Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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A GLITCH IN THE MATRIX: EXPLORING THE ROLE OF PROSTATIC COLLAGEN IN LOWER URINARY TRACT DYSFUNCTION

By

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Chapter 1: Communicating Research to Non-Science Audience for the Wisconsin Initiative for Science Literacy

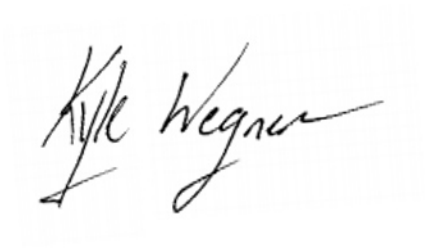
Wegner, K. A.

Preface

My scientific career was born under the Wisconsin Idea, and I have made it my mission to always find ways to demonstrate the real-world value of my scientific endeavors. However, I have found that people cannot value what they do not understand or appreciate as applicable to their lives. It is our responsibility as UW scientists to hone our skills beyond benchwork and learn how we can best share our enthusiasm for science with the public. Scientists who can articulate the core values of their research to those with limited scientific backgrounds invite audiences to form their own opinions, ask their own questions, and discover their own inner scientists. It is my honor to present my research in the following chapter in a form that I feel best communicates its value without the need for extensive background knowledge.

I would like to also extend a special thank you to the Wisconsin Initiative for Science Literacy (WISL) for their efforts to promote literacy in science, mathematics, and technology.

On, Wisconsin!



Your bladder's active nightlife.

Normal urinary function is one of the least discussed but most valued aspects of human health (Rubin et al., 2016). Unfortunately, urinary dysfunction is pervasive in both men and women of advancing age (Girman et al., 1998; Stewart et al., 2003). In fact, nearly all men will experience urinary dysfunction at some point in their lives. This dysfunction has many symptoms but the most commonly observed are:

LOWER URINARY TRACT SYMPTOMS (LUTS)

A weak or slow urinary stream.

A feeling of incomplete bladder emptying.

Difficulty starting urination.

Frequent urination.

Urgency to urinate.

Getting up frequently at night to urinate.

A urinary stream that starts and stops.

Straining to urinate.

These symptoms put tremendous financial and emotional strain on patients and their families. It has been estimated that LUTS treatment carries a financial burden of four billion dollars annually in the United States (Vuichoud and Loughlin, 2015), and LUTS patients suffer from anxiety and depression brought on by lack of control over urinary function (Zhang and Xu, 2018). This finding is especially troublesome given that the projected population of men vulnerable to LUTS will grow by over 12 million individuals by 2030.

Does size really matter?

For decades, LUTS in men was primarily attributed to benign growth of the prostate gland, commonly known as an enlarged prostate or benign prostatic hyperplasia (BPH). Since the prostate gland surrounds the urethra at the base of the bladder, enlargement of the gland can compress the

urethra and inhibit urine flow (Figure 1). Given that most men with LUTS also have an enlarged prostate gland, many experts believed prostate enlargement was the only underlying cause of LUTS. This assumption was further supported by the fact that surgical removal of the prostate is often the most effective treatment for reducing LUTS severity (DiSantostefano et al., 2006; Rassweiler et al., 2006).

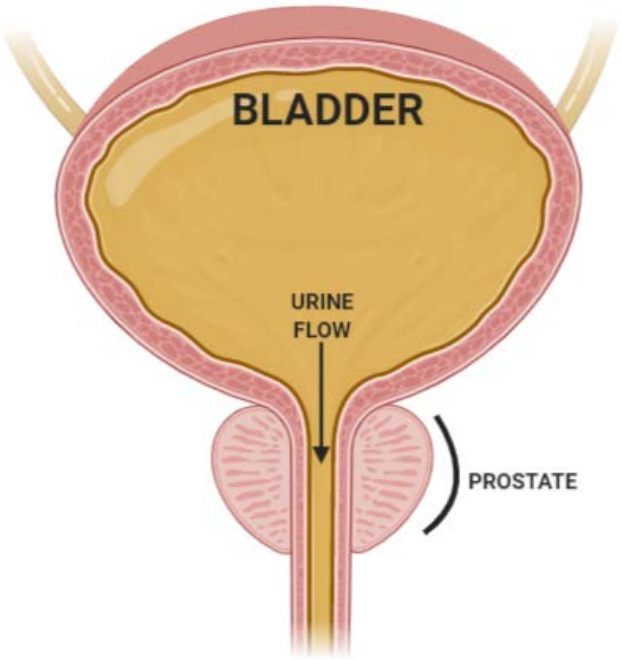


Figure 1. A schematic of the human lower urinary tract. The prostate gland surrounds the urethra at the base of the bladder where the urethra begins (*Biorender, 2017)*.

