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BIOENGINEERED HUMAN KIDNEY FOR TOXICITY ASSESSMENT

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ABSTRACT

Kidney toxicity is one of the biggest threats that causes failure in preclinical safety cases which later gives rise to Nephrotoxicity and is promoted by lots of pharmaceutical companies. We are one the verge of requiring pre- clinical tests and processes so that that we can get more candidates and innovate for them but unfortunately a competent drug has seemed to be more expensive and needs more time. The progression of an innovative new process seems to be on the horizon which is the development of a long lasting 3D human kidney which can also test nephrotoxicity. Simple human kidney epithelial cells and fibroblast cells can be developed through extracellular matrix which later gave rise to a developed model. Production and culturing of fibroblast was possible which includes collagen-matrigel mix conciliate supreme morphogenesis for the cells. The physical characteristics relied on the transportation of Aquaporin 2. Added amount MTT and LDH, GGT-1, Cytokeratin 8.18. 19 cytotoxicity assays displayed a serious impact on the 49F and NRK52E cells, notably on at 10 uM and 100 uM concentration levels.

INTRODUCTION

Clinical Significance

The kidney, liver and lung, are the most important organs to seek out targeted toxicity after a new drug is given to the body but from research we see that kidneys might be more vulnerable to threats. The kidneys possess a small amount of the whole body mass and get 20-25% of the cardiac output, which also states that xenobiotic are controlled in systemic circulation. Kidneys can also distill tubular fluid, which impacts the elevated levels of xenobiotic and its effects and so we see the various reason for vulnerability of kidney (Raju *et al*., 2011). The 3R-principle, represents the switch of animal models and to reduce animal use as stated by William Russell and Rex Burch (1959) so we see that a new process is due. This 3D model can bypass this tissue, so the impact of the drug is seen instead of the physiological processes and also regular 2D tissue models does not successfully replicate drug in vivo (Pampaloni *et al*., 2009). So we see that 3D tissue cultures can be utilized properly.

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Specific Aims

The aim is to make a method for a 3D human kidney model in vitro in cisplatin which is used to treat solid tumors, is examined to see nephrotoxicity on the tissue and so we have two special aims to reach our goal.

Primary Specific Aim: Create a Relevant Tissue Model

To achieve our goal the proper ratio of epithelial to fibroblast cells must be made possible. A perfect optimal seeding density for fusion of human kidney epithelial cells (NRK 52E) and (NRK 49F) are identified and is going to be used and is called morphogenesis of epithelial cells (Shimazu *et al*., 2001). Its plasticity and flexibility of the matrix molecules is because fibroblasts and other cells (Daley *et al*., 2008). So we see why fibroblasts are necessary also a proper ECM, which includes the relevant materials for the tissue structure method. The hypothesis is collagen type I, Matrigel™, also sodium hydroxide, in the projected surroundings and if the test fails then the concentrations of the seeded cells are converted and also ECM components and finally Epidermal Growth Factor (EGF) will be given which helps in cell growth. The outcome of this aim will be assessed using a Hemotoxylin and Eosin (H and E), Carmine-aluminum sulfate stain, and IHC stain for the expression of aquaporin II, γ-glut amyl trans peptidase, and cytokeratin 8,18,19. Table 2 describes the binding patterns

associated with the stained proteins. Present stains or markers allows for testing of cell density and divide into branching figures with similarity to tubules.

Secondary Aim: Assessing the Effects of Cisplatin on Healthy Kidney Model

Secondary targ*et al*lows cisplatin on human kidney cells, in both the 2D and 3D surroundings and her the hypothesis is that cisplatin will greatly lessen the number of cells and reduce the competence of cell interaction which happens because of cisplatin to help in breaking down the cell membrane and getting rid of the physical connections of the cells. Histological stains, such as H and E stain and Carmine-aluminum sulfate stain. Will be utilized and IHC test the impact of cisplatin on the tissue proteins of nephrons. MTT assay is needed which calculates the functionality of cells and to find out the cytotoxicity of cisplatin on cells the 2D surroundings and this assay will evaluate the cytotoxicity of cisplatin on the NRK52E and NRK49F cells. The assay protocol changes the water-soluble MTT agent into an insoluble Formosan which is later solubilized and then the absorption amount is calculated to find the functional cells.
In case of 3D environment, an LDH (lactate dehydrogenase) analysis is done where tissues untreated with cisplatin will work as a control for other tissues. The LDH test is done to check the plasma membrane damage and if it shows death of cell. LDH is a stable, cytoplasmic enzyme. After damage on the membrane of cells LDH is given to the culture supernatant and after the supernatant is gathered the number of cell deaths can be known and because of a coupling reaction LDH is oxidized to produce pyruvic acid and NADH which then reacts with diaphorase to produce the Formosan a red color is made which is the measured to calculate the damage Below is diagram of the principle.

