Rochester Institute of Technology

A Thesis Submitted to the Faculty of
The College of Imaging Arts and Sciences
In Candidacy for the Degree of
MASTER OF FINE ARTS

A Multimedia Instructional Animation and Website
On Anti-Glomerular Disease

By
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18 November, 2010

Abstract:

Immunology is a field of study that is expanding in the biomedical community. Current research has given the study greater consideration in understanding the nature and origin of disease.

The purpose of this project was to explore the immunology of a particular type of kidney disease: Anti-Glomerular basement Membrane disease. The kidneys provide a visual landscape that is anatomically unique. The nephron, in particular, provides anatomical structures that lend themselves nicely to engaging visual graphics. The animation of Anti-Gomerular Basement Membrane Disease provides a visual format for understanding structures and processes that have not been previously presented in a visual media. With the visualization of this disease, perhaps it and other immunological processes can be more thoroughly understood.

Thesis Statement:

The objective of this thesis project will be to create a 3D animation of kidney disease, specifically Goodpasture's Syndrome, otherwise known as Anti-Glomerular Basement Disease. It will focus on the physiology of the glomerulus and of the basement membrane. It will also illustrate the progression of Anti-Gomerular Basement Membrane Disease.

The target audience for this project will be biomedical students. By viewing the animations, students will then be able to identify the anatomical structures of the nephron, identify the pathological markers for Anti-Glomerular Basement Membrane disease, and explain the process of nephritis that occurs with onset and progression of the disease.

This project aims to create images and animations of a subject that was not previously available. The animation will reflect current research as it relates to Anti-Glomerular Basement Membrane Disease, and its pathology.

Models of the nephron and glomerulus will be created in Maya software. These models will be animated in Maya and Adobe Flash software.

Preliminary Research

I met with Dr. Richard Doolittle from RIT's School of Life Sciences to discuss how this work might be incorporated into the 3D Visualization Project he has overseen. I have also contacted Becky Kendall from the National Kidney Foundation to discuss how my work might be utilized by the Foundation. Becky has shown some interest and will be consulting with the other members of the Foundation to see if there could be a use for that type of project.

Introduction

The modeling of Anti-Glomerular Basement Membrane Disease project encompassed: 1. An examination of the anatomical structures of the glomerulus, including a general model of the nephron, and more detailed view of the three layers of the glomerular wall. 2. A view of the glomerular wall in the diseased stage. 3. The antigen. 4. The responding elements of the immune system. 5. Some key features of glomerular filtration.

The animation component of the project consisted of 1. A depiction of the normal physiological process of glomerular filtration though the three major layers of the glomerular wall. 2. An animation of the glomerular wall in the diseased state. 3. A depiction of the pathological process of the disease from the identification of the antigen through the degradation of the glomerular wall.

Research and course work were done in the areas of immunology and histology. Consultations with medical professionals in the area of nephrology were made prior to the thesis work. Illustrations were created for an educational exhibit for the National Kidney Foundation. Research was conducted in the development of this project and consultations were established with Mary Ann Sloand RN, MSN, and Rebecca Kendall of the National Kidney Foundation, and Dr. James A Sloand, MD, nephrologist at University of Rochester.

Study of Other Artists

Styles of several artists were studied as a source of inspiration. Among them were Craig Foster of Foster Medical Communications. He was referenced as a source of inspiration for lighting and drama in 3D animation. Foster's models of anatomical structures were also viewed for their highly organic textures. Another artist whose precision in textures were studied was Jane Hurd of Hurd Studios. Hurd's animations also display a fine fluidity in microbiological and molecular processes. The animations of both studios are highly engaging and instructional, key features for all medical animations.

In 2008 a thesis was produced by fellow Medical Illustration classmate, Betsy Skrip. This thesis project focused on imaging of the respiratory airways. Ms. Skrip created a model of the basement membrane of the respiratory membrane. The structure of the basement membrane located in the lungs is virtually the same as that of the glomerular wall. So the Skrip model of the basement membrane was used as a source of reference for the model that was made for the Glomerular basement membrane when it came time to focus on the glycoproteins.

Evolution

This project evolved over time. In the use of Maya software, the greatest changes took place with the use of textures and shader networks. For example, the erosion of the basement membrane was first attempted using the Booleans tool. Booleans proved to be an unpredictable tool, and cumbersome. This process required each perforation of the membrane to be made one at a time. A fractal bump map was used in place of the Boolean method. The bump map was then animated to demonstrate the decomposition. This was a far more rapid process. In addition, this method saved file space.

Another challenging task involved the importing and animation of Protein Data Bank models. The geometry for these models when brought directly into Maya was so complex and the files were so large, it would not allow any other models in any of the scenes to be brought in. Bringing the model into Cinema 4D helped to reduce the size of the file. In addition, the function 'reduce' was used in Maya to further simplify the geometry and reduce the size of the file.

One of the other components of the project that changed was the narration. The animation was set up to provide a description of the major elements that were involved in the disease. The script was abbreviated with revisions, fewer details were included in the final cut. For example, information pertaining to the antigen was simplified.

Many of the revisions made to the project were made with the intention of providing the most clarity and engaging the viewer.

Research

Preparatory Studies

Research was first conducted on the anatomical structures of the kidney. Illustrations were made of the kidney, with clear emphasis given to each structure. Images were created of the kidney in a healthy state, in the intermediate stage of kidney disease, and in the diseased stage of kidney disease.

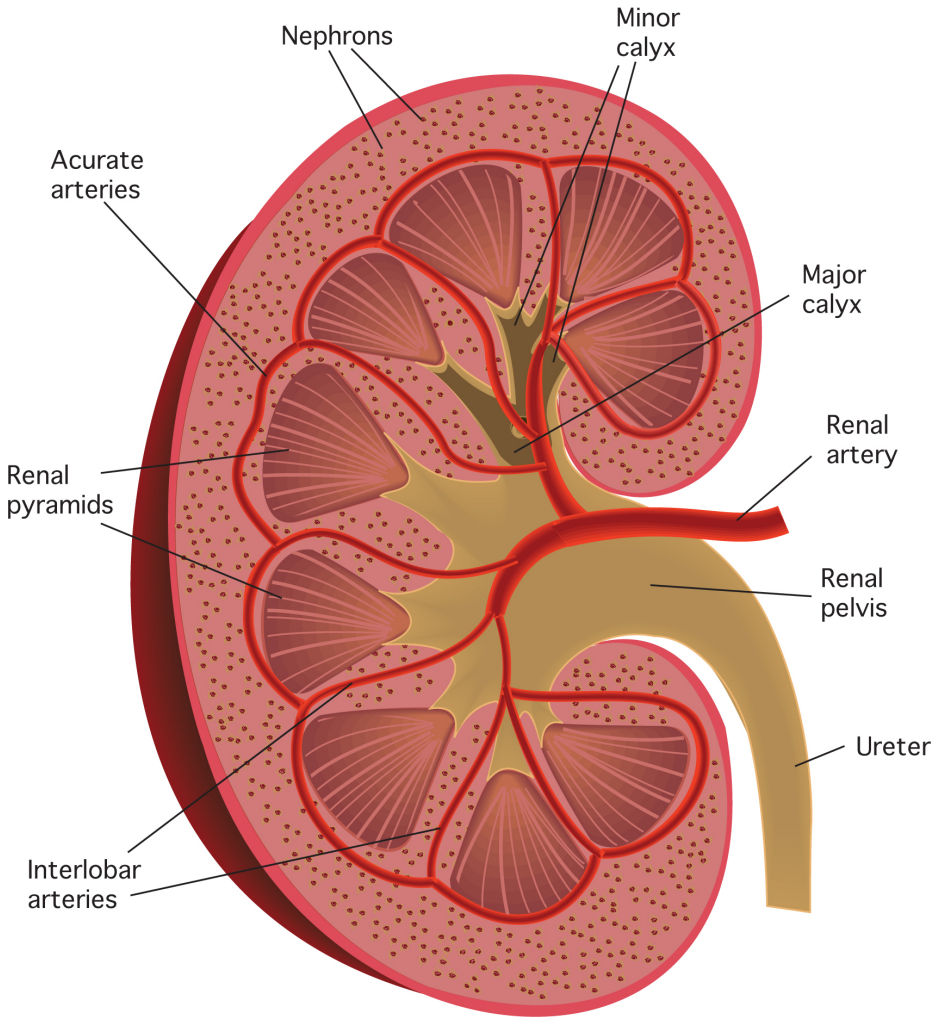

Figure 1. Healthy kidney

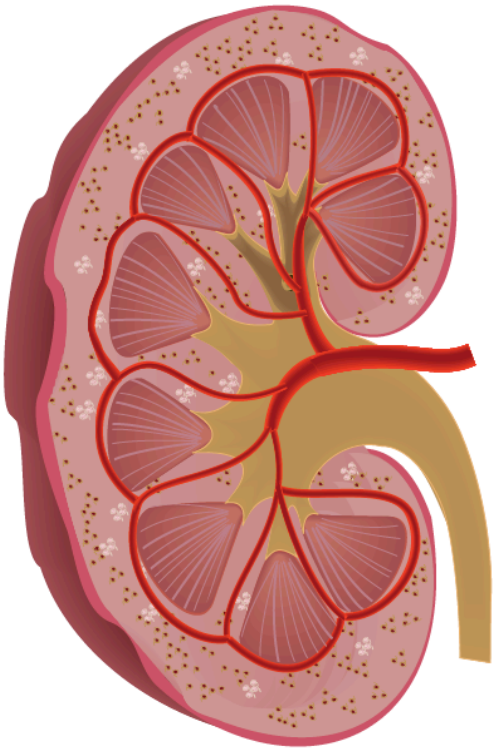

Figure 2. Intermediate stage of kidney disease

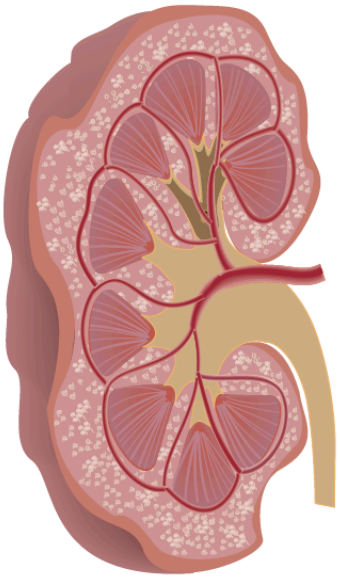

Figure 3. Diseased stage

Microbiology

Preliminary research was also done on the nephron: its microbiological structures, and physiology. The nephron is the smallest functioning unit in the kidney. It works to manufacture urine. Blood enters the nephron through the afferent arteriole, into the renal corpuscle. Blood branches into the smaller arterioles that make up the glomerulus, which is encapsulated by the Bowmans capsule.