Given the historical emphasis on prostate size as the sole cause of LUTS, most pharmaceutical treatments were designed to reduce prostate size or alleviate pressure on the urethra. Even when combined, these therapies fail to improve LUTS in 60% of patients and cause adverse events in 14% of patients (DiSantostefano et al., 2006). The lackluster efficacy of prostate-shrinking therapies points toward other underlying causes for LUTS aside from prostate enlargement. In fact, prostate size is actually a poor predictor of LUTS (Lepor, 2005).

If not size, then stiffness?

In patients, prostate stiffness correlates with the severity of LUTS (Ma et al., 2012). The main contributor to stiffness in the prostate gland appears to be the presence of collagen fibers. Collagen is a fibrous structural protein found in the extracellular matrix (ECM) and is the most prevalent protein in the human body. The ECM provides the tissue “scaffold” on which cells attach

and supports three-dimensional tissue structure. Higher abundance of collagen fibers correlates with increased tissue stiffness and decreased elasticity (Borges et al., 2005; Ma et al., 2012). Most "fibrotic" diseases (liver fibrosis, pulmonary fibrosis, kidney fibrosis) are characterized by a pathological accumulation of collagen fibers. Similar to how a scar forms, collagen in these tissues is produced during a wound healing response. In fibrotic disease, a variety of triggers continually activate this response and eventually the excess collagen becomes so dense the organ fails.

Under normal conditions, the prostate gland consists of a loosely connected network of collagen fibers which allow the urethra to expand during urination. Interestingly, recent studies have revealed that this is not true for many men with LUTS. Prostate collagen abundance and stiffness are both greater in men with LUTS compared to men without symptoms (Ma et al., 2012). This finding has opened a new field in urology focused on understanding the role of prostate *stiffness* rather than studying prostate *size* alone. To support this new area of research, new tools are needed to accurately quantify collagen in the prostate and the effects on urinary function. The goal of this dissertation work was to address this need and support new hypotheses in urologic research.

How do we measure prostate collagen content?

A lack of methods to quantify collagen abundance was a major roadblock in understanding the composition of prostate ECM. Existing methods produced unclear results and relied on decades-old staining methods. To enhance visualization of the collagen network in the prostate, we optimized a new staining and quantification protocol for collagen (Wegner et al., 2017b). We stain slices of prostate tissue with a dye called picrosirius red (PSR). PSR specifically attaches to collagen fibers and can be imaged using specialized fluorescent microscopes (Figure 2.). Researchers can then see where collagen fibers are located and measure their abundance.

