

The Effects of Glucocorticoids on Migration and Tight Junction Proteins in Human Retinal Pigmented Epithelial Cells

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April 2022



Allegheny College
Department of Biology

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**A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College**

*I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code
and to maintain the integrity of both myself and the College community as a whole.*

Pledge

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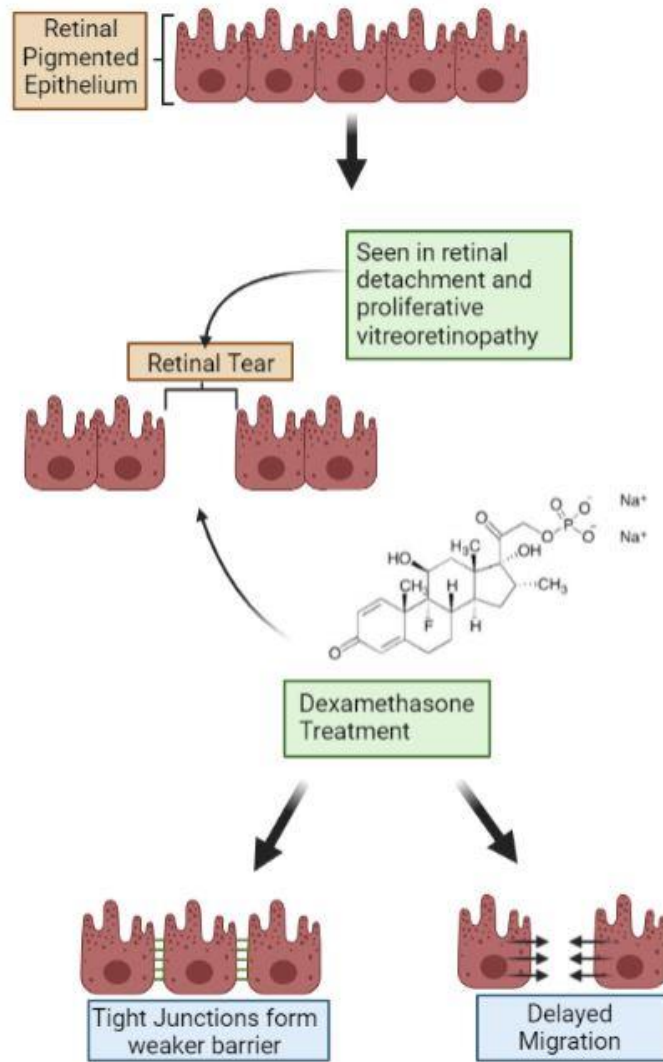
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Abstract

Retinal detachment affects roughly 1 to 10,000 people per year affecting the retinal pigmented epithelium within the retina. The retinal pigmented epithelium is known for maintaining a strong and protective barrier; however, retinal detachment causes this barrier to be weak which can lead to tearing of the retinal pigmented epithelium. Glucocorticoids are natural steroid hormones within the human body that respond to stress in a circadian rhythm and are known to regulate nearly every system throughout the body. Synthetic glucocorticoids, such as dexamethasone, are used to treat numerous auto-immune diseases and inflammatory diseases, including ocular diseases. This study investigates the effects of the synthetic glucocorticoid, dexamethasone, on migration and tight junctions in retinal pigmented epithelial cells. To investigate glucocorticoid actions in retinal pigmented epithelial cells, a wound healing assay was used to analyze the migration of cells under the presence of dexamethasone and immunocytochemistry along with immunoblotting was used to analyze tight junctions under the presence of dexamethasone. Dexamethasone significantly decreased migration in the retinal pigmented epithelial cells while dexamethasone treatment did not show any effect on tight junction distribution at the cell membrane. The results demonstrate that dexamethasone delays wound healing probably by impacting the migration of retinal pigmented epithelial cells. Together, this study provides insights on the functions of synthetic glucocorticoids on retinal pigmented epithelial cells.