As blood passes through the glomerulus, and the capillaries of the glomerular tuft, pressure forces fluid and solutes out into the capsular space. The filtrate at this point is devoid of plasma proteins. As filtrate leaves the renal corpuscle, it goes through a process of change, passing through three major sections. The tree major sections are the proximal convoluted tubule, the loop of Henle, and the distal convoluted tubule, where water and organic molecules are reabsorbed and fluids and waste are secreted. (Martini and Bartholomew,2000).

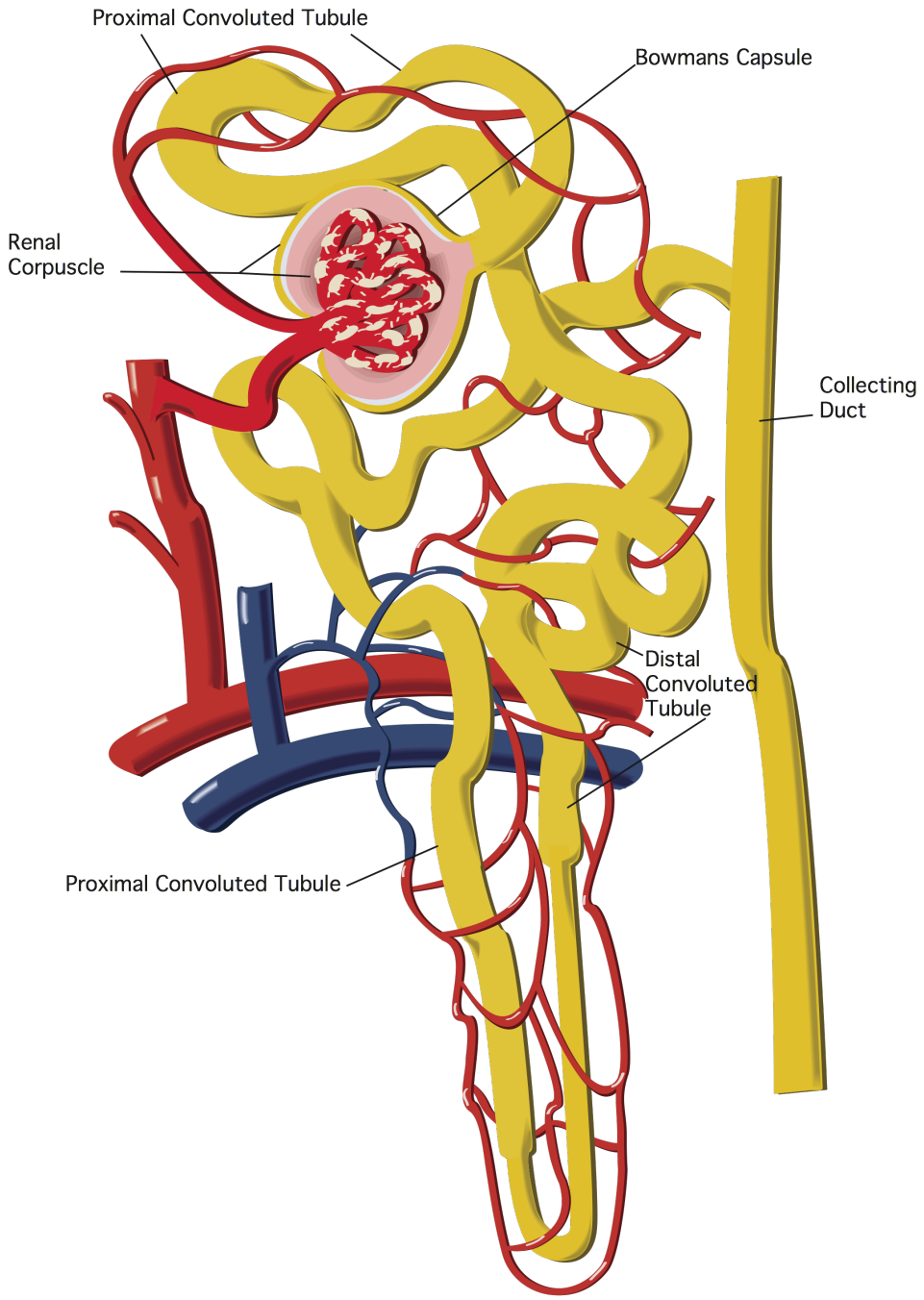
Proximal Convoluted Tubule
Bowmans Capsule
Renal
Corpuscle
Collecting
Duct
Distal
Convoluted
Tubule
Proximal Convoluted Tubule

Figure 4.

The production of urine in the nephron

Martini and Bartholomew (2000), state "The primary purpose of the production of urine is to maintain homeostasis by regulating the volume an composition of blood." (ibid. 524) The nephron does this by filtering solutes, reabsorbing selective molecules, and secreting other undesirable substances. From the urinary space of the renal corpuscle, filtrate moves through the proximal convoluted tubule. Here, water is reabsorbed, along with ions and other organic matter. The filtrate then leaves the proximal convoluted tubule and enters the loop of Henle. The loop of Henle consists of two sections, the descending limb, and the ascending limb. In the descending limb, more water is reabsorbed, as well as sodium ions. In the ascending limb water and chloride ions are reabsorbed. In the distal convoluted tubule, ions, acids, drugs and toxins are secreted. Variable reabsorption of water and sodium ions occurs. The tubular fluid exits the nephron from the collecting duct, where water is reabsorbed, along with sodium, potassium, hydrogen and bicarbonate ions are either reabsorbed or secreted. The collecting duct receives tubular fluid from several nephrons, which merge into the papillary duct. Urine then travels through the papillary duct into the minor calyx, then eventually into the ureter of the kidney. (ibid.).

Glomerular Filtration

The diameter of the afferent arteriole leading into the glomerulus is larger than that of the efferent artery, which leads out of the glomerulus. This leads to a higher blood pressure within the glomerulus. Blood pressure in the glomerulus forces water and solutes through fenestrations into the urinary space. The force of filtrates moving into the urinary space is counteracted by the flow of blood through the glomerular capillaries. This is called filtration pressure. In a healthy kidney, filtration pressure is about 10 mm/Hg. As pressure changes with blood that circulates, the diameters of the afferent and efferent arterioles will respond accordingly. But in certain diseases that occur in the kidneys, hemorrhaging results, and progressive organ failure is a consequence. (ibid.).

Glomeular filtration is also a vital function of the kidneys. ' In the course of a day, the glomeruli generate about 180 liters of filtrate, roughly 70 times the total plasma volume' (ibid. 526). As important as generating filtrate is, the process of reabsorption of water and solutes through the renal tubules is equally as important. But "if filtration does not occur, waste products are not excreted, pH control is jeopardized, and an important mechanism for blood volume regulation is eliminated."(ibid.).

Table 1 (Martini and Bartholemew 525)

Differences Between Urinary and Plasma Solute Concentration

Components	Urine	Plasma
IONS (mEq/l)	147.5	138.4
Sodium(Na+)	47.5	4.4
Chloride(Cl-)	153.3	106
Bicarbonate(Hco3-)	1.9	27
Metabolites and Nutrients (mg/dl)		
Glucose	0.009	90
Lipids	0.002	600
Amino acids	0.188	4.2
Proteins	0.000	7.5g/dl
Nitrogenous Wastes(mg/dl)		
Urea	1800	305
Creatinine	150	8.6
Ammonia	60	0.2
Uric Acid	40	3

Modeling the Gomerulus

The Bowman's capsule and the glomerulus were modeled to demonstrate glomerular filtration. A tube was created using curve and an extruded NURBS circle. Control vertices were manipulated from the curve to create a tubule structure that curved and twisted. Bowman's capsule was created by selecting each hull and scaling them to form the shape of the capsule. The capsule and tubule were duplicated and offset by -0.187. The two copies of the capsule and tubules were grouped. A light brown Lambert shader was attributed to the Bowman's capsule model, and given a transparency that would allow the viewer to see the glomerulus model inside.

The capillaries making up the glomerulus were also modeled with curves and lofted NURBS circles. Control vertices were manipulated so that the capillaries twisted and folded on top of each other. The separate capillaries were grouped. A red Blinn was then applied to the glomerulus. This Blinn was given a stucco texture to simulate the visceral epithelium. There were two color channels applied to the stucco texture: a red channel, and a light gold channel. The same stucco texture applied as a bump map to the glomerulus to give the visceral epithelium added dimension. A transparency was also given to the glomeruls's Blinn so the capillaries would appear semi transparent.

The visceral epithelium was further highlighted with podocytes throughout the glomerulus. These podocytes were created using polygon spheres. Vertices were pulled and manipulated using the move tool. This process was used to create the foot processes that projected from the podocytes and stretched across the capillaries.

For the shader of the podocytes, a layered shader was used. For one layer, a Lambert was used and colored lavender. A transparency was applied using the contrast node. A sampler info node was then attached to the contrast node. The network of this shader would create a soft glow film like shade that is typical of some microbiologic structures. The second shader in the layered shader consisted of a Lambert with a gold color. The layered shader that was created for the podocytes, would later be used for the visceral epithelium in the larger model that highlights the three layers of the glomerular wall.

Animation of filtration

To give a general representation of glomerular filtraton, particles were used in Maya. Curves were created through the vessels. Curve flows were applied to the curves. Particle spheres were used for the individual particles a lavender Blinn was used for the particles. A medium transparency was given to them. And a 9.0 specular roll off was given to the Blinn as a well as a low eccentricity of .030 to give the particles a reflective highlight. In the animation, particles, which represented solutes emanated from the glomerulus. A curve flow was also created leading from the glomerulus to the tubule in order to indicate filtrate leaving the renal capsule.