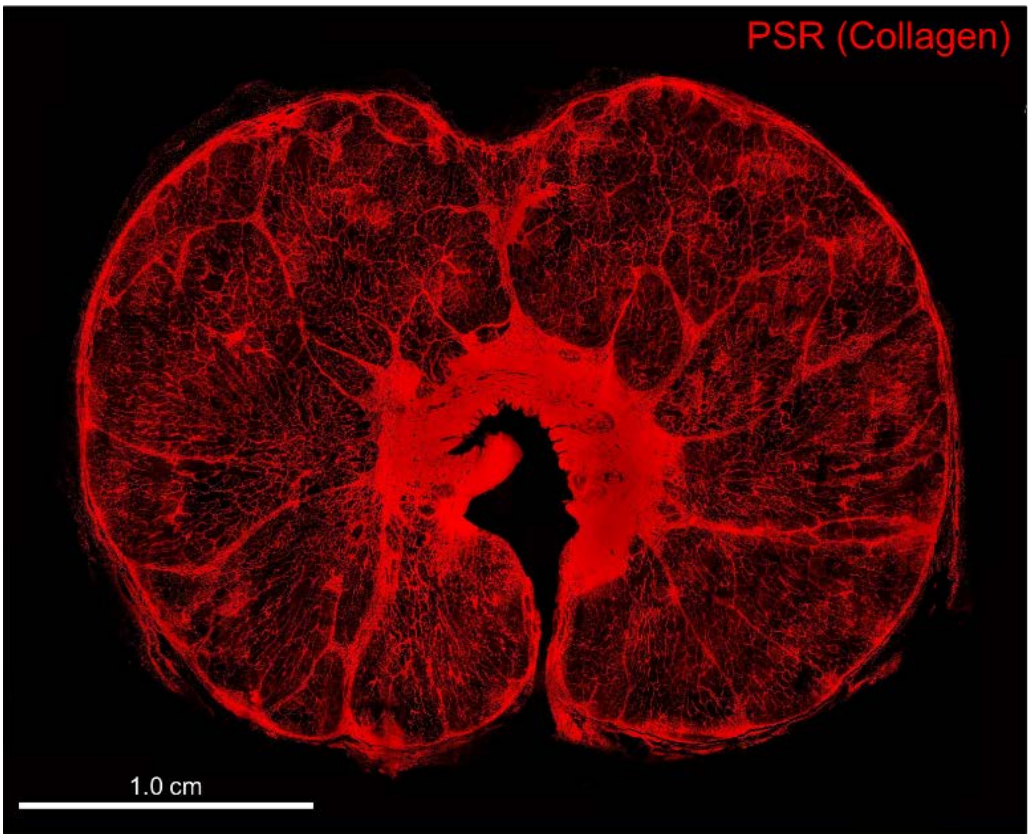


Figure 2. An example of PSR-stained collagen in a dog's prostate gland. This prostate gland was collected from a domestic dog euthanized for medical reasons unrelated to this study. Interestingly, the dog and human prostate are remarkably similar. This prostate was cut into thin slices and stained with PSR. Using a fluorescent microscope, we can visualize the collagen fibers which are seen in red.

Traditional methods of quantifying the amount of collagen in an image were often based on scoring systems. These systems required extensive training for the scorers and ultimately were subjective measurements, leaving them susceptible to bias during scoring. In many cases, scorers would disagree about how to score a particular tissue sample. To accurately and objectively quantify collagen, we trained a computer program to scan images of PSR-stained tissues, identify PSR-stained fibers, and measure the area of any fiber structures within the tissue (Bredfeldt et al., 2014). This software objectively and rapidly quantifies collagen fiber density, length, diameter, and orientation (Figure 3.). This type of analysis has never been performed in the prostate and we

can now identify subtle changes to the collagen network that are overlooked using traditional tissue scoring.

