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Real-Time Immune Complex Imaging in the Kidney Glomerulus Utilizing Dynamically Hyperpolarized MRI Contrast Agents and Artificial Intelligence Guided Microfluidic Design

A University of Pittsburgh Student’s Approach to Making a Transition to Early-Stage Prognosis of Renal Diseases

SPECIFIC AIMS and BACKGROUND

The human animal is a complex, organic, dynamic system which constantly maintains a delicate balance with its environment using the mechanism of homeostasis. The kidney glomerulus complex plays a vital role in the body's ability to maintain homeostasis. By utilizing a finely tuned system, which perfectly demonstrates the classic "Structure equals function," methodology which is present throughout the natural world, the kidney is able to perform selective filtration. To further expand upon the structure, the glomerulus is composed of a high-pressure capillary network which is housed within another structure known as Bowman's capsule. Within this specialized chamber small solutes, such as glucose, are permitted to pass into the urinary filtrate, while large molecules, such as albumin, are retained within circulation. The filtration barrier relies upon a multi-layered design, which flawlessly integrates fenestrated endothelial cells, specialized basement membranes, and unique podocyte foot processes to ensure the continuous control of selective permability. Similar to other structures found in the body, such as the infamous blood-brain barrier or lesser known blood-cochlear barrier, the capillaries of the glomerulus use both physical structure and electrochemical properties to regulate the clearance of metabolic waste while preserving essential plasma proteins. An important note is that the function of the kidney is heavily intertwined with the immune surveillance and response of the body. Typically, the glomerulus is able to withstand a certain degree of inflammation and immune surveillance; however, when the formation of an immune complex overwhelms the clearance mechanisms of Bowman's capsule, these complexes can be deposited within the previously mentioned glomerular basement membranes. Deposition can cause a cascade effect, resulting in leukocyte activation, immune recruitment, and even local cytokine release. The ultimate destination of the cascade is structural damage. Dysfunction of the kidney glomerulus complex is associated with diseases such as lupus nephritis, IgA nephropathy, and post-streptococcal glomerulonephritis. Current clinical interventions rely upon late-stage detection of relevant biomarkers, such as serum creatinine, or upon histological data gathered from kidney biopsy. While both of the mentioned techniques can be definitive in helping identify kidney malfunction, the diagnostic techniques allow for the silent progression of disease states. Roughly 10-30% of lupus nephritis patients will eventually progress to end-stage renal disease requiring dialysis. Also of note, diseases such as chronic kidney disease (CKD) and end-stage renal disease (ESRD), which can be caused by inflammation of the glomerular basement membrane, are responsible for significantly increased cardiovascular risk, a large area of research currently. Despite our ever expanding knowledge of the underlying processes which govern the natural world, current tools and systems fall short in their ability to detect early immune-mediated injury. There remains no non-invasive, real-time imaging system capable of detecting glomerular immune complex deposition at an early, or reversible stage.

The long-term goal of this proposal is to develop a dynamic, real-time diagnostic tool for early detection of glomerular immune complex injury. We hope that by combining dynamic hyperpolarized magnetic resonance imaging (DHP-MRI) with artificial intelligence (AI) driven microfluidic analysis, doctors and physicians will be able to identify and characterize immune complex deposition in vivo, long before any damage or dysfunction can occur. The central hypothesis of this proposal is that immune complex accumulation produces distinct binding and flow disruptions within the glomerular filtration system, which can be visualized using the previously mentioned emerging technologies. We hope that early-detection can help aid in the

continued development of proactive therapies, which may also help reduce the morbidity associated with CKD progression.

AIM 1: Develop and validate dynamically hyperpolarized C^{13} or N^{15} labeled immune complex probes for glomerular imaging,
Hypothesis: By utilizing DHP technology and classical C^{13}-MRI techniques, the 10,000:1 signal-to-noise amplification of DHP will be able to provide real-time data on the immune activity present within the kidney glomerulus complex.
Approach: Synthesize both C^{13} and N^{15} antigen-antibody complexes. Said complexes will be characterized based on polarization efficiency, amplification of the signal-to-noise ratio, and binding ability. The contrast agents must be validated ex-vivo using perfused kidneys.