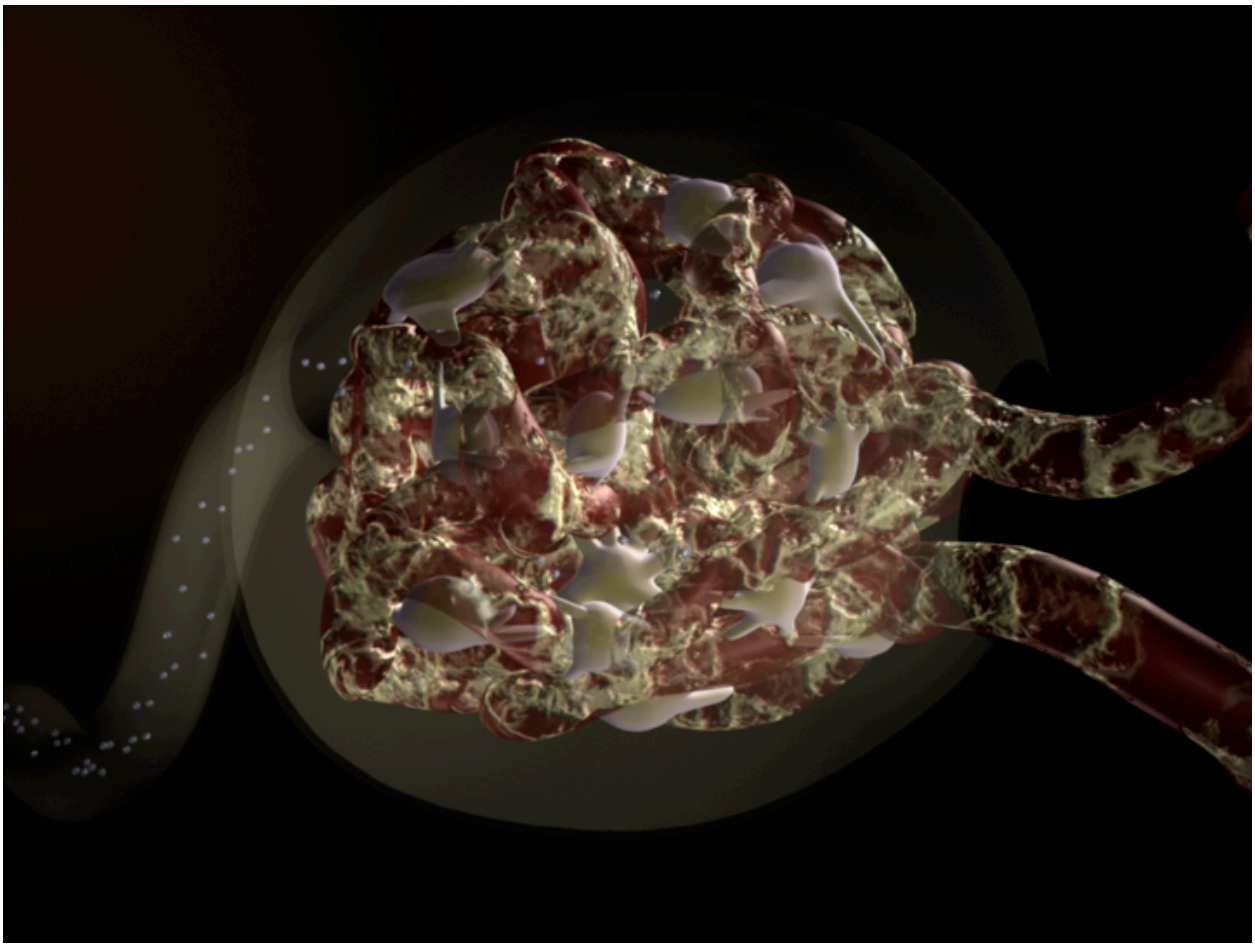

Figure 5.

Research on the anatomical structures of the glomerular wall

The three layers of the glomerular wall - the visceral epithelium, the basement membrane, and the fenestrated endothelium - make up a highly complex and selective structure that allows water and solutes to permeate, and filters proteins and other macromolecules based on size and charge. (Kumar et. al. 2007)

The fenestrated endothelium comprises fenestrata 70 to 100 nm in diameter, which discriminate between protein molecules that are larger than those dimensions. The basement membrane consists of glycoproteins including laminin, nidogen, perlecan, integrin, and type IV collagen. This complex layer is largely anionic, and works to permit cationic protein molecules passage into the capillary lumen. The glygoprotiens, particularly collagen IV, provide strength, structure and integrity to the glomerular wall.

Finally, the visceral epithelium includes podocytes with foot processes. These foot processes are bridged by filtration slits, which are 20 to 30nm apart. The network of the visceral epithelium further supports the basement membrane and discriminates among proteins that continue to pass into the urinary space. (ibid.).

Modeling the glomerular wall

Using Maya software, a section of a glomerular vessel was created. The intent was to create a section of a glomerular capillary that was open at both ends. The inside of the vessel was made visible by cutting out a lateral section of the tube. First a line curve was made in an s-formation to show the natural curvature that a capillary would have. Next a semicircle was made using the circle curve tool. A sweep of 224 was used for an open circle shape. Then, a radius of 175 was used, in order to build a vessel through which the components of the vessel could be seen flowing. The next step was to extrude the circle with the line curve to create the first NURBS surface that would be the outer wall of the basement membrane. That NURBS surface was offset by a distance -.400 to form the inner wall of the basement membrane. The edges for the outer wall were lofted with the edges for the inner wall this closed object became the basement membrane. The basement membrane was given a Lambert shade with a predominantly red color. A bump map was applied to the Lambert that would later be animated. The inner wall of the basement membrane was duplicated and off set by -0.35. This would make up the outer surface of the fenestrated membrane. The outer surface of the fenestrated membrane was offset by -.400. The edges on the outer surface of the fenestrated membrane were lofted with the edges of the inner surface. Next, the texture for the fenestrated membrane was explored. The first technique that was attempted utilized the Boolean tool. This technique proved to be tedious, as the Boolean tool can be unpredictable in its performance. Many holes would need to be made. Further more, the Boolean method did not show the proper texture that is reflected in current scanning electron micrographs of glomerular fenestrated endothelium.

So the more viable approach to creating the fenestrated membrane was to use a texture file in a shader network and applying the shader to the geometry. A jpeg of a texture was generated in Photoshop. This consisted of a jpg image of a bright red background that had a bump texture to it, with black holes. The black holes were eliminated using the eraser tool, so that the texture appeared to have transparent holes instead of simply black holes. The red background was colorized with the paint bucket tool, a saturation of Red-132, Green-52, and Blue-67. This produced a color that was more authentic to a blood vessel. The image was saved as a TIFF file.

The texture was then referenced in Maya as both a texture image, and a 3d texture. The TIFF was applied to the color portion of the shader network. It was also applied to the transparency portion of the shader. A bump map was also incorporated into the shader. This bump map would be later used in the animation to demonstrate degradation of the fenestrated endothelium through the progression of Anti Glomerular Basement Membrane Disease.

The visceral epithelium was an important part of the model for several reasons. First, the visceral epithelium is one of the major features of the glomerulus that makes the glomerular arterioles distinct from any other arteriole in the body. Secondly, the degradation that occurs as Anti-Glomerlar Basement Membrane Disease progresses is a particularly dramatic part of the disease progression. The section of the animation that shows the degradation of the visceral epithelium would become an engaging section, offering a critical level of understanding of the disease and ability for the target audience to identify the disease.

The visceral epithelium was created against the outer surface of the basement membrane in order to maintain the shape and curve of the entire vessel. Polygons were used to create the podocytes that made up the visceral epithelium. Crude spheres were first created 8x8 subdivisions. Faces were selected and extruded from the crude spheres to form the larger foot processes. Animation skeletons were then created. Long foot processes were skinned to an Inverse Kinematics rig system. The joints composing this rig were manipulated to wrap against the outer wall of the basement membrane. Faces from the larger foot processes of the podocytes were then selected and extruded to form the smaller foot processes. Faces and vertices were selected and manipulated to further wrap against the vessel, and expand the network of the visceral epithelium. Geometry was smoothed.

A texture for the podocytes was then created. The same shader that was used for the podocytes in the model that contained the entire glomeruls was also used in the visceral epithelium in the model of the glomerular wall. There was one difference in this shader, however. The second shader in this layered shader consisted of a Lambert with a gold color and a granite bump map. The bump map would be adjusted in the animation to simulate the degradation of the visceral epithelium.

Blood flow; research and modeling

In normal blood flow the leukocyte to blood cell ratio is about 1:700. (23 July 2010) http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/B/Blood.html

Normal Blood flow was demonstrated in the side view of the glomerular capillary. Several elements of normal blood flow were created. First a blood cell was created using a NURBS sphere. Vertices were manipulated and shaped. A shader network was applied using a network similar to the one used for the visceral epithelium. However, the bump map that was applied was a fractal bump map that was not animated.

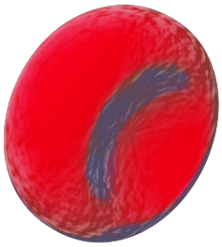

Figure 6.
Blood cell

The blood cell was used as an instancer replacement on a curve flow that was traced through a curve followed through the vessel lumen. Two other curve flows were used to simulate other solutes flowing through the vessel.

A neutrophil was modeled using another reshaped sphere for its lobed nucleus. A similar shader network to the blood cell was used for this part of the neutrophil model. For the cytoplasm surrounding the neutrophil nucleus, a simple sphere was used. The shading network for the cytoplasm consisted of a fractal bump map, a color blend node with transparency, fractal 2d texture and sampler info node to simulate granules.

Figure 7.
Neutrophil

A curve following the shape of the vessel was created. The leukocyte model was duplicated and animated and moved strategically along the curve to establish a flow for leukocytes that would eventually be brought into the scene for the side view of the vessel.

Protein Retained in the Blood Flow Through the Glomerulus

"Albumin is the major plasma protein that must be retained in the blood and normally only traces are filtered" (Hay 1991 p.393) A model of the protein albumin was created in Maya and integrated into the animation where blood flow is shown through the vessel. A model of albumin was imported into Chimera from the Protein Data Bank. In Chimera it was converted to a surface model, and exported as a Virtual Reality Modeling Language (VRML) file. From there, the VRML file was opened in Cinema 4D, then saved in OBJ format (a common format for most 3D applications). It was then imported into Maya as an OBJ file. The geometry was reduced to decrease the size of the file. A shader network was applied to the model. A layered shader was used. For one of the layers, a light pink color was used as a semi transparent shader. A blender was applied to the transparency for that layer. And a fractal bump map was also applied. The other layer included a light blue Lambert.

