Effect of Physiological Flow on Mesangial Cells: Implications for IgA Nephropathy

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Abstract

- Immunoglobulin A (IgA) nephropathy can lead to kidney failure; characterized by IgA accumulation
- Mesangial cells: substrate in glomerulus that helps regulate filtration
- Mesangial role in kidney disease is not yet understood
- Transport of fluids and proteins into and within the mesangial are poorly understood
- Many cell types change phenotype when grown in the presence of physiological flow, versus static conditions
- Mesangial cells produce extracellular matrix and in IgA nephropathy they proliferate and deposit extra matrix

We hypothesized that mesangial cells cultured in the presence of IgA and subjected to physiological flow will show alterations in the deposition of matrix, a first step by making type I collagen gels and testing them for their permeability. I conducted several studies in which the flow of water through these collagen gels was measured using a perfusion chamber. The long-term goal of this study is to determine how matrix deposition by the mesangial cells changes when cultured in flow conditions.

Introduction

Mesangial cells are one of the specialized cells in the kidney. They are found in the mesangial region of the kidney (Figure 1) [1]. They help regulate the filtration process of the kidney and also provide support for the glomerular structure. They are involved in the kidney’s response to injury (nephropathy) [1]. Although mesangial cells have been studied in the past by other groups to try to determine their role in the development of long-term renal diseases, there is still very little known about how mesangial cells actually play in renal diseases. Furthermore, the transport of fluids and proteins into and within the mesangial region of the kidney has not been well studied [1].

IgA nephropathy is a medical disorder in which immunoglobulin A (IgA), a specialized antibody, builds up in a patient’s kidney (Figure 2) [2]. When exposed to this IgA nephropathy, their kidneys are no longer able to filter excess waste and products from their blood, resulting in the presence of protein and blood in their urine. In IgA nephropathy, IgA complexes accumulate within the mesangium. IgA nephropathy is the most prevalent primary chronic glomerular disease of the kidney in the U.S. The exact number of cases of IgA nephropathy is not known, since a definitive diagnosis requires a kidney biopsy. There is no disease-targeted therapy. As a result, many patients are prescribed medications that suppress the patient’s levels of angiotensin II and proteinuria, as well as control the patient’s blood pressure.

Studies have shown that cells grown in the presence of physiological flow exhibit different properties than those grown in static conditions [3]. Mesangial cells produce extracellular matrix molecules, and a key feature of these cells is their ability to respond to physiological flow (Figure 3) [1]. Studies have shown that mesangial cells in the conditions of flow may provide us with a better idea of how mesangial cells function.

Methods

Collagen gels were made following procedures established by a student who previously worked in the lab. To make 4 mm thick (146 mcL) gels, we followed a protocol first by making type I collagen gels and testing them for their permeability. I conducted several studies in which the flow of water through these collagen gels was measured using a perfusion chamber. The long-term goal of this study is to determine how matrix deposition by the mesangial cells changes when cultured in flow conditions.

Perfusion Chambers: In this project, I used a perfusion chamber designed by previous students in the lab (Figure 4A) [4]. The gels were tested in three different sizes of perfusion chambers (1.1 mm, 2.3 mm, and 4.0 mm). The smallest perfusion chamber seemed to contain the gel and hold it in position which allowed for optimal water flow through the gel. The gel volume that did not contain perfusion chambers was combined in order to make complex matrix.

Preliminary Data: The gels were placed in the smallest perfusion chamber, 1 mm diameter, and the chamber was then placed into a container filled with water and hooked up to a setup of tubing (Figure 5). The tubing was attached to a wooden board and hooked up to the sink allowing water to flow through the tubing. Any bubbles that appeared in the tubing were removed from the tubing to ensure that when measuring the distance the water traveled per second would not be disrupted with an air bubble. Once the tubing was clear of all air bubbles and were lined up perpendicular to the ground, 500 µL of cell culture was added to the top of the tube to ensure that no water evaporated out of the system. A switch was opened which set the water flowing toward the perfusion chamber. Gravity was used to flow flow through the chamber to test the permeability of the collagen gels.

Transwell Plates: 6 wells Transwell plates were tested for their ability to allow the flow of fluids through the pores. The wells of the plates had filters with 0.4 um diameter pores which served as a model, for the pores observed between the mesangial cells in the kidney. Water flowed through the filters to determine the flow rate of the water through the pores. Collagen gels were then generated in the upper chambers of the Transwell plates.

Computational Modeling: In order to observe the effects that the basement membrane thickness, volume fraction of matrix, and different sizes of IgA had on the pressure, a computational model developed by Sarah Hunt was used. The Matlab code Sarah has written simulates the flow through the mesangial matrix. Different inputs were used and the outputs of the code were observed using Paraview.

Results

The results of a collagen filled perfusion chamber were analyzed to find the hydraulic resistance. This was done through many equations (Figure 6) where Equation 2 was applied to the data to a linear fit where a best fit line of the data was applied (Figure 6).

\[ \frac{\Delta P}{\Delta L} = \frac{1}{K} \frac{1}{A} \frac{1}{\text{Diameter}} \]  
(Equation 1)

\[ \frac{\Delta P}{\Delta L} = \frac{1}{K} \frac{1}{A} \frac{1}{\text{Gel}} \]  
(Equation 2)

The best fit line was y=0.005x+0.009 with a R2 value of 0.9994.

Using the area of the tubing, the density of water, gravity, and the slope of the best fit line, the hydraulic resistance was calculated to be 2.848 cm/m².

Transwell Plates: The Transwell plates did not serve to be optimal because the fluid did not flow freely through these chambers. Despite this, collagen gels were generated in the upper chambers of the Transwell plates, but were not preserved in PBS for future experimentation. This showed that the cells could solidify in the upper chambers of the wells; which can be applied to future studies.

Conclusions

During the course of these experiments, a better procedure was developed for testing the permeability of collagen gels in the perfusion chambers. The new procedure established that certain precarations must be taken before the gels are to be tested and certain materials must be available before performing the trials. When the commercially purchased mesangial cells are purchased and tested, the procedures developed through these trials will most likely be used to ensure that the flow is properly measured through the device and across the gels. The long-term goal of this study is to determine how matrix deposition by the mesangial cells changes in individuals with IgA nephropathy as the flow changes. It is hypothesized that conditions cultured in the presence of IgA and subjected to physiological flow will show alterations in the matrix deposition due to alterations in flow rate. Plasma derived from the blood of individuals with and without IgA nephropathy will be added to culture the mesangial cells. By using fluorescently tagged IgA, the movement of the IgA complexes will be monitored as they flow through the chambers. Changes in IgA deposition that occur in the presence gradients are then monitored. By time-lapse photography, differences in the way that the mesangial cells appear when cultured in the presence of serum with and without IgA nephropathy will be detected. In future studies, the matrix porosity will be monitored; the porosity will be quantified and possibly the different molecules that are secreted by the mesangial cells during the experiments will be identified.

References