AIM 2: Build and calibrate a glomerulus-on-a-chip microfluidic system to model flow-mediated immune complex deposition.
Hypothesis: Immune complex binding patterns can be predicted based on flow patterns, shear forces, and charge interactions. We hope that by integrating an AI model within our system, it can be leveraged such that our machine-learning algorithm could be created and trained to help predict renal disease progression.
Approach: Construct a microfluidic chip which mimics glomerular geometry and pressure/concentration gradients. We will introduce human immune complexes under physiologic flow parameters, and then train a machine learning model to predict deposition sites and then create and refine new imaging protocols.

AIM 3: Apply and integrate the methodologies in AIM 1 and AIM 2 into an in vivo animal model of post-infectious glomerulonephritis.
Hypothesis: Early detection of deposition correlates with histological injury and could precede filtration dysfunction of Bowman's capsule.
Approach: Induce mild immune-complex mediated damage in rabbits. We hope to use the previously mentioned technologies to longitudinally image immune complex deposition. We hope to also compare our findings to classical urinalysis and histopathological analysis of kidney tissue, in order to verify our new system works at every level.

Impact: If successful, this proposal would establish a platform for real-time, non-invasive detection of immune complex-mediated glomerular injury. We hope that success would instigate a dramatic clinical shift towards earlier diagnosis and individualized risk assessment for autoimmune related renal diseases and post-infectious neuropathologies. To expand further, we hope that our research will aid in gaining further insights into barrier function, countercurrent flow systems, and microvascular immune injury, which may have implications in the treatment of other organ systems, specifically the brain and lungs.

SIGNIFICANCE

Chronic kidney disease affects over 37 million Americans, and is projected to become the 5th leading cause of death by 2040. Patients who suffer from kidney damage are dependent upon the healthcare system, as their condition requires continuous dialysis. Patients of different

socioeconomic status can be left without treatment, as dialysis can be costly and requires a significant dedication of time. The foundational knowledge of human physiology offers many precedents to be examined for further exploration. At its core, CKD and other renal diseases are all due to a failure of filtration. Proper filtration requires strict molecular selectivity, which relies upon exclusion based on size and charge, hydraulic pressure, and countercurrent flow to amplify the effect of local concentration gradients to ensure small physiological changes are properly regulated. This is an expertly balanced system, meant to be robust to the challenges presented by life. Immune complex deposition can subtly disrupt this balance before the effects can be seen in the filtration rate. By capturing the subtitles that precede total failure of filtration, early detection is no longer a hopeful dream, but a possibility. While this work may seem trivial or non-novel due to the prevalence of modern techniques, I believe we as a society can do better, and should begin to push the boundary of what is possible by leveraging emerging technologies. Additionally, I believe findings here could have further implications in human physiology. For example, our understanding of barrier function could be translated to our understanding of the blood-brain barrier. Such an insight could aid neuroscientists in identifying early indicators of stroke or other traumatic brain injuries. Another example relating to barrier function could be seen in the pulmonary system. Leaky capillaries are characterized by malfunctioning membranes, and their continuous degradation is responsible for disease progression and mortality. To summarize, we hope this proposal addresses a fundamental gap in current nephrology, which is its inability to detect early-stage disease progression, non-invasively and in real-time.