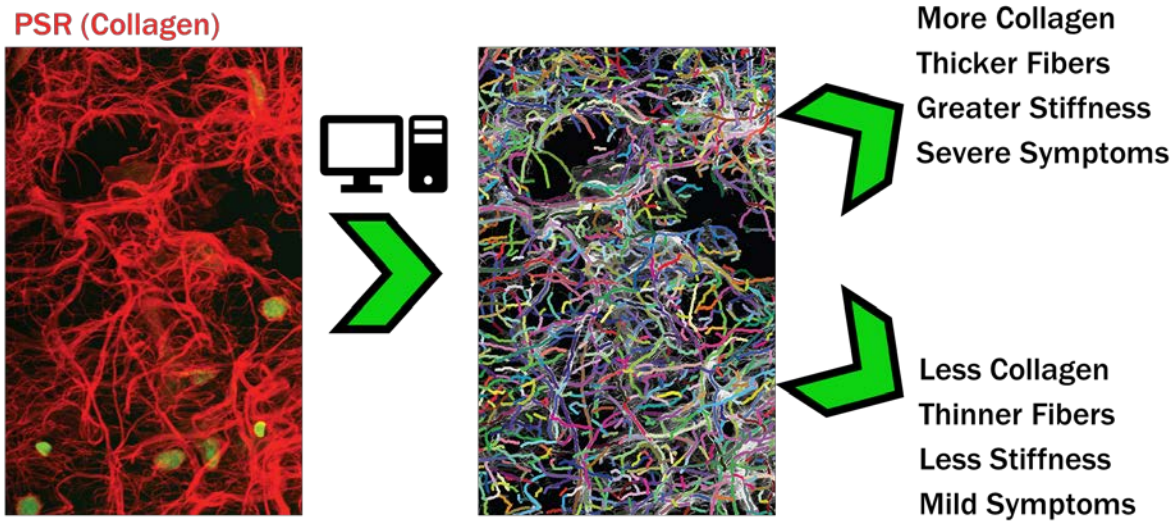


Figure 3. We can use specialized computer software to track fibers in PSR-stained tissues. The computer measures the abundance of the collagen as well as the size and shape of the fibers.

Using animal models to understand the effects of excessive prostate collagen.

Human studies have shown a correlation between the presence of prostate collagen and LUTS, but a cause and effect relationship cannot be established from human samples alone. To fully understand the mechanisms of collagen production and effects on urinary function we must turn to animal models. Mice are uniquely suited as a biological model to examine this relationship because prostate collagen accumulation in mice is also associated with urinary dysfunction (Gharaee-Kermani et al., 2013; Lee et al., 2014; Nicholson et al., 2012; Ricke et al., 2016; Wong et al., 2014). Characterizing urinary dysfunction in mice is challenging because mice cannot communicate the presence or absence of symptoms and little is known about normal mouse urinary behaviors. We addressed these hurdles by devising an affordable, non-invasive, and repeatable assay to quantify urinary function. We used the filter paper void spot assay (VSA) to track urinary

function over time (Bjorling et al., 2015; Hill et al., 2018; Keil et al., 2016; Wegner et al., 2018). In this assay, mice are placed into a standard mouse cage lined with paper for a four-hour period. During this time, the mouse urinates on the paper. The mouse is then removed from the cage, and the paper dried and imaged under an ultraviolet light. To enhance the utility and practical application of this assay, we developed novel computer software to automate the quantification process. Our software identifies any changes in total urine spot number/area, total area per urination event, % area of largest spot, and % urine area in cage center and corners. Using this data, we can track urinary behavior and identify any changes in real time (Figure 4.).