Long Term Goals

The model of the kidney may be given into the FDA method as evaluation system prior to the drug is given to an organism and so by studying the screening method scientists lessen and fitter the implementation of organisms for testing and this also can help to control tissue culture for a long time if it is controlled in a perfusion bioreactor which in turn can rectify in vivo conditions can give data on the impact of cisplatin. Finally if the drug has seen progression then. After this model has been effectively established using cisplatin as the test drug, the tissue may be utilized as an example to seek out nephrotoxicity in various organisms.

BACKGROUND

Renal Anatomy and Physiology

Renal Anatomy

The kidney is one of the most unique organs in the human body which helps in the excretion and filtration of metabolic waste materials for example ammonium and urea, keeping balance of acid-base and other fluids, starting the production of red blood cells and on top of that the distribution of important electrolytes is also carried out by kidney. Reabsorption of amino acids, glucose is possible, as they also have hormonal functionality by stimulating Vitamin D, erythropoietin, and release of calcitriol, and finally it also maintained blood pressure by controlling the water absorption (Eknoyan, 2009). Upper surface of kidney consists of a strong connective tissue which also has a membrane of fibroblasts and collagen fibers and also my fibroblasts are present in the inner layer. Inside structure is categorized mainly into to three sections such as the renal medulla, renal cortex and the renal pelvis and the outside most layer called the renal cortex acts as the boundary of the organ. Cortex consists of renal corpuscles which are sort of spherical in shape and is the origin of the nephron and possess a group of capillaries known as glomerulus and various other tubules. There's a sort of funnel know the renal pelvis which directs the route of the urine on to the ureter which is later excreted. Renal medulla, the innermost layer plays a huge role which is consists of parts or sections such as renal pyramids where each has a straight and a parallel segments of nephron and is relied upon to control proper levels of water and salts by a switching process (Zhongping *et al*., 2009).

Renal Physiology

The primary functional unit of kidney is called nephron which has a unique structure where each one of them has a glomerulus enclosed by a Bowman's capsule, descending limb ascending and is also responsible for absorbing water right before the filtrate is given to the ureter and this is done by a gathering or a collecting tubule. Glomerulus acts as tissue for filtration and has a group of capillaries which makes a round shape or a sphere. The units for filtration are made by a big layers of endothelial cells which are linked by diaphragms. Just below these big layers is the base of the glomerulus which uniquely has a negative charge and just on the on the other side are glomerular epithelial cells. Glomerulus acts as a gate and its structure has a hand for passing required molecules and there are three layers through which the molecules has to go through (Sands, 1999). Mainly bigger molecules like hemoglobin and albumin are not allowed to go which in turn pass to bloodstream and smaller sized molecules such as potassium and sodium ion can pass with ease (Li *et al*., 1995). Glomerulus is also responsible for getting about 20-25% of the cardiac output by the afferent arteriole prior going through the filtrate which is a bi layered epithelial mug or a cup to the capillaries of the Bowman's capsule and after that it reaches the proximal convoluted tube.

The main site for reabsorption is the proximal part which is thick and surrounded by epithelial cells which arises from parietal epithelial layers of the Bowman's capsule and the tubule is categorized into three parts such as S1 and S2 which advocates the proximal convoluted tubule and also the S3 which is the straight proximal tubule whose source is from the proximal tubule from the parietal epithelial layer of Bowman's capsule, the proximal tubule is subdivided into three segments—the S1 which has a rough surrounding and vacuolar lysosome and also mitochondria because active transport is takes place and S2, where the layering is regular and possess different size and capacity of lysosomes (Sands, 1999). S3 is more simpler and is more durable because of metabolic activity that is reperfusion/hyproxia or which is also related to distributor or linked collection (Khan *et al*., 2002). The enhanced sensitivity in the S3 layer might be a cause of reduced cystolic NADP+-dependent isocitrate dehydrogenase

