National Institutes of Health

National Institute of Diabetes and Digestive and Kidney Diseases

Unraveling the Secrets of the (Renal) Interstitium

Virtual Meeting
March 8–9, 2023

EXECUTIVE SUMMARY

The National Institutes of Health (NIH) sponsored a scientific workshop on March 8 and 9, 2023, titled "Unraveling the Secrets of the (Renal) Interstitium" that was hosted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The purpose of the workshop was to discuss the interstitium, a compartment located in the kidney and other organs largely ignored by the research community due largely to technical difficulties in studying it.

Although the renal interstitium (i.e., interstitial cells and the matter around them) has gained some attention in the context of the pathophysiological process of fibrosis, much less is known about its role under normal physiological conditions, in development, or in directly initiating disease pathology beyond the activation of fibrotic pathways secondary to epithelial cell changes. Investigators have moved away from thinking of the renal interstitium as a mere passive compartment of the kidney, but much of its function remains "dark matter." In fact, even the structure of the renal interstitium is not completely understood, most likely due to the lack of technologies to visualize and study it. Much is unknown about the interstitium, yet it may contain important keys to understanding renal function.

An External Organizing Committee (Drs. Ben Afzali, Sarah Calve, Thomas Carroll, Helene Langevin, Neil Theise and Rebecca Wells) was recruited to work with NIDDK staff members (Drs. Eric Brunskill, Danny Gossett, Chris Ketchum, Christine Maric-Bilkan, and Anna Sadusky) to construct the meeting agenda (See Appendix). The objectives of the workshop were to—

- Define the current state of the field.
- Define the physiological significance of the renal interstitium and its role in modulating renal function.
- Leverage knowledge from other organs to identify knowledge gaps and research opportunities.
- Identify the need for developing novel tools and technologies for imaging and interrogating the interstitium.
- Define a framework for moving the field forward.

Following opening remarks from the Director of the NIDDK Division of Kidney, Urologic and Hematologic Diseases, leading international experts in the field presented their research across six scientific sessions. These sessions encompassed a fresh look at the interstitium, elements of the renal interstitium and their function, the renal interstitium in urine concentration, tools and approaches for studying the interstitium, mechanical properties of the interstitium, and the renal interstitium in development and aging. In discussion, participants identified areas for future exploration and opportunities for cross-disciplinary collaborations.

Session 1: A Fresh Look at the Interstitium

Speakers outlined known features of the interstitium and identified areas where further exploration is needed. Presentation topics included continuity of interstitial spaces across tissues and organs, the structure and development of large interstitial spaces, liquid–liquid phase separation (LLPS) in tissue biology, and the interstitium as an extrarenal sodium storage compartment. The speakers in the session identified the following knowledge gaps and research opportunities:

Research Gaps and Opportunities

- The contents and organization of interstitial spaces, as well as the roles of cell contractility and active extracellular matrix (ECM) organization, are poorly understood. A big-data multi-omics approach (e.g., matrisomics, glycomics, proteomics, metabolomics) would help researchers examine interstitial spaces in multiple tissues.
- More work is needed on the mechanics of interstitial spaces, connectivity of the collagen network across interstitial spaces, and time and length scales of signal and force transmission.
- An area for further research involves the contents or functional relevance of interstitial spaces during development, as well as during other periods. Furthermore, the add-on functions of hyaluronic acid (HA) must be characterized (e.g., body wide vs. tissue specific).
- Collagen in interstitial spaces shows measurable piezoelectric activity, but the activity's magnitude, length scale, and relevance to cell behavior and signaling are unknown.
- Physiological probing of intracellular and endogenous phase separation remains challenging, but LLPS sensors are promising new tools. Areas for future study include unearthing kidney biomolecular condensates, linking kidney-relevant stimuli and intracellular phase separation, and investing in tools for tissue-level probing.
- The kidney is involved in salt balance, but it remains unclear how the sodium–chlorine–urea gradient is generated across biological barriers (e.g., skin, kidney, intestines). The anatomy of normal solute gradient formation must be established. Genetic targeting of physiological controls could provide insight into this topic.