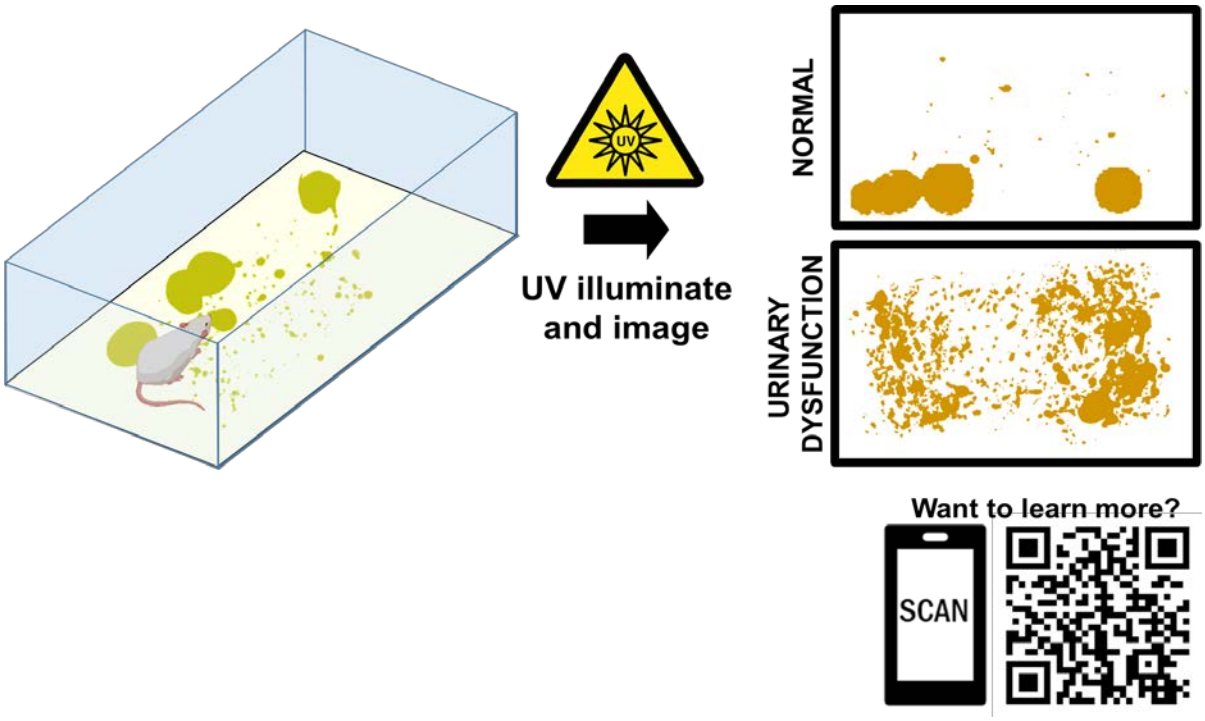


Figure 4. The VSA method for measuring mouse urinary function. Using simple, cost-effective tools (paper, UV light source), we can monitor the urinary behaviors of mice in real time as they age or as disease progresses.

How do we identify which cells are producing collagen?

We now have precise methods for quantifying prostate collagen content and evaluating urinary function in mice. The final addition to these methods is a technique for identifying which

cells are responsible for making excess collagen in the prostate. Many different cell types exist in the prostate and all are important for the prostate to function. Much like a criminal investigation, finding disease-causing cell types requires dedicated efforts towards finding incriminating evidence. However, once the burden of proof has been met, pro-fibrotic cells can be precisely targeted with pharmaceutical agents to stop them from making too much collagen.

To find the cellular culprit behind excess collagen production, we devised an identification system based around immunohistochemistry (IHC). IHC is a methodology for visualizing proteins made by specific cell types within a tissue. For example, smooth muscle actin is a protein commonly produced by myofibroblasts, a common collagen-producing cell type in fibrotic disease. We use IHC to fluorescently mark smooth muscle actin-producing cells and image them in thin tissue slices. Once imaged, we can identify the location and abundance of any cells expressing smooth muscle actin in the tissue slice. By using increasingly complex combinations of protein-markers, we can get more and more refined cell identification. Like a fingerprint, the protein signature of a cell type is commonly used to assign an identity to a cell. However, different research groups don't always agree on which markers best label a specific population of cells. A universally accepted tool is needed to bring consensus amongst research groups on the true identity of the cell. To meet this need, we used IHC to create a dichotomous key for cell identification in the prostate (Wegner et al., 2017a). Dichotomous keys are often used by naturalists to identify different plant or animal species. A dichotomous key is a series of yes-or-no observations based on appearance to narrow down potential species identities until only one remains. We used the same technique, but substituted appearance for protein expression (Figure 5.). This tool makes it possible for researchers around the world to identify cells based on the exact same features and directly compare data from different labs.

Another feature of our IHC dichotomous key is its compatibility with PSR-based collagen detection. We altered the PSR method slightly to use it on the same tissue slices as IHC for cell identification (Wegner et al., 2017b). We can now measure the amount of collagen within a tissue and visualize what kinds of cells are present in areas with particularly dense collagen. This is a