(IDPc). IDPc synthesizes reduced NADP (NADPH), which are important elements for production of glutathione (GSH), which is the most regular antioxidant in the cells of mammals (Kim *et al*., 2009). The proximal tubule absorbs sodium potassium glucose and amino acids onto the bloodstream after it gets the desired filtrate from Bowman's capsule and it reabsorbs about 80% of the initial filtrate (Li *et al*., 1995). As we know that the proximal tubule is greatly permeable to water where sodium is reabsorbed and it goes against the electrochemical reagent Na+-K-/ATPase a pump which is present in the plasma membrane of lateral fold water goes into channels of aquaporin back into the circulation system and the proximal tubule also control levels concentration levels of chlorine, ammonia, potassium, a bicarbonate including hydrogen ions. Filtrate gets more distilled by going through a developing gradient as the descending limb are not good for reabsorption as a result water is diffused out of nephron and goes into the bloodstream. After the filtrate has passed from the loop Of Henle it reaches into the ascending limb of nephron which of course is impermeable to water. This part though is permeable to ions like Na+, Cl-, and K+, but they go out of the nephron and reach the medullary interstitium by the help of the Na+-K+/ATPase pump.

Distal tubule is responsible for further distillation of the filtrate since NaCl is likely to be actively reabsorbed into systemic circulation by NaCl (NCC) which is a co-transporter and an Epithelial Na+ server (ENaC). This part also plays a role for regularly absorbing bicarbonate ions with the secretion of hydrogen ions and giving rise to a very acidic urine liquid. Also the distal tubule is the place for changing ammonia into ammonium ions for making it easy for the body to excrete it and getting rid of the bad effects of the compounds. Aldosterone which is a hormone originating from adrenal gland after getting refreshed by angiotensin II, which serves to gather sodium and also releasing potassium so enhancing water keeping along with blood pressure of the living body. Collecting duct acts as final site for urine distillation and volume control. Antidiuretic hormone or (ADH), is a regulator hormone secreted by posterior pituitary gland, prompts translocation of the aquaporin servers of the nephron's plasma membrane layer, which in turn enhances the permeability of the collecting duct.

MATERIALS AND METHODS

Materials

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, and penicillin-streptomycin (pen-strep) was bought from Gibco (Invitrogen) and NRK 52E and NRK 49F, at Passage 0, from American Type Culture. Primary antibodies were purchased from Abcam (Grand Island, NY). FBS (fetal bovine serum), (Eugene, OR). Epidermal Growth Factor (E. coli derived) (EGF) and Cisplatin (cis-Diamineplatinum (II) dichloride) and Triton-100X were purchased from SigmaAldrich (Natick, MA). Collection (ATCC) (Rockville, MD). DMEM (Dulbecco's modified Eagle's medium),Growth Factor Reduced BD Matrigel™ Matrix and Human TaiCollagen Type I (3.87 ug/mL) was collected from BD Biosciences (Franklin Lakes, NJ) and LDH Cytotoxicity Detection Kit was received from TaKaRa Bio (Shiga, Japan).

2D NRK 52E and 49F Cell Culture

Preparation of Proliferation Media

For sterilization, materials were pipetted into a 500 mL filter flask with a pore size of 0.2 um. 500 mL of Gibco® DMEM was put on to the filter flask. It was then supplemented with 5% FBS and 1% Penicillin-Streptomycin and then flask was fixed with the vacuum and filtration was on its way while consequential media was utilized for 2D cell enlargement and tissue growth culture and prior to interaction it was warmed in a water bath at 37°C. Another media was utilized for culturing tissues and cells for the MTT analysis which had extra EGF at a concentration level of 0.02% of the volume (100 uL EGF/ 500 mL media).