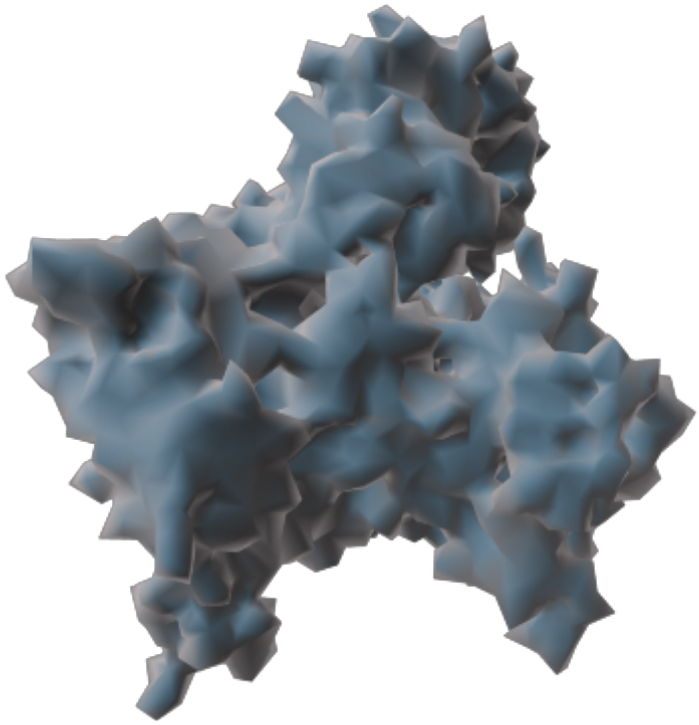

Figure 8.

Albumin molecule

Albumin was also added to the animation of the blood flow.

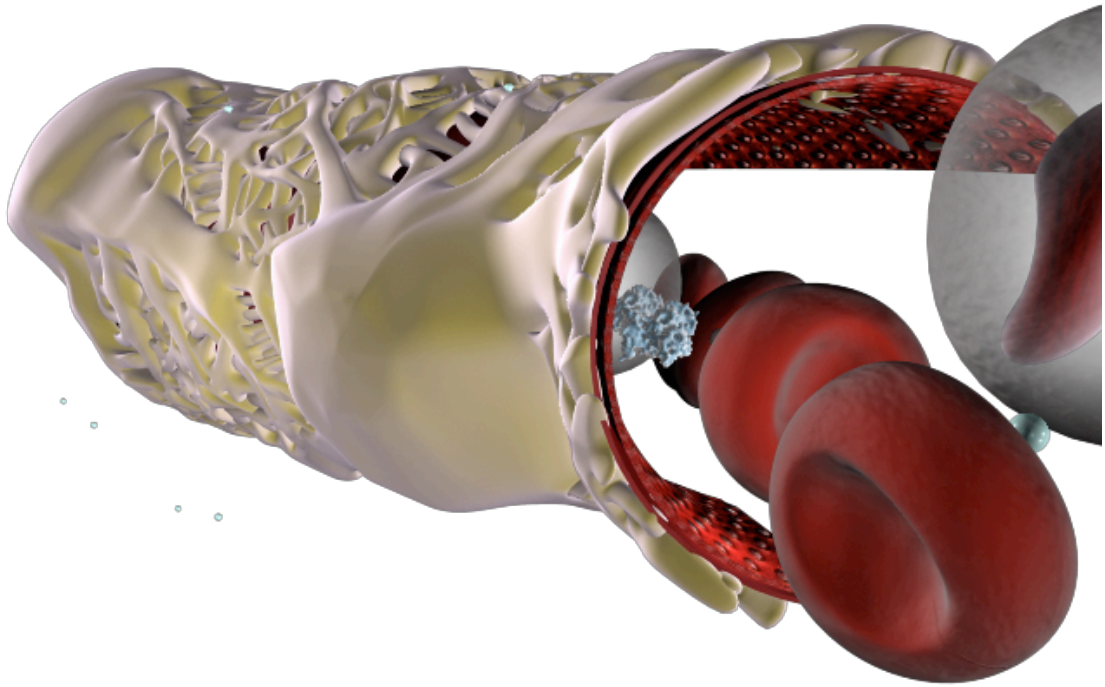

Figure 9. Normal blood flow through glomerular vessel

Research on Glycoproteins

Glycoproteins are particularly important in the study of Anti-Glomerular Basement Membrane Disease. It is within the network of the glycoproteins that the antigen for the disease is found.

The basement membrane is comprised of glycoproteins, which interact with one another, including laminin, nidogen, perlecan, integrin, and type IV collagen. There is interaction among these protein molecules.

(Kumar et al., 2007 and Kramer, 2005)

Modeling the Glycoproteins

Collagen IV was the first to be created, as it would be the protein molecule that would contain the antigen that activates Anti-Glomerular Basement Membrane disease. Polygon modeling was used to create three helices for the Collagen IV model. Spheres were used to create the carboxyl terminals of the Collagen IV model. The three helices and three spheres were grouped so that all six pieces combined to form the Collagen IV molecule. The Collagen IV model was duplicated numerous times and laid out to form the grid.

Laminin molecules were modeled in Maya using polygon helices that were bent and reshaped using deformers, and manipulating edge loops. Nidogen molecules were modeled using extruded NURBS surfaces, and reshaped by manipulating vertices. Manipulating edge loops, as well, shaped them. For the perlican molecule, heparan sulfate chains were modeled using using extruded NURBS surfaces. The core proteins were modeled using NURBS spheres. Finally, integrin molecules were modeled using polygon sphere and cylinder shapes. Manipulating edge loops also reshaped these molecules. (Alberts et al.,2000 and Hay, 1991) were used as primary references for the grid network for the glycoproteins.

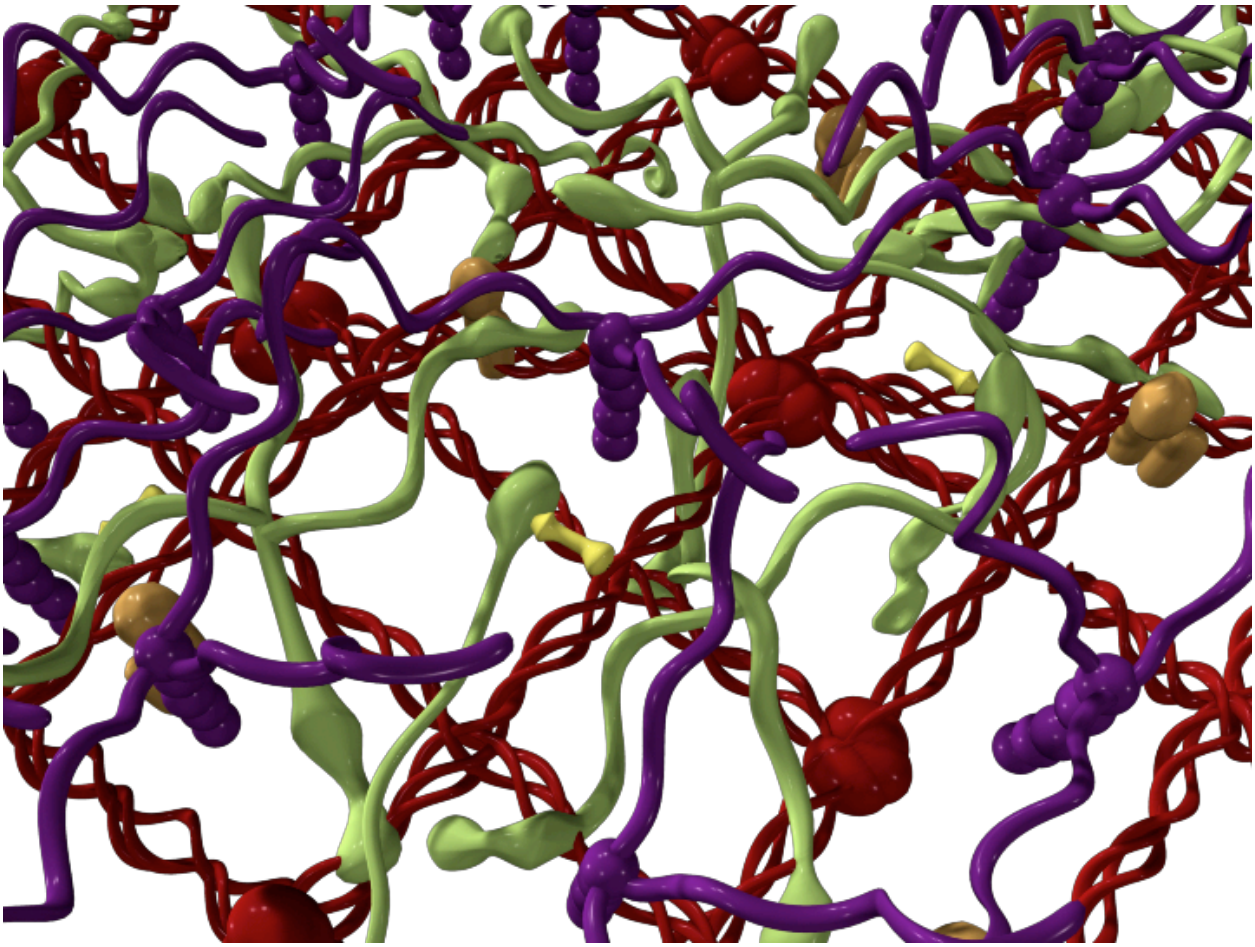

Figure 10. The Glycoproteins

This complex layer is largely anionic, and works to permit cationic protein molecules passage into the capillary lumen. The glycoprotiens, particularly collagen IV, provide strength, structure and integrity to the glomerular wall.

Modeling Beta Globulin

Beta globulin is one of those proteins that the basement membrane will allow to pass through due to its cationic properties. A model of beta globulin was created and used with the layer of glycoproteins to demonstrate normal clearance of some proteins through the basement membrane. The model of beta globulin was made in the same method as the method that was used for the albumin model. This model was imported into the scene with models of the glycoproteins. The beta globulin model was then animated to pass through the glycoprotein grid by key framing its position from the right of the stage to the left of the stage.