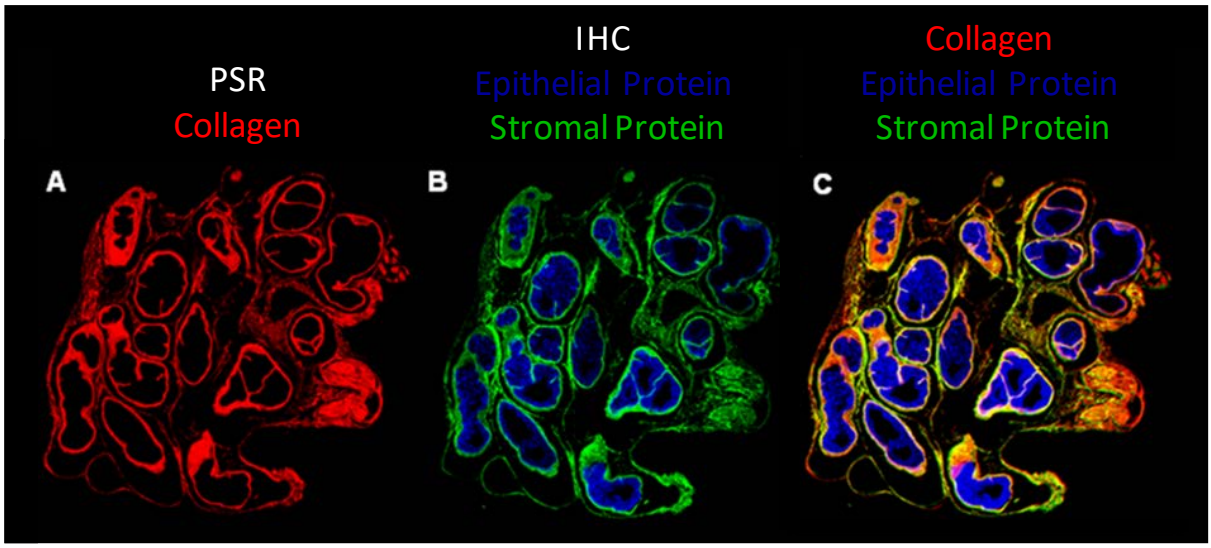


Figure 6. A mouse prostate section stained to label collagen and specific cell types. In this image we used PSR to stain collagen fibers (Panel A, collagen in red). We then perform an IHC experiment on the same tissue slice to find specific cell types of interest (Panel B, epithelial cell protein in blue and stromal cell protein in green). These images can be overlaid and we can measure which cell types are most abundant in areas with dense collagen (Panel C). We use this technique to hunt down collagen-producing cells in the prostate.

powerful tool for learning which cells types are most common in prostate slices with high collagen content and gives us insight into which cell types are likely producing collagens.

What does this mean for men with LUTS?

Now armed with the array of techniques described above, we can finally address the role of collagen in the prostates of men with LUTS. Using PSR collagen detection we identified a subset of LUTS patients that have very dense collagen immediately surrounding the urethra (Figure 7.). We also observed that the amount of collagen appears to increase with age in some

men. By classifying patients into two groups—those that have too much collagen or those with a normal amount—we can better predict which patients might respond to anti-collagen therapies.

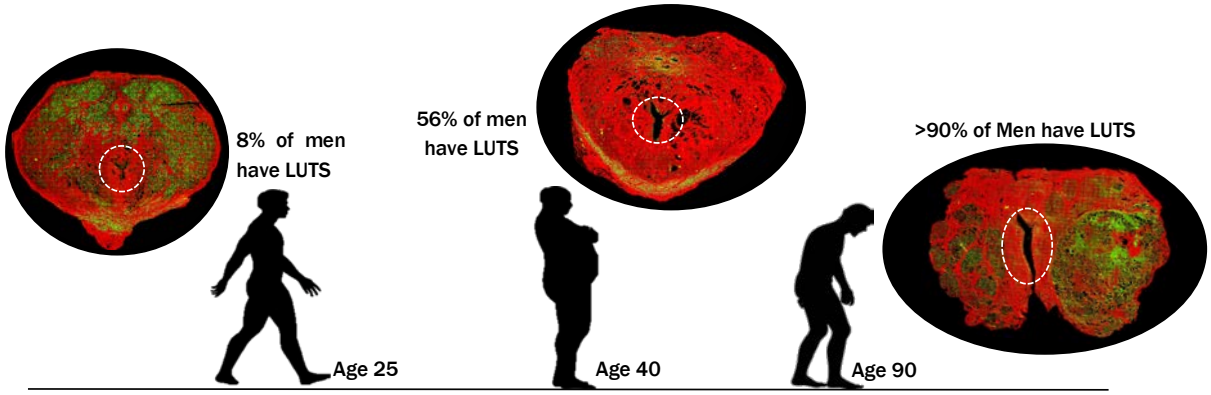


Figure 7. A timeline of prostate collagen content. We have found that as men age, the collagen content of the prostates appears to increase. This is especially evident in the prostate tissue immediately surrounding the urethra (urethra identified by white dashed circles). Many men seeking treatment for LUTS can be characterized by this dense region of collagen.