NRK 52E and 49F Cell Expansion and Passaging

Kidney was thawed in water at 37°C and to wash off the Dimethyl sulfoxide (DMSO), which were mixed when frozen and finally were mixed with 8 mL of cell culture media. Final solution was spun for 5 minutes at 1250 rpm at 25°C. The reminder of the DMSO were aspirated and cells was then suspended with 5 mL of culture media where the 5 mL cell solution was distributed to a T-185 tissue culture flask and more 20 mL of culture media was given to flasks. Cells were later cultivated at 37°C in 5% CO2. The cells were examined for convergence under a microscope 3 times per week and then culture media was altered. Old media was withdrawn and switched for 25 mL of fresh culture media which was added with precision. As the cells got 80-90% convergence, they were passed at. Gibco® 0.25% Trypsin (1x) was warmed in a water bath at 37°C. Cells were cleaned with 15 mL of Phosphate Buffered Solution (PBS) to get rid of excess components. Washing as complete and the PBS was removed and 5 mL of warmed Trypsin was added to the flasks which were incubated at 37°C, 5% CO2, for a time frame of 5 minutes, where joint proteins decayed and cells broke free from the wall. Flasks were examined for proper cell detachment under microscope after 5 minutes. Culture media, 10 Ml in volume was needed to stop Trypsin reaction and the final solution was distributed to a 15 mL spinning tubule and the solution was spun for 10 minutes at 1250 rpm at 25°C. Remainder of Trypsin and culture media was removed, when cell pellet was achieved and suspended with 5 mL of new culture media. The cell suspension was perfectly mixed, and 2 mL was added to a fresh T165 flask. 25 mL of new media was given as the cells were stored at 37°C, 5% CO2.

Tissue Construction

12-well plate was needed for structuring the tissue. The cellular layer, was meant for providing a uniform extracellular surroundings. and for each well a total volume of 150 uL was augmented which had 50% matrigel (75 uL per well), Collagen Type I (1 mg/mL), and 2M NaOH (0.023 M) while the remainder was with PBS and after proper measures 150 mL was added to every well. The plate was then cultured at 37°C, 5% CO2 so that cellular layer becomes rigid. NRK 52E and 49F cells seeded in 165cm2 flasks having a convergence of 8090% was displaced and needed to trypsinized and after proliferation was withdrawn 10 mL of PBS was added for ensuring that all components was removed and no blocks in

trypsin reaction. Cells were trypsinized with 5 mL of Trypsin and incubated at 37°C, 5% CO2, for a time frame of 5 minutes. The cells were floating freely and the reaction was halted by mixing 10 mL of warmed proliferation media to the flasks. Proliferation media and trypsin solution were pipetted up and down for checking disconnection and the solution was spun at 25°C at 1250 rpm for 5 minutes. The media and Trypsin solution was removed, as the cell pellets were formed and were re-suspended in cellular proliferation media with EGF in a volume given by the cellular layer remaining volume needed (see below). The cellular layer was then made and each well, a total volume of 400 uL of the cellular layer solution was augmented which comprised of 50% matrigel (200 uL per well), Collagen Type I (1 mg/mL), and 2M NaOH (0.023 M) while the other volume was The remaining volume was NRK 52E and 49F.The NRK 52E cell line was then attached to a density of 100,000 cells per tissue, and the NRK 49F were connected at a density of 50,000 cells per tissue. Right after 400 uL of the cellular suspension was given to each well, the plate was cultured at 37°C, 5% CO2 for 30 minutes and incubation, 0.5 mL of proliferation media with EGF was which is adjusted to the top of the tissue, and more l 1.5 mL was given to the bottom of the well. In Figure 6 we see broad structure of seeded tissue. Media distributed with EGF was converted three times every week at first two times of tissue culture.

Dosing with Cisplatin

Tissues were filled with cisplatin and re attached to a precise volume of 1 mM (5.14 mg/mL), which works as a pack for gradual dilutions. Media present at the lowest stage of every (1.5 mL) was then separately pipetted and then sterilized and called as eppendorfs and put in -80°C for later analysis (Figure 14). NRK 85, 89, and 93 were kept in culture with the usual EGF media as low control. NRK 86, 90, and 94 placed with EGF media which has with 1% Triton, working as high control NRK 87, 91, and 95 were achieved with media with 0.01 uM cisplatin; NRK 88, 92, and 96 were with media 0.1 uM cisplatin; NRK 97, 101, and 105 were refined with media 1.0 uM cisplatin; NRK 98, 102, and 106 were refined with media with 10 uM cisplatin; and NRK 99, 103, and 107 was polished with media with 100 uM cisplatin. NRK 100, 104, and 108 was then refined with EGF regulated proliferation media and disconnected after two weeks have passed. This mechanism was repeated on Day 3 and Day 7 of the cisplatin incubation time and after three weeks of final culture time that is1 week after first cisplatin-exposure the remainder of the tissues were then put down.