Session 2: Elements of the Renal Interstitium and Their Function

Presenters discussed various elements of the renal interstitium, as well as associated functions, across several physiological systems. Presentation topics included renal physiology and pathophysiology, immune–stromal cell interplay in tissue inflammation, antibactericidal immune responses in the kidney, lymphatics, and renal nerves in kidney physiology.

The function of the renal interstitium is linked to the neuronal, lymphatic, immune, and endothelial systems; the interplay among these systems necessitates further exploration. The afferent kidney nerves appear to drive the sympathetic nervous system to many organs, potentially leading to disease. The kidney lymphatics have not been well studied to date but likely function as the waste-disposal system. The epithelial compartment has direct immune function and might help orchestrate immune topology.

Research Gaps and Opportunities

- To date, interstitial cells in the developing kidney have been largely ignored. Better tools are needed to target these cells.

- Barriers to understanding the role of immune–stromal cell interplay in tissue inflammation include the lack of model systems, limited access to patient samples, trauma of tissue dissociation, limitations of single-cell RNA sequencing approaches, limitations of computational tools, spatial transcriptomic solutions, increasing cost, and trainee recruitment.
- Single-cell sequencing can be used to generate a kidney cell atlas, and *ex vivo* perfusion can be used to generate a 4-D response cell atlas and can help researchers move away from mouse models.
- More work is needed to understand the location of renal sensory nerves, what the nerves are sensing, and the role in physiological and pathological states.
- Gaps related to antibactericidal immune responses in the kidney include the heterogeneity of immune cells in the human kidney, localization to specific anatomical structures, influence of kidney-specific tissue cues, and non-immune cell contributions to kidney defense.
- Studies that employ 3-D imaging of the kidney can provide new insights for clinical diagnosis and development studies. This approach can be used to understand the role of lymphatics in kidney disease. Potential therapies that enhance lymphatic formation and angiogenesis would be beneficial.
- Functional studies are needed to identify the roles of lymphatics in kidney development, health, and disease; characterize cellular and molecular interactions with lymphatics in health and disease; and develop targeted lymphatic therapies for kidney disease.

Session 3: Renal Interstitium in Urine Concentration

Panelists discussed the role of the renal interstitium in urine concentration. Presentation topics included the generation and maintenance of the osmotic gradient, the role of renal urea transport in urine concentration, the role of interstitial medullary cells and HA in urine concentration, mathematical modeling of the renal concentration mechanism, and kidney epithelial cells as active mechano-biological fluid pumps.

Urea transport plays a key role in the urine-concentrating mechanism, but how urine is concentrated in the inner medulla remains an unresolved question. The properties of the renal interstitial space change with body hydration status and during kidney pathology, both of which affect the transport properties for fluid. HA could be important for the change in transport through its physicochemical properties; medullary interstitial HA differs among animal models with different urine-concentrating abilities.

Research Gaps and Opportunities

- Researchers have hypothesized that the interstitial urea osmotically balances the high urea concentration in the inner medullary collecting duct lumen, preventing the osmotic diuresis that otherwise would occur in association with the large amounts of urea that are present in urine.
- To date, few studies have examined concentration of solutes in the interstitium. Additionally, the relative contribution of different mechanisms for water conservation (e.g., pumps, HA) remains largely unknown.
- Studies of urine concentration provide a systemic view of active global fluid circulation. More work is needed to understand the effects of interstitial pressure and ionic environment, cell pressure and osmotic sensing, and pressure and cell stress across cell types.
- Mathematical models can be used to explore the process of urine concentration (e.g., role of the 3-D structure of the medulla), as well as other dynamics (e.g., HA, membrane transporters, epithelial cells as active fluid pumps).

- Single-cell transcriptomics and imaging techniques can help researchers better understand the roles of renal inner medullary interstitial cells in the concentrating mechanism. Additionally, microfluidics and single-cell techniques can provide insight into the role of HA in water reabsorption and solute transport.