Using IHC we identified a specific cell type that is found in the collagen-rich region around the urethra. These cells have expression profiles matching that of a fibroblast. Fibroblasts are abundant throughout the body and are known to produce collagen (Narayanan et al., 1989). We suspect that this fibroblast cell type is the one responsible for producing excess collagens in the prostate. Interestingly, a cell type with a similar protein expression profile also exists in the mouse prostate and becomes more numerous in mice that have excess prostate collagen. Whether it is the sole culprit behind collagen accumulation or not, this cell type clearly plays a crucial role in the pathological production of collagen in the prostate gland and is a prime candidate for further experiments to create new LUTS therapies. Taken together, the data generated from this dissertation should convince both scientists and clinicians that prostate size is not the whole story and that we should consider new techniques to treat LUTS that may be more effective in many patients.

Works Cited

BioRender, 2017. https://biorender.com/.

Bjorling, D.E., Wang, Z., Vezina, C.M., Ricke, W.A., Keil, K.P., Yu, W., Guo, L., Zeidel, M.L., Hill, W.G., 2015. Evaluation of voiding assays in mice: impact of genetic strains and sex. American Journal of Physiology - Renal Physiology 308, F1369–F1378. https://doi.org/10.1152/ajprenal.00072.2015

Borges, L.F., Taboga, S.R., Gutierrez, P.S., 2005. Simultaneous observation of collagen and elastin in normal and pathological tissues: analysis of Sirius-red-stained sections by fluorescence microscopy. Cell and Tissue Research 320, 551–552. https://doi.org/10.1007/s00441-005-1108-6

Bredfeldt, J.S., Liu, Y., Pehlke, C.A., Conklin, M.W., Szulczewski, J.M., Inman, D.R., Keely, P.J., Nowak, R.D., Mackie, T.R., Eliceiri, K.W., 2014. Computational segmentation of collagen fibers from second-harmonic generation images of breast cancer. J Biomed Opt 19. https://doi.org/10.1117/1.JBO.19.1.016007

DiSantostefano, R.L., Biddle, A.K., Lavelle, J.P., 2006. An evaluation of the economic costs and patient-related consequences of treatments for benign prostatic hyperplasia. BJU Int. 97, 1007–1016. https://doi.org/10.1111/j.1464-410X.2005.06089.x

Gharaee-Kermani, M., Rodriguez-Nieves, J.A., Mehra, R., Vezina, C.A., Sarma, A.V., Macoska, J.A., 2013. Obesity-induced diabetes and lower urinary tract fibrosis promote urinary voiding dysfunction in a mouse model. The Prostate 73, 1123–1133. https://doi.org/10.1002/pros.22662

Girman, C.J., Jacobsen, S.J., Tsukamoto, T., Richard, F., Garraway, W.M., Sagnier, P.P., Guess, H.A., Rhodes, T., Boyle, P., Lieber, M.M., 1998. Health-related quality of life associated with lower urinary tract symptoms in four countries. Urology 51, 428–436. https://doi.org/10.1016/s0090-4295(97)00717-6

Hill, W.G., Zeidel, M.L., Bjorling, D.E., Vezina, C.M., 2018. Void spot assay: recommendations on the use of a simple micturition assay for mice. Am. J. Physiol. Renal Physiol. 315, F1422–F1429. https://doi.org/10.1152/ajprenal.00350.2018

Keil, K.P., Abler, L.L., Altmann, H.M., Bushman, W., Marker, P.C., Li, L., Ricke, W.A., Bjorling, D.E., Vezina, C.M., 2016. Influence of animal husbandry practices on void spot assay outcomes in C57BL/6J male mice. Neurourol. Urodynam. 35, 192–198. https://doi.org/10.1002/nau.22692

Lee, S., Yang, G., Mulligan, W., Gipp, J., Bushman, W., 2014. Ventral Prostate Fibrosis in the Akita Mouse Is Associated with Macrophage and Fibrocyte Infiltration. J Diabetes Res 2014. https://doi.org/10.1155/2014/939053

Lepor, H., 2005. Pathophysiology of Lower Urinary Tract Symptoms in the Aging Male Population. Rev Urol 7, S3–S11.