Carmine-Aluminum Sulfate Stain

Tissues are cut in half with a scalpel and placed in a cassette and kept in 10% buffered formalin at 4°C for the rest of the night and half of it was cleaned with PBS right after 24 hours for approximately10 minutes and this was done a couple of times before it was finally filtered in Carmine solution (1g Carmine, 2.5g KAl(SO4)2, 500 mL dH2O) for that night at normal temperature and then again tissues are cleaned with PBS for 20 minutes after a 24 hour time frame and seen through a Leica DMIL microscope.

Histology

Again tissues are cut in half and placed carefully at10% buffered formalin at 4°C for 48 hours' time period then they are placed paraffin wax, and cut into horizontal cross parts by a Leica RM2255 microtome which was set to section in 8.0 uM increments and they were glued to polarized slides and dried overnight on a plate set to 37°C.The samples were exposed to H and E and IHC for and γ-glutamyl-transpeptidase (GGT1), Cytokeratin 8,18,19 (CK8/18/19), and Aquaporin 2. Aquaporin 2 was examined with rabbit polyclonal (1:100) and goat polyclonal to rabbit IgG-Cy5 conjugate (1:200). GGT1 and CK8/18/19 were probed with mouse monoclonal (1:100) and goat polyclonal to mouse IgG-FITC united (1:100). Bright images of the H and E filled tissue samples were seen by a Leica Fluorescence Microscope and its suitable Leica software. A fluorescent visual microscope Leica DMIL and black and white camera, Leica DFC340X, was utilized to see the IHC sample.

MTT Assay

MTT analysis was done to find out the cell viability of the NRK 52E and NRK 49F cells in relation with the EGF proliferation media after being mixed with cisplatin. NRK 52E and NRK 49F were trypsinized and again suspended to a density of 50,000 cells/mL and after that 100 uL was augmented to a 96-well plate—48 of the wells were acknowledged for epithelial cells and the other 48 wells were categorized for fibroblastic lines. Right after 24 hours the cells were placed to 0, 0.01, 0.1, 1.0, 10, and 100 uM of cisplatin for about 48 more hours and solid cisplatin was hanged in proliferation media with EGF, and 100 uL of media with the required concentration levels of cisplatin was augmented to required wells. Following incubation time 20 uL of MTT dissolved in PBS (5 mg/mL) was mixed well and incubated at 37°C, 5% CO2 for about 5 hours and the media and MTT solution was aspirated while 100 uL of DMSO was mixed to lyse them. The cells processed incubated in DMSO for 15 minutes at 37°C, 5% CO2 for another 15 minutes and the absorbance was seen to be 560 nm by a Spectra Max M2 which is a device for viewing molecular objects.

LDH Assay

LDH analysis helps to see cell death and cytotoxicity of the supernatant at certain points (Day 0, 3, and 7) right after cisptalin is given. The supernatant was put in -80°C freezer which was later heated for 7 minutes and after thawing 100 uL of supernatant was mixed to a well of a 96-well plate by a micropipette. LDH analysis reagent (100 uL), product of TaKaRa LDH Cytotoxicity Detection Kit was finally given to all the wells. Plate was molded with aluminum foil to stop out light and placed at room temperature for 30 minutes and after 30 minutes, the plate was withdrawn from foil and its absorbance was seen at 490 nm and 650 nm by utilizing the Spectra Max M2 (Molecular Devices). The % cytotoxicity was measured by the following: (experimental-control)/(triton-control) x 100.

Statistical Analysis

MTT and LDH analysis was done by PRISM. Distinct value of culture media surroundings was found by a two-way analysis of random or variance (ANOVA). A pvalue ≤ 0.05 was taken and p-value ≤ 0.001 played a crucial role.

RESULTS

Proliferation Media

Tube shaped bodies were seen which was given with EGF as countered to media that had no EGF.

Tissue Construction

Tissues made with a composition of 52E and 49F cells, placed onto a silk scaffold in an ECM surroundings comprising of matrigel and collagen showed cystic bodies. On the other hand tissues made with only 52E or 49F showed cells engagement to the scaffolding, so not allowing other bodies to take shape and from these results we seem to be given an idea of a fusion of 52E and 49F cells are required to make a model.

Carmine-Aluminum Sulfate Stain

To see the branching tubular stature of the tissue, the Carmine-aluminum sulfate stain was implemented as shown below are proper images for the tissues. To find the total growth and the application of the tissue the degree of its epithelial branching parts might be utilized. Tube bodies showed reduced characteristics conditions with excess of 0.01 uM of cisplatin given to the media where concentration levels not thought to be of significance in case of tubulogenesis of the 3D tissue. The high control is seen in Figure 6 h where the tissues were vulnerable to proliferation media possessing 1% Triton, so that other cells can be destroyed and in Figure 6 c-h we see the breaking of the plasma membrane layer because of apoptosis.