Session 4: Tools and Approaches for Studying the Interstitium

Speakers highlighted tools and approaches for studying the interstitium and identified areas in which further technological development is needed. Presentation topics included techniques to visualize ECM architecture, intravital microscopy imaging of the interstitium, metabolic labeling of the ECM, and mapping the human stroma.

To date, researchers have not established a unified method for 3-D tissue imaging, and multiple methods likely will be needed for imaging of the interstitium. Second-harmonic generation microscopy can be used to visualize the structural heterogeneity of collagen structures *in vivo*, and tumor cell–derived fluorescence and scanning electron microscopy can be used to visualize interstitial interfaces and fluid spaces directly. Laser-capture microdissection and RNA sequencing of the interstitium allow researchers to obtain a specific transcriptomic signature. Opportunities for integration among different technologies are present (e.g., mapping, spatial registration, alignment, systems biology).

Research Gaps and Opportunities

- Sample opacity has limited the capacity for imaging of morphology deep within tissues. Potential approaches include cryosectioning, light scattering, and multiscale imaging. Additional limitations include artifacts and lack of antibody penetration, and a combination of multiple methods will be needed.
- The ECM is dynamic and critical for cell function, and approaches to mimic the properties of the ECM can help researchers understand how cells are able to shape their local environment. Correlative fluorescence and 3-D electron microscopy tomography can be used to characterize tissue–space relations. To date, imaging of the ECM has been relatively unexplored. An unanswered question is whether cells can invade tissue without degrading or remodeling ECM structures.
- Hydrogels can be engineered to mimic the properties of the ECM, and modifications with cadherin-mimetic peptides can help researchers visualize cell–cell interactions. Analyzing newly secreted matrisome components may help researchers probe questions regarding spatial and temporal signatures *in vitro* and *in vivo*. Research opportunities include exploring the relationship between nascent matrisome composition and local mechanics, as well as cell-type–specific metabolic labeling.
- Transcriptomic studies can provide valuable insight into the molecular identity of stromal cells, as well as cell types and cell states. Stromal cell states can be mapped with disease. Protein markers for cell types and states must be defined and incorporated into multiplex assays. Neighborhoods of cell clusters and niches can be developed and associated with biological relevance.

Session 5: Mechanical Properties of the Interstitium

Presenters outlined mechanical properties of the interstitium. Presentation topics included physical forces around cells and their effects on growth and function in health and disease, mechanical properties of the kidney ECM, dynamic regulation of interstitial fluid by fibroblasts, and fibroblast cytoskeletal remodeling in connective tissue tension.

Cells are influenced by various mechanical cues that initiate tissue and cell polarity. The morphology of fibroblasts changes in response to sustained stretching of loose connective tissue. This polarity also might exist at higher levels of tissue organization (e.g., tubules, blood vessels). The kidney is heterogeneous, and compartmentalization must be considered. Analyses can be performed at the nano, micro, tissue, and organ scales.

Exchanges and separations within the renal medulla are mediated through the interstitium. For example, the interstitium actively influences capillary–interstitial–lymphatic transport and participates in control of interstitial pressure via the cytoskeleton and integrin system. A dynamic balance exists in which the connective tissue cells can place more or less strain on collagen fibers.

ECM complexities involve viscoelasticity, nonlinear elasticity, tension–compression elasticity, disease, time-dependent effects, and spatial dynamics. Mechanical properties of the renal ECM also can be affected by disease processes. Injury leads to ECM stiffness, ultimately leading to organ dysfunction and disease. In some cases, however, decreased ECM stiffness could indicate early injury. Increased ECM stiffness is driven by increased matrix density, decreased matrix degradation, nonenzymatic cross-linking, and enzymatic cross-linking.

Research Gaps and Opportunities

- Changes in ECM mechanical properties potentially could affect kidney function at the level of the entire nephron. More work is needed to determine whether disease-mediated changes in kidney ECM stiffness are compartment-, time-, or disease-specific. Researchers could target ECM stiffness to mitigate kidney interstitial injury.
- Movement of fluid requires pressure, and various approaches have been used for measuring interstitial pressure. More work is needed to determine whether internal kidney tissue stress and strain and interstitial fluid pressure vary in response to external forces applied to the kidney. Fibroblast–collagen gels and fibroblast spheres can be used to study interstitial pressure.
- Fibroblasts might play a role in regulating kidney interstitial fluid pressure and preventing fluid shifts caused by stretching and compression. If so, this protection could be impaired in fibrotic tissues. More work is needed to determine whether fibroblasts play a role in regulating kidney interstitial fluid pressure and prevent fluid shifts from kidney stretching and compression.