Graphical Abstract



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Introduction

The Anatomy of the Human Eye

The human eye, the organ of the visual system, provides vision while receiving and processing visual information. There are many structures that are incorporated into the three layers of the eye. The external layer consists of the sclera and cornea providing protection while the transparent nature of the cornea allows for light to enter the eye. [1]. This anterior chamber of the eye contains a clear liquid known as the aqueous humor. The liquid brings nutrients to the eye while also disposing of waste and any excess materials [1]. The intermediate layer consists of two layers, anterior and posterior. The anterior layer is composed of the iris and ciliary body, giving the eye its color, and controlling the size of the pupil. The lens is a transparent structure that works in combination with the cornea to transmit light to the retina and changes shape to alter the focal length in order for the eye to focus on objects at varying distances [1]. Between the lens and the retina contains vitreous humor, a colorless fluid. This liquid remains in the posterior layer and contains water, salts, collagen, and sugar to provide nutrients to the lens and retina [1]. The posterior chamber of the eye contains the choroid which is a vascular tissue present between the retina and the sclera, responsible for transferring nutrients throughout the eye [1]. The internal part of the eye contains the retina known to send chemical and electrical signals to the brain in order to perceive visual information. Photoreceptor cells, bipolar cells, and ganglion cells make up the neural retina. Within the back of the retina is the macula which is composed of a very high concentration of photoreceptor cells. The macula is responsible for the fine detail that people see and colored vision. Photoreceptors cells receive the light signal, and this is carried to the brain by the axons of the ganglion cells converging to form the optic nerve which leaves through the back of the retina (Fig.1) [1]. The axons of the ganglion cells are long fibers that process the image when light enters the eye and transmits the image to the brain through the axons [2]. Bipolar cells are a type of interneuron that connects the ganglion cells to the photoreceptor cells allowing for input and output of visual signals [2]. Photoreceptor cells are neurons

that transform light into electrical signals in order to process an image. Photoreceptors are further divided into rods and cones. Rods are known to be functional at night as they are highly sensitive to light. Cones are known to process color and have the ability to detect a large spectrum of light photons [2]. The retinal pigmented epithelium protects and provides support for the photoreceptor cells allowing the neurons to interpret the visual information [3]. Retinal pigmented epithelium, located between the neural retina and the choroid, is a layer of epithelial cells containing tight junctions and connections known to serve as a barrier as well as a transportation system for water and nutrients [1,3]. The retinal pigmented epithelium also plays an important role in photooxidation by disposing of the membranes of photoreceptor cells. Because the retina receives frequent and direct exposure to light, photooxidation occurs causing toxicity to the neuronal cells within the retina [4,5]. Rods and cones contain an outer segment that is constantly being regenerated at a very high rate. The retinal pigmented epithelium aids in this process due to the phagocytic ability of these cells. This layer of cells is responsible for engulfing the outer segment of the rods and cones and degrades this outer segment through phagocytosis. This process is regulated by circadian rhythms as well as changes in light conditions [5].

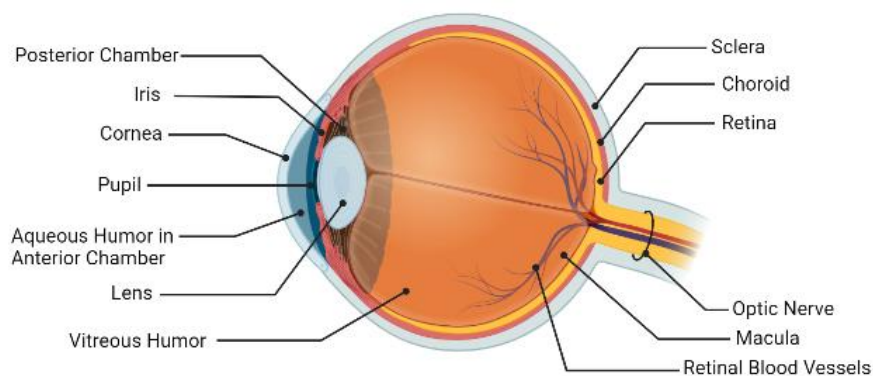


Fig. 1 The Anatomy of the Human Eye. Created with BioRender.com.

The retinal pigmented epithelium is a single layer of cells located in the back of the retina (Fig. 2). The main function of these cells is to form the tight junctions and connections that make up the blood-

retinal barrier. The barrier is very crucial due to its functions of regulating nutrient intake and regulating the different molecules, such as glucose, vitamins, and other nutrients within the retina [6]. Certain diseases of the eye can disturb this barrier and cause disruption in function [7]. For example, diabetic retinopathy is a disease that causes blindness due to high levels of blood sugar in those diagnosed with diabetes. This disease disrupts these connections and tight junctions within the barrier, affecting the regulation and function of this blood-retinal barrier. In diabetic retinopathy and other retinal diseases, the epithelium within this barrier is broken down causing apoptosis and degeneration of the cells [8].