Tissue Characterization Stains

H and E stain displayed that the tissues readily exposed to all concentration levels of cisplatin had dead spaces which is a sign of cellular apoptosis and those cells seemed to be changing to tubules but empty spaces enclosing the bodies were dying and more deep spaces were seen where more significant exposure to cisplatin was present when differed to undiagnosed more analysis might be needed later on to support the claim. To acknowledge the model of the kidney an antigen stain was utilized which was made by an engineering structure as this stain was seen in perfect conditions of tissues so regular transportation of proteins were forecasted and IHC was also used to acknowledge it. Positive outcomes were seen from Aquaporin 2 and GGT1 spots. Aquaporin 2 stain displayed ductal structures, and GGT1 stain showed the designed proximal tubule. The CK8/18/19 stain had huge numbers of non-certified fluorescence. The sign for nuclei of cell was a Dapi blue stain or mark.

MTT Assay

From the outcomes we see that MTT analysis had a reduced competence of cell feasibility in perfect cases and 10 uM and also including normal and 100 uM stages. Epithelial cells had less feasibility that other fibroblast cells.

LDH Assay

from the LDH analysis it's seen that 100 uM cisplatin had high rate of cytotoxicity but fascinatingly lower levels of concentration had more cytotoxicity than expected (Fukuishi and Gemba, 1989). Statistical data were not of that importance in case of the control to the 10 uM and 100 uM culture specifics on Day 3 of culture ($p < 0.05$).

DISCUSSION

Tissue Construction

Ceil densities and ECM materials are important to make a relative model of kidney as it was necessary to acknowledge both of those fibroblasts and epithelial cells to make relevant 3D kidney model (Daley *et al*., 2008). Inflammatory hydrolytic degradation products and mechanical power and the components and stature of ECM had to be specifically achieved through proper mechanics as morphogenesis of epithelial cells is reactive for reduction of their lifetime and also the materials are co productive and easily interacts so it maintains their characteristics to resolve morphogenesis of NRK 52E and 49F cells (Subramanian *et al*., 2010). After more research we see that EGF would help to elongate of the progression of the kidney and helping to structure of tubule bodies (Subramanian *et al*., 2010). From as Figure 4 tubulogenesis in tissue comes in contact with EGF related media but no presence of tubular bodies were spotted in absence of EGF so we see to create NRK 52E and 49F cell mix for making a multicellular tubulogenesis and morphogenesis with branching in a single solitary cell and can be related to EGF's power and proliferation in human proximal tubule cells and synthesis of protein (Villegas *et al*., 2005). These tissues were grown in a static culture for only four weeks to increase durability and to give a string platform and we see that perfusion enhances the distribution of mass relocation and also to sustain the culture as it replicates in vivo (> 8 weeks) (Subramanian *et al*., 2010). Lastly, a TUNEL might aid to calculate cell deaths and injury to the DNA as H and E indicates large amount of cell destruction which are due to the impact of apoptotic signaling cascades and right before engaging with cisplatin LDH test can be done with the media to test cytotoxicity levels of final component.

Tissue Characterization

IHC Staining

For specific tissue formation an IHC stains for GGT1, Aquaporin 2, and CK8/18/19 was done which showed proper transfer of the GGT1 and Aquaporin 2 antibodies but However, the CK8/18/19, displayed non-specified stains of tissue even though it plays a role of a marker there even though there were patches of green color within the cell but the possibility of production of epithelial is not proved plus non-specific is also a marker for non-production of those epithelial cells and if those epithelial cells does not behave as cells then re structure is necessary and to convert the densities of cells. No deep green fluorescence coloration is seen among the tubular bodies so it has to done again with specific attention to blockage of the protocol. Aquaporin 2 and GGT1 showed promising characteristics as GGT1 mark gathered close to the cell nuclei proving a positive outcome for formation of proximal tubule and Aquaporin 2 stain had smaller number of indicators so we define the tissues more efficiently. Proteins for example Aquaporin1, Collagen IV, E-

cadherin, can bring about precise specifications but some other cells may show other types of genes. Other markers or stains E-cadherin can also promote epithelial cellular junction structure while Laminin and Collagen IV interpret the polarity and the membrane of the basement (Subramanian *et al*., 2010). To find information about the model of the kidney and its specifications is necessary as tubules play a pivotal role. From a study done we saw how 3D human kidney model displayed correct tubulogenesis with the help of a group of IHC stains or markers for which also had N-cadherin, E-cadherin, and Na+-K+/ATPase pump or motor so we see that we are one the verge of achieving a positive outcome for our aim (Subramanian *et al*., 2010).