INNOVATION

Traditionally, detection of kidney injury has relied on surrogate markers of filtration failure, such as reduced estimated glomerular filtration rate (eGFR). Such events occur late in the disease process when damage is irreversible. We believe that by focusing on small-scale, early molecular events, specifically those relating to the accumulation of immune complexes, new tools can be developed to image changes in real-time, without the need for invasive biopsies. The ideal result would be the identification of subtle shifts in flow, molecular charge, or binding dynamics, which could act as relevant biomarkers that are predictive of functional decline. With the prevalence of the COVID-19 virus in recent years, this work could also aid in helping identify and diagnose patients with post-COVID nephropathy. Additionally, this technology could see translational use in autoimmune disorders such as diffuse alveolar hemorrhage, where the barriers that are responsible for capillary exchange start allowing blood to leak into the alveoli. Another field where this technology could be applied is transplantation medicine. With the complex and subtle reactions of the body's immune-mediated response, this technology could aid in verifying the healthy integration of transplanted organs into host tissue. This has huge implications in the field of bioengineering, where new synthetic, biologically active polymers which can integrate with the host-tissue are being developed daily. Overall, we hope that by focusing on the disease pathology present within the kidney, our understanding of immune complexes will be advanced, such that it can be applied to other tissues and organs.

APPROACH

Specific Aim 1: Develop and validate dynamically hyperpolarized C^{13} or N^{15} labeled immune complex probes for glomerular imaging,

A. Rationale: DHP-MRI enables amplification of low-abundance molecules by over 10,000:1 relative to thermal equilibrium MRI. Traditionally, this technology is applied for real-time metabolic imaging. A classical example is for imaging of pyruvate, due to the fact that the hyperpolarization takes place at a specific point on the molecule, such that depending on the current metabolic activity of the tissue it is injected into, its products will still be tagged but have distinct nuclear magnetic resonance (NMR) profiles, making it ideal to identify the form of metabolism taking place, as well as its rate. We acknowledge that this technology has not been leveraged for targeted imaging of the kidney glomerulus complex, and so there may be unforeseen barriers to applying it. Regardless, we hypothesize that DHP-MRI technology can reveal early glomerular immune accumulation with high sensitivity and spatial resolution, thus allowing real-time monitoring of the kidney.

B, Study Design:

B.1-1a. Molecular Probe Synthesis and Labeling:

For the selection of our molecular probe we will choose an antibody. The ideal selection would be a monoclonal anti-IgG or an engineered fragment crystallization region (the region of the antibody which binds to cell receptors) which has preferential binding to IgG immune complexes. We hope that by linking our probe to the portion of the antibody which is responsible for immune activation, we can image the earliest abnormality in behavior. It is of extremely important note that the C^{13} or N^{15} label is included within a non-critical amino acid residue. We suggest utilizing lysine side chains as they can be easily synthesized and hyperpolarized.

B.1-1b. Characterization of DHP Probes:

We must ensure the natural bioactivity of the antibody remains intact. Before proceeding with testing in perfused kidneys, one must ensure our probes integrity using size exclusion chromatography and mass spectrometry validation. Binding could be easily assessed using a classical ELISA assay. We also must ensure that the signal-to-noise amplification is intact using NMR spectroscopy.

B.1-1c. Initial Imaging Ex-Vivo using Perfused Kidneys:

We must isolate rabbit kidneys and perfuse that with oxygenated plasma at physiologically relevant pressures (approx. 80-100 mmHg). Once correctly perfused, kidneys should be infused with DHP-labeled antibody probes**.** It is important that these are introduced through the renal artery if possible, in order to properly mimic the in-vivo environment. C^{13} MRI can be performed using either 3T or 7T magnetic fields, with H^{1}/C^{13} coils. Imaging should take place over a time-span of 0-5 minutes post-infusion.

B.2-2a. Probe Success Criteria:

We hope for a polarization level greater than 20% along with a x10,000 improvement in signal-to-noise ratio over a non-DHP control.

B.2-2b. Imaging Success Criteria:

Success in the imaging portion of this experiment would ideally yield detection of immune complex deposition post-infusion. It is of important note that damaged and undamaged kidneys are used to prevent false positives.

B.3. Anticipated Results:

We hope that if all aims are successful, detection of immune complex deposition is possible within the ex-vivo damaged kidney, within 3 minutes post-infusion.

B.4. Possible Complications:

Binding ability of the antibody is abolished by the DHP process.

B.5. Alternative Strategies:

Instead of tagging a marker for the immune response with DHP, we could tag a histamine to act as an inflammatory marker. While this would have great utility in-vivo, it is not the best option for ex-vivo modeling. Additionally, it may not allow our proposed system to detect early-stage disease progression as early as intended.