Session 6: Renal Interstitium in Development and Aging

Panelists discussed the role and functions of the renal interstitium in development and aging. Presentation topics included the role of the stroma in kidney organoids, identification and characterization of cellular heterogeneity within the developing renal interstitium, the origin and role of the renal stroma, 3-D mapping of the interstitial matrix during murine kidney development, and dynamics of the kidney matrisome in time and space.

Interstitial cells are essential for multiple aspects of kidney development. The matrix is an information system for cells and might serve as an outside-in signal that initiates processes associated with aging. Certain matrix changes can serve as indicators of tissue decline. The ECM shows dynamic changes, both early on and later in development. These expression changes have not been correlated with a clear phenotype.

Single-cell RNA sequencing can be used to identify cell clusters in the stroma. Additionally, spatial transcriptomics can be used to define cell-type relationships over developmental time. These studies have been used to identify distinct anatomical regions. The embryonic renal stroma creates unique niches that are critical for development and differentiation. In adults, the stroma plays multiple roles in renal function

(e.g., structural support, contractile forces, hormone production, immunogenesis). Therefore, it is likely that the adult stroma also is heterogenous. This differentiation likely is important in maintaining different epithelial cell types.

Research Gaps and Opportunities

- Kidney organoids can be improved by addressing challenges related to interstitial populations. It may be important to evaluate whether an appropriately patterned interstitium could assist in the development of high-fidelity kidney organoids from parenchymal stem cells that more accurately model mature kidney tissue. New tools and markers will be needed.
- The embryonic stroma plays multiple roles in the development of kidney parenchyma and is molecularly heterogenous. Interstitial populations might influence parenchymal cell differentiation with trophic factors or metabolites. Gaps related to stromal development include establishment of interstitium and stromal cell heterogeneity during normal development, key signaling pathways between stroma and epithelial cell types that drive kidney formation, and recapitulation of stromal development and heterogeneity in organoids. Machine learning can be used to better characterize stromal identities.
- Gaps related to homeostasis include functional classes of stromal cell types, dynamics and turnover of kidney interstitial cells in time and space, and ECM changes in aging and disease. Additionally, the turnover of the kidney matrix, and its potential regulation by interstitial cells, remains unknown. A kidney matrix signature might herald functional decline.
- More work is needed to understand the structure of the developing ECM and stroma. Previous studies have been performed primarily via cryo-sectioning or electron microscopy. 3-D rendering could provide better resolution of structure patterns. Additionally, global proteomics provides a way to identify components within the matrix, which are dynamic in the contexts of development and disease. Mapping can help researchers understand matrix changes in time and space.
- Areas for future study include multimodal integration, single-cell analysis, and resolution of degradation, production, and spatial dynamics. Additionally, studies of other organ systems can provide insight into the interactions among relevant aspects of biology. Studies of neuronal cell types can provide insight into the roles of stromal cells. New ontology would be helpful in conveying this information to the scientific community.

Summary and Conclusion

The interstitium is considered the “newfound organ” because it encompasses a variety of contexts and functions. Until recently, the interstitium remained largely unknown. Current evidence suggests that the interstitium functions as a conduit, barrier, filter, storage unit, and communicator across physiological systems. Modern imaging approaches have brought to light the importance of studying the interstitium to better understand its dynamic structure and functions. During the meeting, speakers discussed the dynamics of the interstitium in the context of various cellular elements, including interstitial cells, immune cells, HA, ECM, lymphatics, and nerves. The interstitium represents a potential meeting point for cross-cultural medical engagement. In the future, more cross-disciplinary work focused on the interstitium will be needed to better understand how these different elements interact with one another in the context of its numerous functions.

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Reference

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