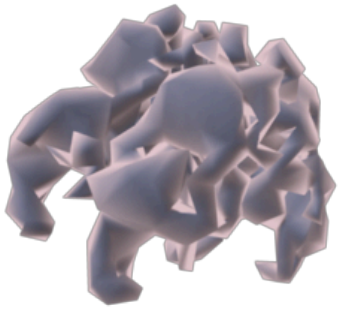

Figure 11. Beta Globulin Model

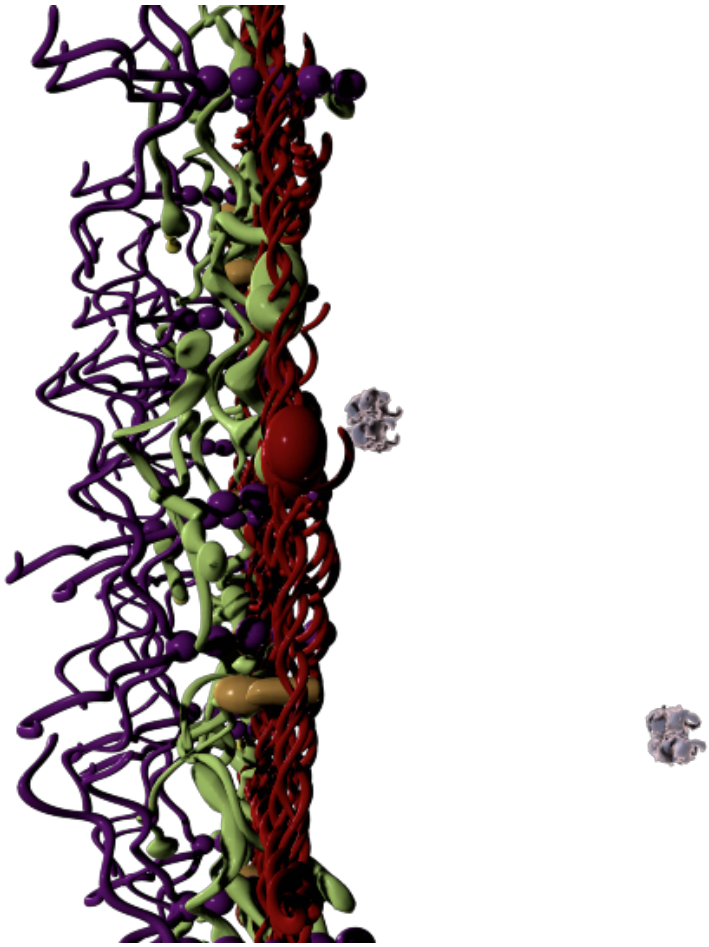

Figure 12. Beta globulin molecules passing through glycoproteins.

Modeling Cross Section of Nephron

A cross section of the nephron was an important view for the anatomical structure of the nephron. It included all the critical structures that were being highlighted in the animation. The cross section view provided the target audience with another reference point for understanding the nephron and its physiology. Additionally, this view was also a critical reference for comparison with the image of the crescent formation of leukocyte infiltration in the nephron that is later observed in the advanced stages of Anti-GBM disease.

A cross section of a nephron was created using NURBS and polygons. Smaller cross-sections of the capillaries that make up the glomerular tuft were created. The basement membranes and fenestrated endothelium were created using lofted NURBS cylinders. Duplicates of the fenestrated endothelium were placed inside the basement membrane. Vertices for all cylinders were manipulated to make distinct shapes of what would be all the separate arterioles in the nephron. Polygon cylinders were used to create foot processes for the visceral epithelium.

The foot processes were situated on the exterior of the arterioles forming a ring around each arteriole. The same shaders that were used for demonstrating the three sections of the glomerular wall were also used in the images of the cross section of the glomerulus.

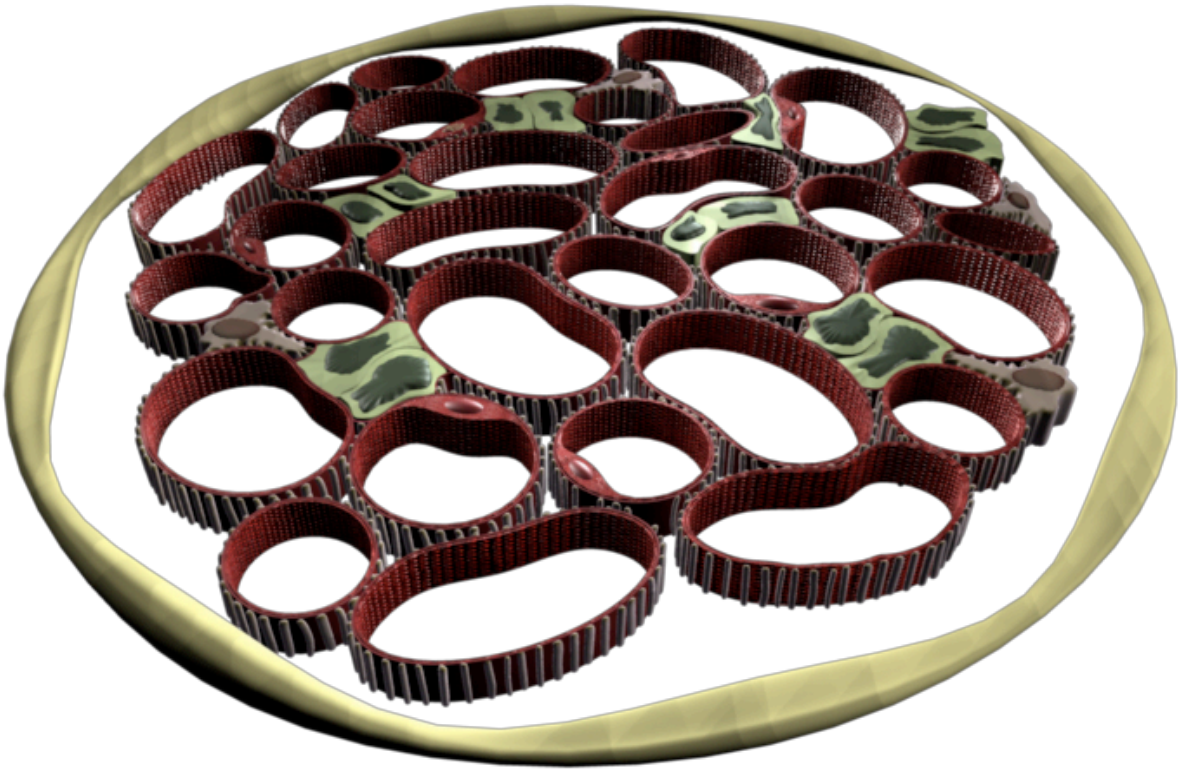

Figure 13. Cross section of nephron

Research on Anti Glomerular Basement Membrane Disease

Goodpasture's disease, or Anti-Glomerular Basement Membrane (GBM) disease, is a rare renal disease that, left untreated, leads to the progressive deterioration of the glomerular filtration barrier. Its estimated occurrence is about one case per 2 million per year in European populations, which are mostly Caucasian. (Kluth 1999)

It has been determined that Anti-GMB disease originates within the basement membrane of glomerulus in the nephron. It can also occur in the basement membrane of lung tissue.

At the present time Anti-GBM disease is considered by the medical community to be an idiopathic autoimmune disease. However, research into the origins of the diseased has suggested links between environmental factors, particularly with relation to hydrocarbon exposure, and also among patients with mechanically damaged kidneys. (ibid.). In addition, research as demonstrated some genetic susceptibility. "Associations with DR alleles with positive associations with HLA-DR15 andHLA-DR4, though exactly how these DR alleles predispose to development of the disease remains uncertain." (Kluth 1999).

The Antigen

Research has widely determined the antigen in Anti-GBM disease is located in the basement membrane, particularly the carboxly terminal globular (non-collagen 1 or NC1) domain of the alpha 3 chain of collagen IV.

Researchers from the Laboratory of Medicine and Pathology at the University of Minnesota Medical School (Butkowski et. al.1989) have indicated that the specific antigens recognized by antibodies of patients with Anti-GBM disease are M24, M26, M28+, and M28+++. M28+++ was found to be the most reactive. (ibid.).

Modeling and Animating the Antigen

In the animation, highlighting the NC1 domain of the alpha 3 chain of collagen IV identified the antigen. The antigen was identified by applying a bright orange Blinn, lowering the eccentricity, and increasing the glow intensity to .370.