Specific Aim 2: Build and calibrate a glomerulus-on-a-chip microfluidic system to model flow-mediated immune complex deposition.

A. Rationale: Methods for studying immune-mediated glomerular injury rely on physiological markers and do not capture the dynamic processes associated with fluid shear disruption or countercurrent exchange. An ex-vivo, engineered microfluidic system in the form of an “organ-on-a-chip” model, could allow for real-time, quantitative, and manipulable assessment of such dynamic processes. Combining such a platform with AI driven analysis could allow for the automated detection of niche disturbances and binding patterns before they are visually apparent. This has great utility as a tool for increasing the sensitivity and accuracy of predictive imaging.

B. Study Design:

B,1-1a. Organ-On-A-Chip Model:

Here I will expand upon the specific considerations that must be handled in order to accurately model the kidney glomerulus complex. The system will be designed in AutoCAD. While seemingly trivial, this is important, as we need the countercurrent multiplier feature of the glomerulus to be properly modeled. Once the design is complete, it can then be printed onto a high-resolution transparency mask. This will allow us to use SU-8 negative photoresist spun onto silicon wafers, in tandem with photolithography to form the base of our master mold. Following this, one can then use polydimethylsiloxane (PDMS) and soft lithography techniques to create the base model. It is important that it has multiple inlet ports for immune complexes,

plasma analogs, and immune cell suspension. We must include parallel microvessels that simulate the afferent and efferent arterioles. Semi-permeable PDMS will act as the membrane between blood and filtrate compartments, while nano-scale slits of approximately 100-200 nm will be used to mimic podocyte chambers. To ensure the most accurate physiological environment, we should culture both glomerular endothelial cells on the luminal side, and podocytes on the opposite side. Additionally, we could include mesangial cells in adjacent compartments for other matrix deposition studies.

B.1-1b. Integrating the AI Model:

The power of this proposal is the ability for it to be automated by AI; however, integrating it is not trivial. One must use high-speed fluorescence microscopy with relevant fluorescent markers in order to track real-time deposition and flow disturbance. If every part of AIM 1 is successful, we could simply employ the use of our DHP antibody. Additionally, we want to embed microsensors within the microfluidic design that can inform our predictive learning model. We need sensors for change in hydraulic pressure and electrical impedance (for permeability). Once the model is properly configured to receive data, it would still need to be trained using datasets of known immune complex deposition patterns under controlled flow conditions. Once the model has been properly trained, it could predict binding zones, quantify flow distribution, and flag abnormalities which may relate to different pathologies.

B.2-2a. Chip Success Criteria:

The device would be considered successful if it is fully functional with countercurrent channels, a semipermeable membrane, and endothelial cells and podocytes forming a confluent monolayer with validated junction markers. Luckily we are using an ex-vivo model and we can use histological analysis to make sure our cultures are developing with the proper cell junctions. Additionally, we hope that stable flow-rates can be maintained with the correct physiological concentration gradients.

B.2-2b. Integration Success Criteria:

We hope our model can achieve a greater than 90% sensitivity and specificity for detecting early deposition within 5 minutes post-infusion.

B.3. Anticipated Results:

We hope this aim will yield a fully functional kidney-on-a-chip system which can be used to help study the unique processes associated with immune-mediated glomerular damage.

B.4. Possible Complications:

We are not able to accurately culture and accurately simulate the environment present within the glomerulus filtration system.

B.5. Alternative Strategies:

Luckily, we do not need a microfluidic system to train the AI model. We could use the perfused kidneys, detailed in AIM 1; however, this is not sustainable, and the proposal could suffer from small sample sizes or a lack of physiologically relevant data. It would take far more

time, but it would still be possible to train the AI model using the same techniques detailed above.

Specific Aim 3: Apply and integrate the methodologies in AIM 1 and AIM 2 into an in vivo animal model of post-infectious glomerulonephritis.