Ma, J., Gharaee-Kermani, M., Kunju, L., Hollingsworth, J.M., Adler, J., Arruda, E.M., Macoska, J.A., 2012. Prostatic fibrosis is associated with lower urinary tract symptoms. J. Urol. 188, 1375–1381. https://doi.org/10.1016/j.juro.2012.06.007

Narayanan, A.S., Page, R.C., Swanson, J., 1989. Collagen synthesis by human fibroblasts. Regulation by transforming growth factor-beta in the presence of other inflammatory mediators. Biochem J 260, 463–469.

Nicholson, T.M., Ricke, E.A., Marker, P.C., Miano, J.M., Mayer, R.D., Timms, B.G., vom Saal, F.S., Wood, R.W., Ricke, W.A., 2012. Testosterone and 17β-Estradiol Induce Glandular Prostatic Growth, Bladder Outlet Obstruction, and Voiding Dysfunction in Male Mice. Endocrinology 153, 5556–5565. https://doi.org/10.1210/en.2012-1522

Rassweiler, J., Teber, D., Kuntz, R., Hofmann, R., 2006. Complications of Transurethral Resection of the Prostate (TURP)—Incidence, Management, and Prevention. European Urology, European Urology goes Platinum 50, 969–980. https://doi.org/10.1016/j.eururo.2005.12.042

Ricke, W.A., Lee, C.W., Clapper, T.R., Schneider, A.J., Moore, R.W., Keil, K.P., Abler, L.L., Wynder, J.L., Alvarado, A.L., Beaubrun, I., others, 2016. In Utero and Lactational TCDD Exposure Increases Susceptibility to Lower Urinary Tract Dysfunction in Adulthood. Toxicological Sciences kfw009.

Rubin, E.B., Buehler, A.E., Halpern, S.D., 2016. States Worse Than Death Among Hospitalized Patients With Serious Illnesses. JAMA Intern Med 176, 1557–1559. https://doi.org/10.1001/jamainternmed.2016.4362

Stewart, W.F., Van Rooyen, J.B., Cundiff, G.W., Abrams, P., Herzog, A.R., Corey, R., Hunt, T.L., Wein, A.J., 2003. Prevalence and burden of overactive bladder in the United States. World J Urol 20, 327–336. https://doi.org/10.1007/s00345-002-0301-4

Vuichoud, C., Loughlin, K.R., 2015. Benign prostatic hyperplasia: epidemiology, economics and evaluation. Can J Urol 22 Suppl 1, 1–6.

Wegner, K.A., Abler, L.L., Oakes, S.R., Mehta, G.S., Ritter, K.E., Hill, W.G., Zwaans, B.M., Lamb, L.E., Wang, Z., Bjorling, D.E., Ricke, W.A., Macoska, J., Marker, P.C., Southard-Smith, E.M., Eliceiri, K.W., Vezina, C.M., 2018. Void spot assay procedural optimization and software for rapid and objective quantification of rodent voiding function, including overlapping urine spots. Am. J. Physiol. Renal Physiol. 315, F1067–F1080. https://doi.org/10.1152/ajprenal.00245.2018

Wegner, K.A., Cadena, M.T., Trevena, R., Turco, A.E., Gottschalk, A., Halberg, R.B., Guo, J., McMahon, J.A., McMahon, A.P., Vezina, C.M., 2017a. An immunohistochemical identification key for cell types in adult mouse prostatic and urethral tissue sections. PLOS ONE 12, e0188413. https://doi.org/10.1371/journal.pone.0188413

Wegner, K.A., Keikhosravi, A., Eliceiri, K.W., Vezina, C.M., 2017b. Fluorescence of Picrosirius Red Multiplexed With Immunohistochemistry for the Quantitative Assessment of

Collagen in Tissue Sections. J. Histochem. Cytochem. 22155417718541. https://doi.org/10.1369/0022155417718541

Wong, L., Hutson, P.R., Bushman, W., 2014. Prostatic Inflammation Induces Fibrosis in a Mouse Model of Chronic Bacterial Infection. PLoS ONE 9, e100770. https://doi.org/10.1371/journal.pone.0100770

Zhang, A.Y., Xu, X., 2018. Prevalence, Burden, and Treatment of Lower Urinary Tract Symptoms in Men Aged 50 and Older: A Systematic Review of the Literature. SAGE Open Nursing 4, 2377960818811773. https://doi.org/10.1177/2377960818811773