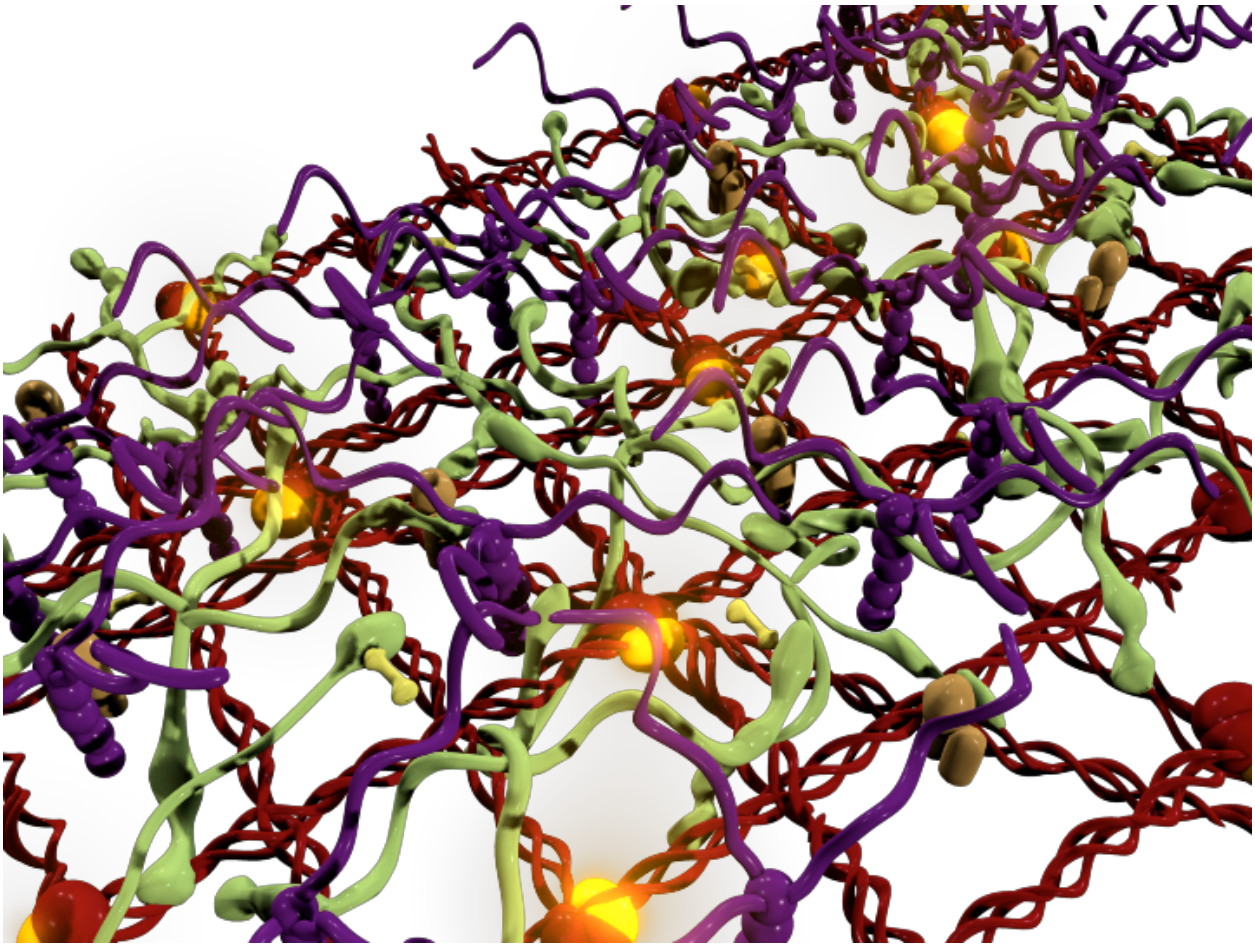

Figure 14. The antigen identified in the basement membrane.

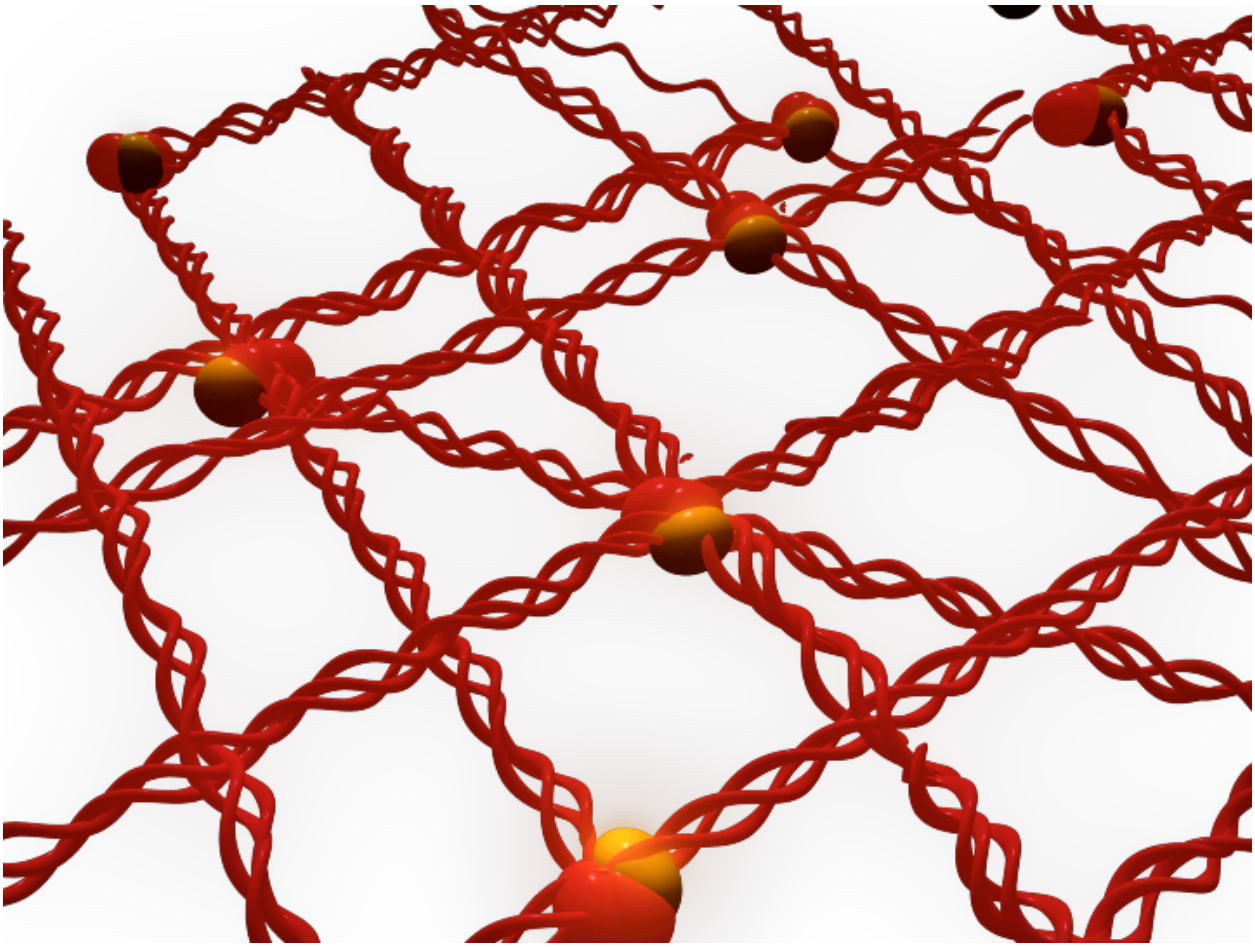

Figure 15. The antigen isolated in the collagen grid

The Body's Response to the Perceived Antigen

IgG antibodies line up at the site of the antigen in the basement membrane in a linear formation that distinguishes Anti-GBM from other diseases. As a response to the perceived invader, antigen antibody complexes also circulate and congregate at the site of the antigen. Both of these events lead to inflammation and thickening of the glomerular basement membrane.

Modeling of the Antibody Response

The scene in which IgG antibodies line up along the site of the antigen in the basement membrane was also an important reference point for the animation. This image marked where the chain of events that lead to the degradation of the three layers of the glomerular wall begins. A smaller section of the glomerulus was assembled. The basement membrane and fenestrated endothelium were both created using the NURBS torus tool.

Textures that were used in previous models with the basement membrane, fenestrated endothelium, and visceral endothelium were also used in this model. Foot processes of the visceral epithelium were created using polygon cylinders. Edge loops and control vertices were manipulated and shaped to simulate the podocyte foot shape. IgG antibodies were also created using cylinders. A light grey Blinn was applied to the IgG molecules. The basement membrane antigen was created using polygon spheres.

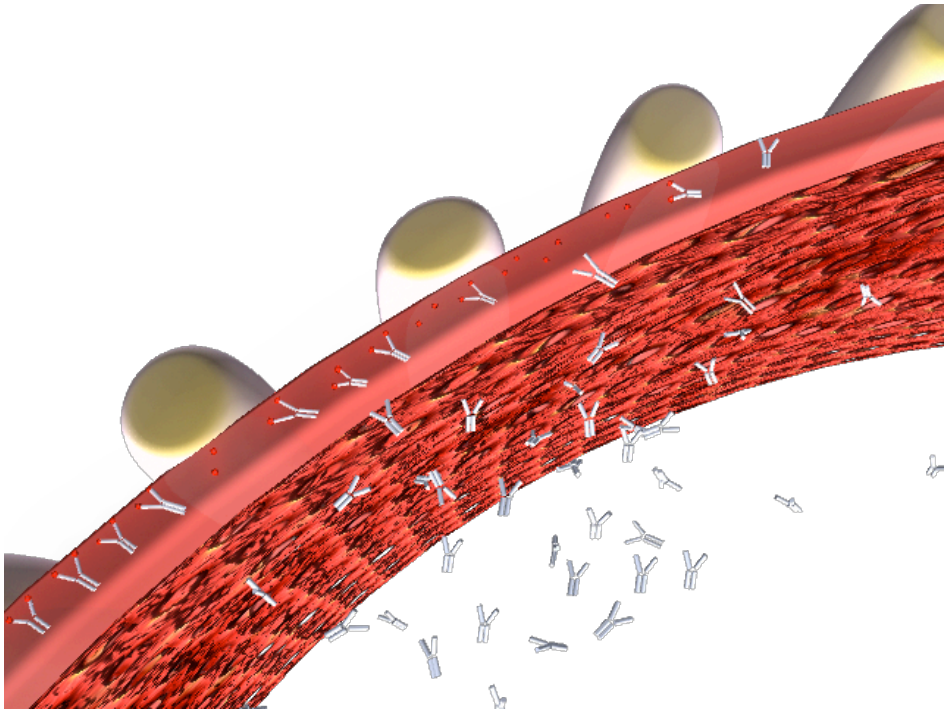

Figure 16. IgG's in linear formation at the basement membrane.

Auto Immune Response

Auto reactive T cells may play a role in the development of Anti-GBM disease. Complement proteins impact on the basement membrane. Anytime antigen-antibody complexes develop, there is activation of the complement cascade that promotes inflammation and direct injury to tissues.

Along with complement, cytokines associated with antibodies and leukocytes work to disrupt the basement membrane and then the other layers of the glomerular basement membrane. Eventually there is a loss of foot processes. This degradation is known as effacement and then detachment.

Modeling and Animation of Complement Cascade and Cytokines

A closer view of the three layers of the glomerular wall was created to show the details of the auto immune response. Polygon cubes were used for the basement membrane and the fenestrated endothelium. A bend deformer was used for each polygon cube shape to add a curve to the shapes, in order to create a vessel wall. NURBS spheres were used for podocyte foot processes. They were elongated and reshaped to simulate the foot processes.