A. Rationale: In order to validate the clinical translational potential of our proposal, it is critical to demonstrate that the DHP contrast agents and predictive learning model can actually predict glomerular immune injury in living organisms. By inducing post-infectious glomerulonephritis in rabbits using streptococcal antigens, we can test our new technologies on a reproducible platform in order to study immune complex-mediated kidney injury.

B. Study Design:

B.1-1a. Animal Model Preparation and Protocol:

The ideal species of rabbit would be the New Zealand White rabbit due to their kidney size. Each subject should be injected intravenously with heat-killed streptococcus pyogenes antigen. In order to boost the animal's immune response, one could use Freund's incomplete adjuvant, which serves as an immune potentiator. The animals should be monitored over a time-span of 2 to 4 weeks for development of glomerular immune complex deposition,

B.1-1b. Imaging Protocol:

To prevent hemodynamic variability the animals should be anesthetized with light isoflurane. Venous catheters can be employed for DHP contrast agent infusion. The contrast agent should not be injected in volumes over 2 mL, but should have a concentration of at least 80 mM to ensure proper contrast with adjacent tissue. Injection can occur linearly over a time span of 10 seconds. We can then move to C^{13} MRI imaging immediately post-infusion. It is of note that we should also be collecting blood and urine samples to verify our results match what we know about conventional markers. Imaging and sample collection should be done on day 0, 7, 14, and 26 following antigen administration. To validate results we must compare dynamic imaging patterns with urinary protein excretion levels, serum creatinine levels, and once the rabbits have been sacrificed, histological assessment (specifically IgG staining).

B.2. Success Criteria:

Success for this aim would mean accurate imaging detection of early immune complex accumulation before the appearance of serum creatinine in urine samples.

B.3. Anticipated Results:

The machine-learning algorithm can accurately predict which rabbits have kidney damage, days or weeks before the appearance of the usual relevant biomarkers within urine samples, with an accuracy of greater than or equal to 90%.

B.4. Possible Complications:

The predictive learning model can not identify new parameters that can serve as an early warning system of kidney damage.

B.5. Alternative Strategies:

The images generated from using the DHP contrast agent could still be extremely useful for training new AI's. New systems and technologies are emerging daily, and utilizing them for image analysis could help researchers uncover new features in systems previously believed to be well understood in human physiology.

CONCLUSION

‘ Human physiology has provided an extraordinarily valuable blueprint for understanding the underlying mechanisms that help maintain life. The body demonstrates a mastery of engineering solutions that it has refined over millions of years. Our current clinical frameworks for diagnosis rely on the foundational work of many great scientists who dedicated their lives to uncovering the features of the human animal. Despite older generations' tenacity, perseverance, and progress, the tools at our disposal are limited by their reliance on older findings. Predictions are often based on static, late-stage indicators of injury and disease progression. The subtle, dynamic changes that precede irreversible damage are largely overlooked by modern techniques. Advances in molecular imaging, microfluidics, and artificial intelligence offer to move beyond previous forms of passive observation, and towards real-time assessment. By applying DHP contrast agents to help image small-scale molecular events in tandem with AI analysis, we can capture new features of the human system, and intervene when injury may still be reversible. The significance of this proposal does not lie it its immediate technical and clinical objectives, but instead its broader contribution to redefining how we detect and manage diseases. The ability to visualize immune complex accumulation dynamically could completely reshape the field of nephrology and transplant medicine. Additionally, by improving our understanding of how membranes and barriers in the body work, a similar system could be created to study other organs, like the blood-brain barrier. Future directions for this proposal could include further integration of novel biosensors within microfluidic chips to improve its clinical translation. Overall, we hope the development of this technology leads to more personalized medicine. To conclude, we hope to bridge the best humanity has to offer, by taking what we have learned from foundational physiology, and combining it with the possibilities offered to us by the emerging technology of our era. This proposal represents not just one student's aspirations, but a significant need that we as a society have. We must begin to transition to a state where the golden standard of care isn't only achievable for those that can afford it, but for all. I believe work like this, using high throughput models and predictive learning models, is what will bridge the gap and allow us to have more personalized medicine.

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