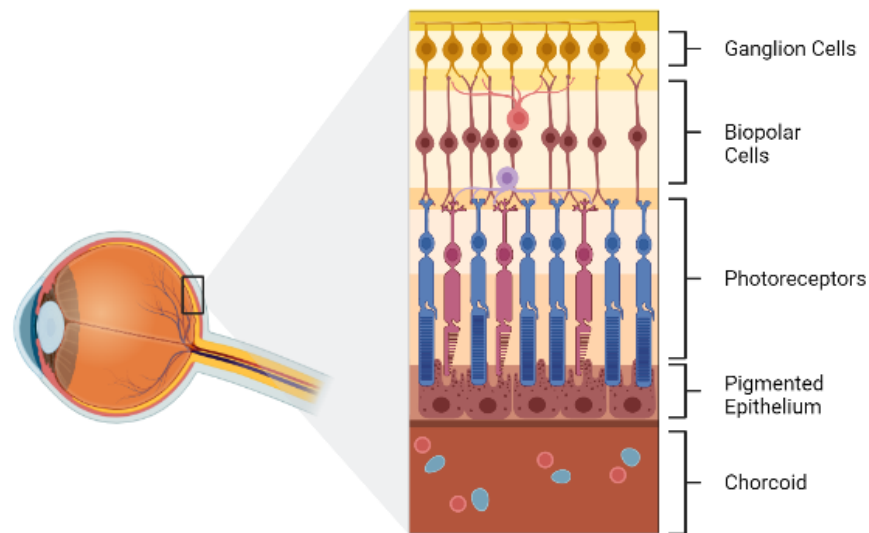


Fig. 2 Structures of the Retina. (A) A diagram of the eye showing the location of the retinal pigmented epithelium. (B) A diagram of the layers of the retina showing the retinal pigmented epithelium located between the photoreceptors and choroid. Created with BioRender.com.

Retinal Detachment

Retinal detachment is the separation of the photoreceptors, bipolar cells, and ganglion cells from the retinal pigmented epithelium within the eye. The detachment can occur when the retina, including the retinal pigmented epithelium, is under stress causing an accumulation of clear and lipid-rich fluid [6]. This fluid, known as subretinal fluid, occurs in the subretinal space located between the neural retina and the retinal pigmented epithelium [9]. Some of the main causes of retinal tears and retinal detachments are aging, eye surgery, trauma, or other ocular diseases [10]. There are three main types of retinal

detachment which are rhegmatogenous, traction, and exudative. Rhegmatogenous retinal detachment (RRD), the most common type of retinal detachment, is caused by liquified vitreous and tractional forces producing a break in the retinal pigmented epithelium and leaks fluid into the subretinal space. In most cases, RRD is followed by proliferative vitreoretinopathy (PVR) in which part of the retina tends to detach from the rest of the eyeball [10]. Traction retinal detachment is when the part of the retina, including the photoreceptors, is removed from the retinal pigmented epithelium by tractional forces but without retinal tears. This type of detachment is very common in diseases such as diabetic retinopathy, sickle-cell retinopathy, and other types of retinopathies [10]. Exudative retinal detachment can be due to the retinal pigmented epithelium being under an excessive amount of stress causing excess fluid to accumulate and tears in the retinal pigmented epithelium as well other parts of the retina. It can also be caused by hemorrhagic retinal detachment when blood accumulates in the subretinal space [10]. Overall, the main cause of any retinal detachment is due to an abundance of stress on the retina which causes the layers to detach from each other. Some types of stress and predisposing factors that may cause retinal detachment are accumulation of fluid in the subretinal space from the vessels or choroid, contractile fibrous tissue forming in the vitreous cavity due to retinopathy or trauma, and vitreoretinal adhesions in association with PVR [10].

Treatment options for retinal detachment and other retinal diseases are very minimal. Currently, the only treatment option for these diseases is injections directly into the eye of the anti-vascular endothelial growth factor (VEGF). This treatment works by reducing the swelling of the macula, located in the back of the retina. It has been known to slow vision loss and even improve vision. Vascular endothelial growth factor (VEGF) is a protein, expressed by endothelial cells and many epithelial cells, including those within the eye, that plays an important role in the production of new blood vessels [11]. However, in patients diagnosed with retinopathy, there is an excess amount of VEGF causing irregular blood vessel formation and inflammation of the capillaries within the retina. This inflammation and irregularity of the

vessels can lead to vision loss and blindness [12]. While anti-VEGF treatment slows the rate of vision loss, it is not cost-effective and does not cure the disease entirely [11]. In the latest research, glucocorticoids have been researched as a treatment option for retinopathy and other vision diseases [12].