Complement components were created using a series of NURBS shapes. For example, the C1 components were created with extruded surfaces and spheres. Control vertices were manipulated to shape the components. Cytokines were modeled with cones. Various colored phongs were used as shaders for the cytokines.

The complement cascade was demonstrated by selecting the individual models and moving them to the surface of the basement membrane and setting those progressive placements along key frames. Cytokines then followed the complement cascade with similar placement.

The basement membrane needed to be animated to demonstrate the impact of the complement cascade and cytokines, which gradually erode the membrane. A noise texture was applied, and then animated by adjusting the bump value. Adjusting the bump value ensured consistency of the holes that were forming in the animation. They remained essentially in the same place, and grew as deterioration took place.

This technique worked effectively for both the close up model, and the larger model of the three layers.

The same texture that was used in other scenes for the fenestrated endothelium was also used in this scene. However, for the animation of the deterioration, a blend node of leather 2D texture and the Photoshop fenestrated texture file was also applied as a bump value. The bump size map was progressively adjusted as a negative value along the timeline.

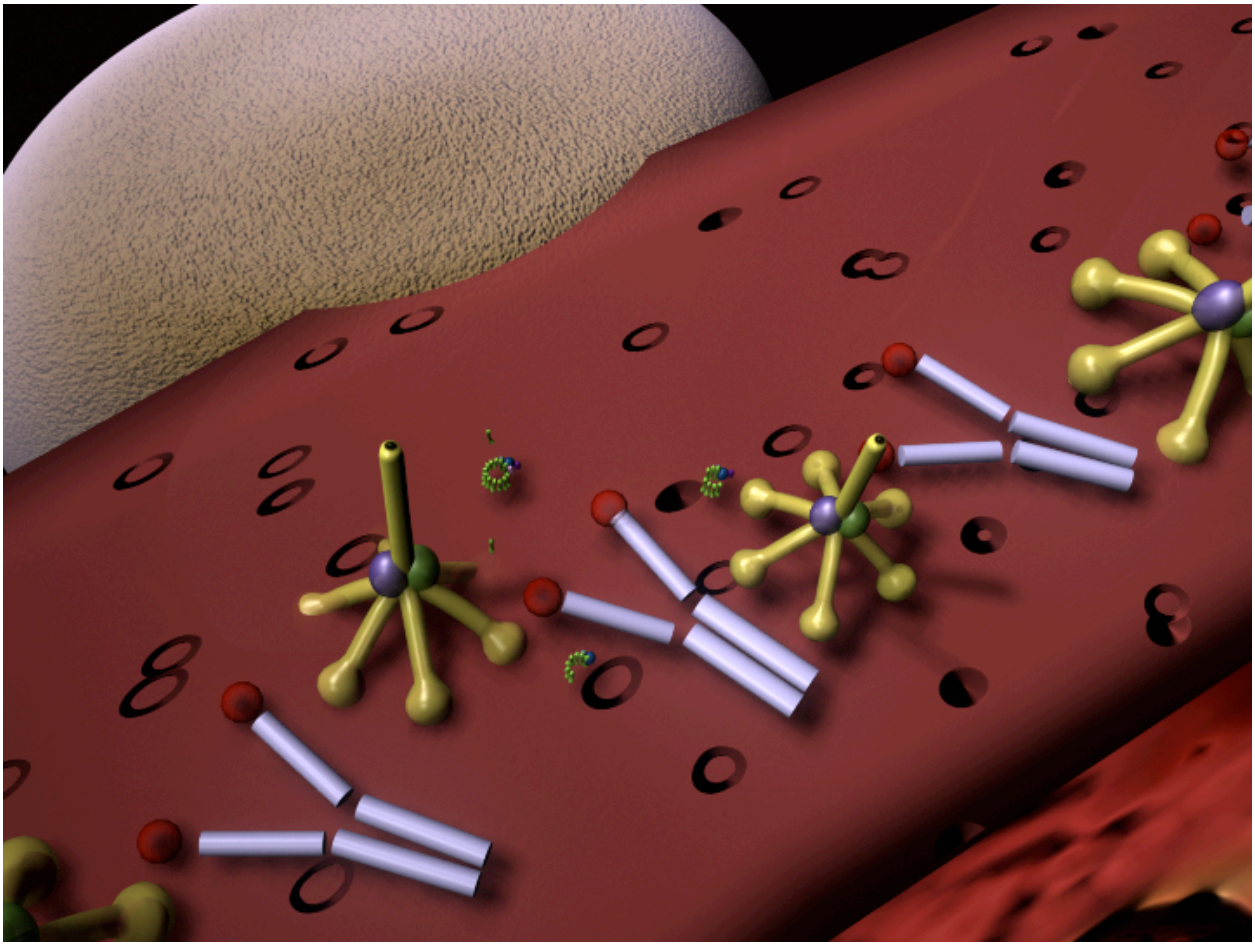

Figure 17. Basement membrane and other layers impacted by immune response.

Animation of Defacement and Detachment

Control vertices were manipulated at progressive key frames along the timeline, until each foot process in the scene was at least partially detached from the basement membrane.

Leukocyte Infiltration

As the total glomerular filtration barrier degrades, leukocytes infiltrate the glomerulus, forming a crescent, which also distinguishes Anti-GBM disease from other renal diseases.

Animation of the Leukocytes into the Glomerular Capsule

The model was then made to demonstrate the infiltration of the leukocytes into the glomerular capsule. The neutrophil model was duplicated and brought into the scene, which demonstrated the complement cascade. Animation deformers were applied to duplicated neutrophils. Neutrophils were progressively moved and manipulated with the deformers. Each movement and reshaping was key framed so neutrophils would appear moving through the glomerular wall.

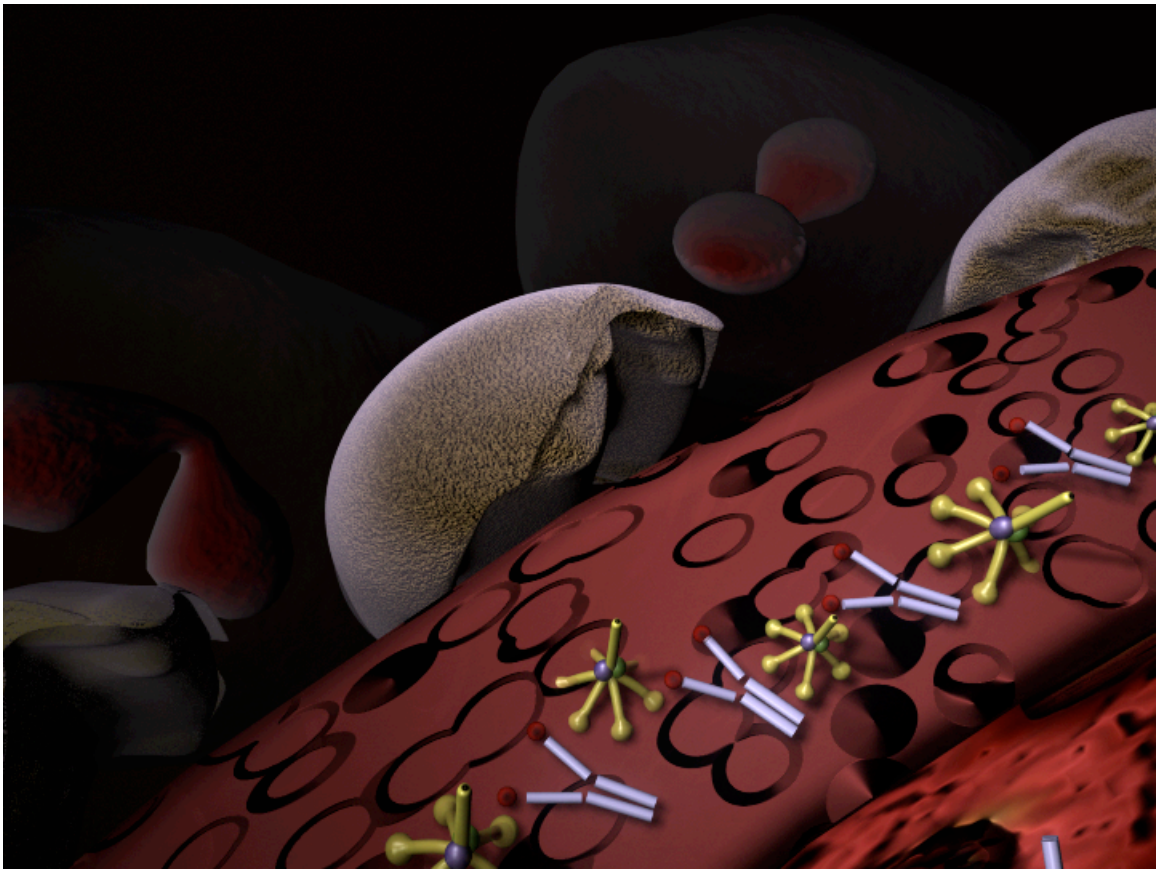

Figure 18. Leukocytes infiltrating the urinary space

Modeling the Crescent Formation

The model of the cross section of the glomerulus was then brought into Maya and saved under a new file named 'leukocyte infiltration'. Control vertices were moved on the glomerular capillaries in order to demonstrate the squeezing of the glomerulus, and the crescent formation leukocytes that move into the urinary space. A still image of the model was used in the animation.