Carmine Staining

Information gathered from the carmine stain shows us about the progression of tubulogenesis and its linkage among cells. Tissues that are vulnerable to concentration levels of cisplatin as low as 0.01 uM had signs of cytotoxicity and tubule devaluation but tissues that are undiagnosed showed tubule like bodies and breakage is seen in single cell links but in place of a single tubule fragment a group is visible with certain use of cisplatin. The reason being the breakage of plasma membrane layer of those epithelial cells and an after effect of cellular apoptosis and happens by the proteolysis of plasma membrane proteins and cytoskeletal (Liu *et al*., 2004). Primary hypothesis had been thought to believe that more tubule like bodies will be seen at greater levels of concentrations (>10 uM), and so the devaluation might be understood. A possibility is that static 3D environment might have reduced the design feasibility and satisfaction which was an outcome of mass distribution, so to re-do the study a cell line has to be drawn and to acknowledge the whole process and its system onto the structure of the bio reactor (Subramanian *et al*., 2010).

Cell Viability and Toxicity in 2D and 3D Environments

*From the MTT analysis, we saw higher levels of concentrations (10 uM and 100 uM), where cisplatin had the feasibility of both NRK 52E and 49F. NRK 52E cells had great effects from concentration amounts of cisplatin (82.5% decrease in cell viability (49F) against 94.1% reduction in cell feasibility (52E)) which can be because of the fact that epithelial cells have megalin receivers, and acts as endocytosis receiver shown in cells which are polarized and tube part of the kidney and it promotes receiver or receptor-mediated endocytosis in case of cisplatin (*Riedemann *et al*., 2007*). It is also related with aminoglycosides, known for nephrotoxic effects (*Schmitz *et al*., 2002*).*

Cisplatin needs other means to go through 49F cells as fibroblasts does not have those megalin receivers. It is thought that a link with hyaluronic binding protein 1 (HABP1/p32/gC1gR), present in membrane of the cell remains unstimulated as cisplatin goes into the cell, but the road is still not understood but it also is closely associated with admittance of cellular apoptosis (Guo *et al*., 1999). Unprocessed information was seen because of LDH analysis in case of 3D surroundings while distinctions between the control and 10 uM and 100 uM were important ($p<0.001$) on Day 3, no other data was claimed legit. From the graph we see the high levels of cytotoxicity and in Figure 15 we see a 3D in vitro human kidney model. Tissues given to 100 uM of cisplatin shows greater cytotoxicity with similarities of 10 uM of cisplatin possessing almost a same curve with less percentage. The current data might show higher vulnerability of the NRK 52E and 49F cells to cisplatin- related cytotoxic after effects and here immortalized cells were utilized. The experiments show us that the greater level of cytotoxicity in case of kidney tissues but as lower concentration levels did not justify the earlier studies, by using tissue distillation or concentration equipment or parameters for example seeding density of cells and ECM materials might had an impact for making a viable design which illustrates the reduction of cell feasibility at high levels concentrations of cisplatin (>10 uM) in case 2D analysis, as the counter opposite of high amounts of cytotoxicity in all 3D LDH tests or analysis. Distinctions are also possible because the 3D and 2D was in cisplatin regulated media for 7 days (336 hours) and 48 hours respectively before the absorbance was calculated and so the extra time length of 288 had greater effects and tissue media in the trans-well plates was replaced every 3-4 day which could have promoted immature death of cell if and if it is not replaced then concentration level might enhance and cause toxicity to those cells, thus it might be understandable about the death capacity of H and E even though they were thought to be in good health. For better results the tissue culture media has to replaced often that is every 2 days and if is introduced to a bioreactor structure it might be in touch with fresh and healthy media and ensure better quality of the final product.

