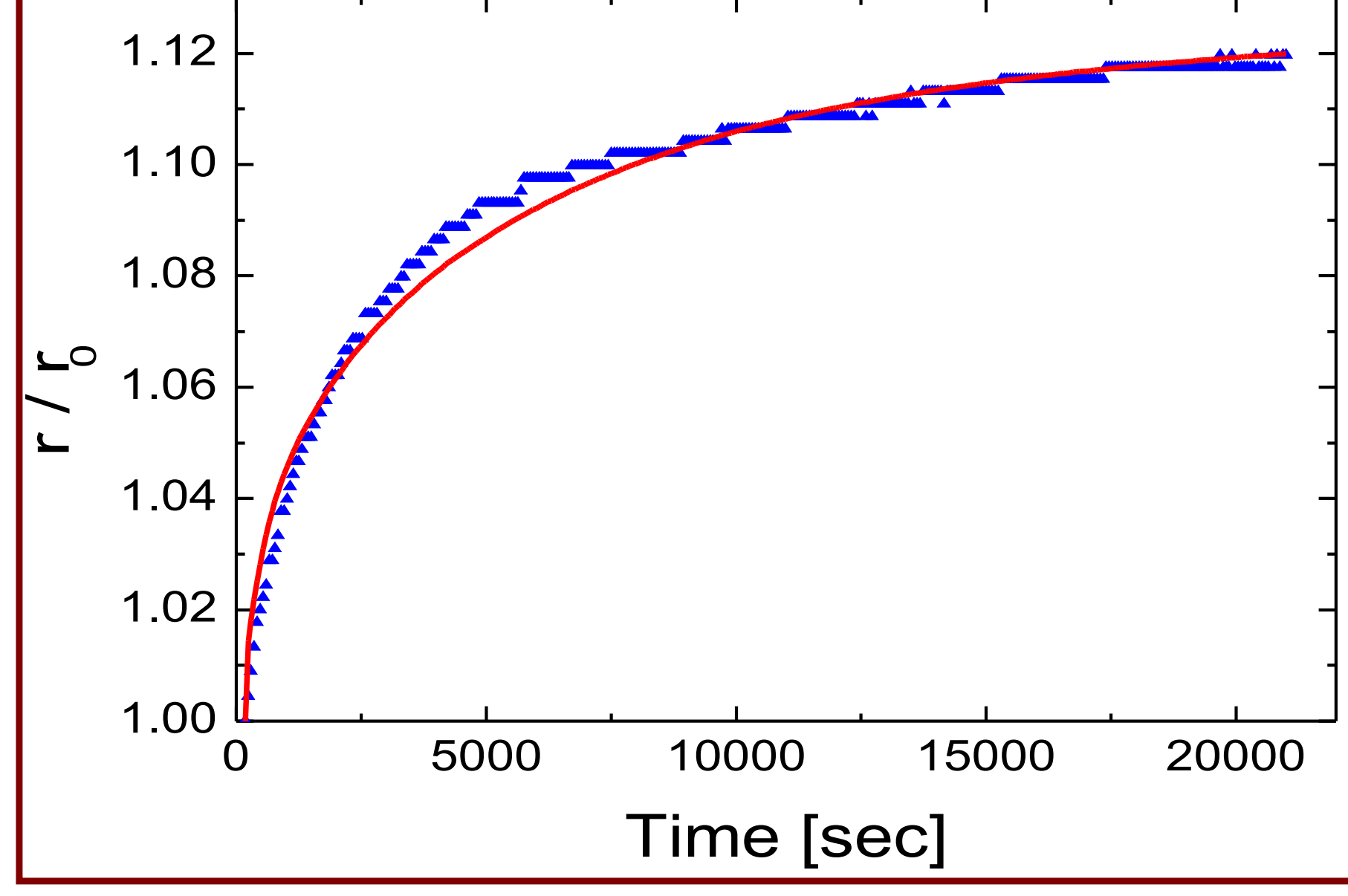


Figure 3: Picture of a lens taken by the microscope- mounted camera (left) and after black and white conversion in MATLAB (right). Red arrow indicates points used for diameter measurement.



Graph 1:
Blue: Experimental Data
Red: Simulated Model
Constants and Model Results:
m=0.3%
r_0=1209 μm
E=62.2 KPa
error=0.0028
zc=500mmol/L
K_M=2.69×10^{-18} m^{2}Pa^{-1}s^{-1}

CONCLUSIONS

- Data will continue to be collected and analyzed until 10 samples are obtained for each hypotonic solution, to more accurately measure the Young's Modulus of Elasticity.
- The same experimental methods will be used for the Alport mouse.
- The Young's Modulus of Elasticity of the wild-type and Alport mouse will be compared and later used in computer modeling of the matrix structure of the basement membrane.
- The osmotic swelling and MATLAB simulation model allowed for the measurement of the elastic modulus without dissection or significant manipulation of the lens. This technique is less complex and expensive than other methods, such as mechanical ring pulling and atomic force microscopy (AFM), and may more reliably measure the elastic modulus of the lens capsule because the lens is remaining in its anatomical state.