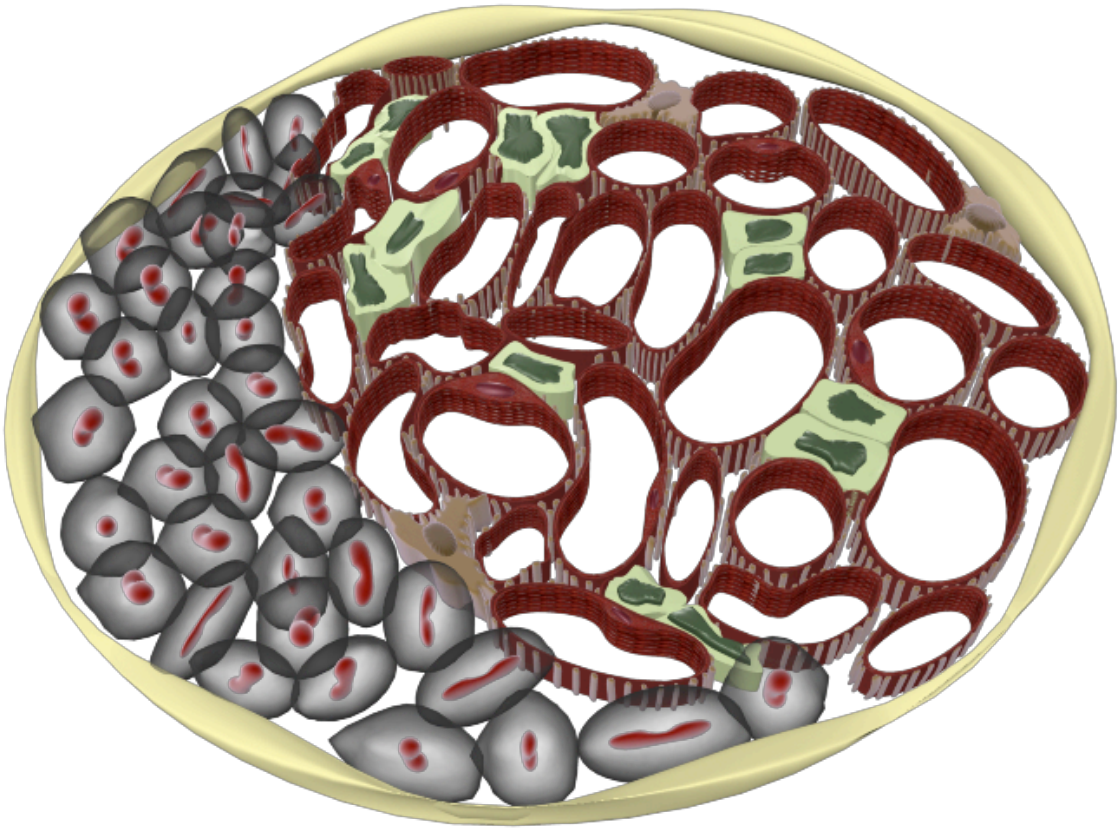

Figure 19. Crescent formation of leukocytes into the glomerular capsule

Final Stage of Anti-GBM Disease

With the deterioration of the glomerulus, filtration fails. Proteins pass through to the urinary space. Without treatment, nephrons deteriorate beyond repair, and total kidney failure is the final result.

Animating the Break Down of Glomerular Wall

The animation of the breakdown of the glomerular wall was the scene that required the largest space, the majority of the project's models, and the most animation activity. Consequently, it required the most time to animate and render. To save render time, blood flow animation was first created outside the final scene. A crude vessel was created in the same position and shaped so that it would work with the vessel in the final scene. Elements were positioned, reshaped, and key framed for the animation.

Particles were created moving in the vessel, and passing through it. Neutrophils and blood cells were also shown moving in and passing through the vessel. And finally albumin was shown moving in and passing through the vessel.

The three layers were separated. Individually, the three layers were shown to be breaking down. This was done by progressively decreasing all the bump maps that were applied to each layer. Deterioration of the visceral epithelium also involved manipulating control vertices to change the overall shape. Vertices were also progressively moved and key framed. The changing shape was made to look as though the epithelium was being eaten away in larger areas by the elements of the auto disease.

One by one, each animated layer was bought back into the main scene. The camera was animated separately so that it would eventually zoom into the three layers and show the progressive deterioration. The camera then zoomed out to show the seepage of blood flow components that were modeled.

In this scene that depicts the final stage of Anti-Glomerular disease, blood, leukocytes, protein, and solutes are shown not only flowing through the glomerular arteriole, but are also seen seeping through the deteriorated wall. The albumin model was included among the components that passed through the glomerular wall.

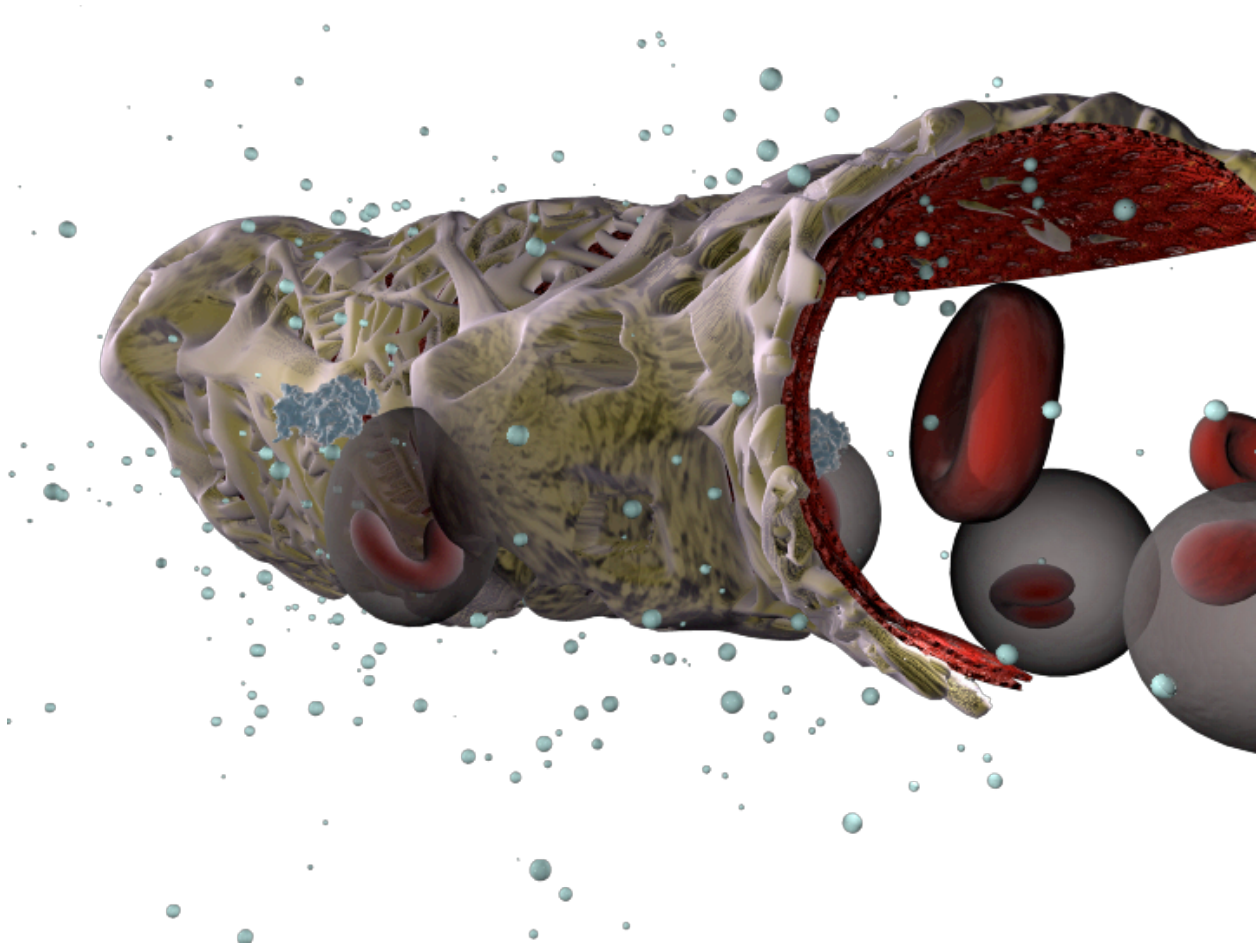

Figure 20. Proteins and neutrophils pass into the urinary space at a dramatic rate.

Anti-GBM Disease Research Conclusion

While Anti-Glomerular Basement Membrane Disease is considered rare, its study is critical. When the disease goes untreated, progression to the final stage can prove to be catastrophic or fatal. In addition Anti-GBM is also known to trigger other renal diseases, such as Alport's syndrome (Kluth and Rees, 1999). Plasmapheresis is a relatively effective treatment for Anti-GBM disease. Plasmapheresis is a process by which plasma is separated from the whole blood of a donor. For the patient suffering from Anti-Glomerular Disease, the donated plasma is then used in a therapeutic exchange for the patient's plasma. Patients with a lower percentage of crescent formation, and therefore a more effective filtration barrier have had greater success with plasmapheresis.

The Animation

The entire animation portion of the project was divided into three sections; 1. The Nephron, which provided instruction on the anatomy and physiology of the nephron; 2. The Disease Progression, which identified the antigen, and demonstrated the progression of the disease; and 3. The Disease, which demonstrated the disease in its final stages.

A script was written summarizing the basics of the research that be broken down into the tree sections. A recording was made using sound booth and Soundtrack Pro software on Mac OS X. Gregory Kulina was the narrator. These recording files were saved as AIF files. The AIF files were brought into Sound Booth software, edited, and separated into the three sections. The files were saved again as AIF files, then brought into After Effects and used to narrate the respective scenes.

All the models and animations were rendered as PNG files in Maya at 360 pixels per inch and using 540 x 720 dimensions. All PNG files were imported into After Effects. Scenes were created in After Effects according to the section of the animation that was being demonstrated. Once each animation was completed in After Effects, it was saved as an FLV file and brought into the website.

The website

The website for the project was designed with simplicity and functionality in mind. The design itself was intended to focus the viewer's attention on the images. Pages were set up with static images that were intended to reinforce the main points of consideration in reviewing the animations. The main menu buttons offer the viewer the option to preview the topics of anatomical views, pathology of the disease, or simply view the animations.

Several layout styles were explored, including menu bars and pull down menus with standard grid layouts. The final format became primarily a grid layout with organic features. The website was can be viewed at: http://mcccostello.com/wholepg512mccd.html

Conclusion

Many research skills were acquired in the development of this project. First, many resources for medical research were accessed, such as medical journal databases and medical library media resources. Relevant contacts were made with people in the medical field and Medical Illustration profession.

Fundamental skills for developing instructional media were acquired. Extensive experience using a variety of software programs was gained. Current 3D modeling, texturing and animation methods were explored and used. Image rendering processes were studied and these skills were honed as well. Various animation programs were utilized. Website development skills were also learned.

The goal of this project was to provide a multimedia instructional lesson on a particular type of autoimmune disease. Ideally this project would provide visual information that was not previously available. It is hoped that the project will provide a greater understanding of Anti-Glomerular Basement Membrane Disease in biomedical education and lead to further research into this disease and autoimmune disease in general.

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