Conclusion

Future Directives

To counter nephrotoxicity in drugs we need replacement methods such as utilizing 3D tissue engineering methods to precisely test the toxicity of the drug at hand and from this project we can see great possibilities to substitute toxic materials and get an early FDA approval note. High control utilization of Triton at 1% so that it can explain the death of cells in the tissue but as it acts a more of a detergent it blocks the layer of phospholipid and destroys bacteria by necrosis which was the opposite as the cells in apoptosis had been vulnerable to cisplatin and so for further studies agents that can kill might come in handy. After this model is acknowledged with the bio reactor we can show its resourceful capacity for longer periods of time and after that it can be more applicable for studying the length effects of cisplatin. The working capacity of the kidney must also be tested when cisplatin is introduced and the transportation of the Na+/K+ ATPase pump or motor might give out more data and working analysis which tests anion distribution of organic anion, glucose and albumin absorption and functionality of proximal tubes could become necessary for further projects. More choices among 2D cellular tests and preclinical analysis can be done so finally we can say that by coming up with this new method and executing it in the FDA processed which will promote more pharmaceutical drugs onto the shops and markets.

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REFERENCES

Raju, S., *et al*., *Nephrotoxicants and Nephrotoxicity Testing: An Outline Of In Vitro Alternatives. Journal of Pharmaceutical Sciences & Research*, 2011. 3(3): p. 110-116.

Pampaloni, F., E. Stelzer, and A. Masotti, *Three-Dimensional Tissue Models for Drug Discovery and Toxicology.* R*ecent Patents on Biotechnology*, 2009. 3(2): p. 103-117.

Shimazu, K., *et al*., *Morphogenesis of MDCK Cells in a Collagen gel Matrix Culture Under Stromal Adipocyte-Epithelial Cell Interaction. Kidney International*, 2001. 60(2): p. 568-578.

Daley, W., S. Peters, and M. Larsen, *Extracellular Matrix Dynamics in Development and Regenerative Medicine. journal of Cell Science*, 2008. 12(3): p. 255-264.

Eknoyan, G., *The Wonderful Apparatus of John Jacob Abel Called the "Artificial Kidney". Seminars In Dialysis*, 2009. 22(3): p. 287-296.

Zhongping, H., *et al*., *Blood–Membrane Interactions During Dialysis. Seminars In Dialysis*, 2009. 22(6): p. 623-628.

Sands, J., *Regulation of Renal Urea Transporters. journal of the American Society of Nephrology*, 1999. 10(3): p. 635-646.

Li, X., *et al*., *A Huntingtin-associated Protein Enriched in Brain with Implications for Pathology. Nature*, 1995. 78(6): p. 398-402.

Khan, K., *et al*., *Pharmacology of Cyclooxygenase-2 Inhibition in the Kidney. Kidney International*, 2002. 61(4): p. 1210-1219.

Kim, J., *et al*., *Role of Cytosolic NADP+-Dependent Isocitrate Dehydrogenase in Ischemia-Reperfusion Injury in Mouse Kidney. American Journal of Physiology*, 2009. 96(3): p. 622-633.

Fukuishi, N. and M. Gemba, *Use of Cultured Renal Epithelial Cells for the Study of Cisplatin Toxicity. Japanese journal of pharmacology*, 1989. 50(2): p. 247-249.

Subramanian, B., *et al*., *Tissue-Engineered Three-Dimensional in Vitro Models for Normal and Diseased Kidney. Tissue Engineering*, 2010. 16(9): p. 2821-2831.

Villegas, G., B. Lange, and A. Tufro, *Autocrine and Paracrine Functions of Vascular Endothelial Growth Factor (VEGF) in Renal Tubular Epithelial Cells. Kidney International*, 2005. 67(2): p. 449-457.

Liu, X., T. Vleet, and R. Schnellmann, *THE ROLE OF CALPAIN IN ONCOTIC CELL DEATH. Annual Review of Pharmacology and Toxicology*, 2004. 44(3): p. 349-370.

Riedemann, L., *et al*., *Megalin Genetic Polymorphisms and Individual Sensitivity to the Ototoxic Effect of Cisplatin. The Pharmacogenomics Journal*, 2007. 8(1): p. 23-28.

Schmitz, C., *et al*., *Megalin Deficiency Offers Protection from Renal Aminoglycoside Accumulation. Journal of Biological Chemistry*, 2002. 277(1): p. 618-622.

Guo, W., *et al*., *Up-regulation of Endothelial Cell Binding Proteins Receptors for Complement Component C1q by Inflammatory Cytokines. The Journal of laboratory and clinical medicine*, 1999. 133(6): p. 541-550.

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