The Role of Glucocorticoids

Due to their effects on anti-inflammatory and anti-angiogenic roles, glucocorticoids have been at the forefront of ocular pharmaceutical research since the early 1990s [8]. Glucocorticoids are natural steroid hormones located throughout the human body. In response to stress, these hormones are released from the cortex of the adrenal glands in a circadian rhythm. These hormones are known to regulate nearly every system throughout the body, including the eyes [13]. The secretion of these hormones is regulated by the hypothalamic-pituitary-adrenal axis. The hypothalamus releases a corticotropin-releasing hormone causing the anterior pituitary to synthesize and secrete the adrenocorticotrophic hormone (ACTH). The adrenal cortex produces and secretes cortisol (natural human glucocorticoids) in response to stimulation by ACTH [13]. Glucocorticoids can also act in a negative feedback loop. The hypothalamus and anterior pituitary can be targeted to inhibit the secretion of corticotropin-releasing hormone and ACTH, respectively, in order to prevent too much glucocorticoid from being released [13].

Synthetic glucocorticoids, such as dexamethasone, are medications prescribed to treat different inflammatory and autoimmune diseases [14]. These glucocorticoids' function and mechanism are to block cytokine function and inhibit leukocyte adhesion induced by VEGF [15]. Glucocorticoids bind to the glucocorticoid receptor to regulate these various functions throughout the body [16]. The *NR3C1* gene which encodes the glucocorticoid receptor is a part of the nuclear receptor superfamily (ligand dependent transcription factors). Once the glucocorticoid binds, the glucocorticoid receptor either represses or activates the transcription by binding to the glucocorticoid response element (GRE) in the DNA of target

genes (Fig. 3). Since glucocorticoids are regulated throughout nearly every system in the body, the sensitivity of glucocorticoids is diverse and is dependent on each system and tissue in the body [13].

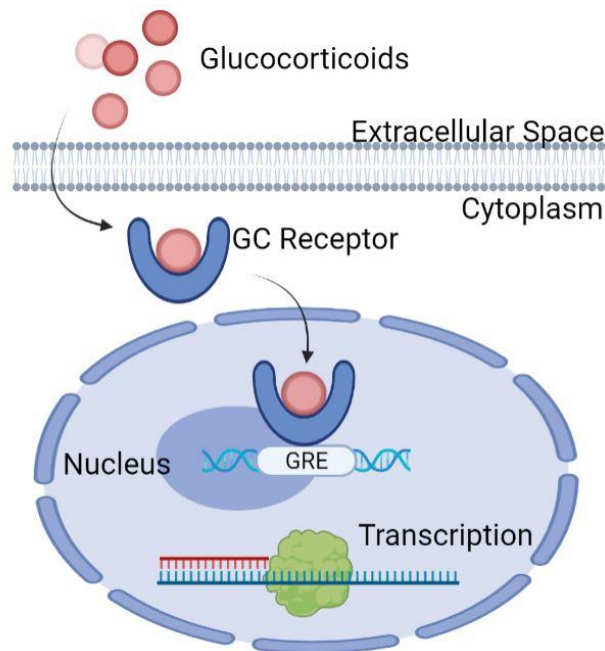


Fig. 3 The Pathway of Synthetic Glucocorticoids. Glucocorticoids bind to the glucocorticoid receptor and travel to the nucleus where the receptor binds with a GRE to regulate gene transcription of the target gene. Created with BioRender.com.

The role and function of the glucocorticoid receptor signaling has been researched in every organ system throughout the body, however the latest research is focused on understanding the molecular mechanisms of glucocorticoids in numerous ocular diseases. In a research study conducted by Kadmiel et al. (2016), the effects of glucocorticoids on corneal epithelial cells were investigated. The main goal of this study was to define the glucocorticoid actions in a human corneal epithelial line. Human corneal epithelial cells were treated with dexamethasone, RU486 (anti-glucocorticoid) and vehicle for 6 hours. Gene expression studies and ingenuity pathway analysis (IPA) were used to analyze the changes in transcription within the human corneal epithelial cells. A wound healing assay was also used to detect the genes associated with movement, cytoskeletal remodeling, and permeability. The glucocorticoid treatment improved the organization of the tight-junction proteins. In addition, the wound healing assay showed that the epithelial barrier function was enhanced when treated with dexamethasone. Glucocorticoids,

specifically dexamethasone, altered corneal epithelial expression, where hundreds of genes, including *IL-6* and *MCP-1*, were downregulated and upregulated, respectively, by activation of the glucocorticoid receptor. These genes are expressed through the inflammatory response as well as migration of the cells. The changes in these functions affect both wound healing and the epithelial cell barrier function within the human corneal epithelial line [17]. *IL-6* has been found to be expressed during migration in numerous other cell lines including breast cancer cells, astrocytes, and human aortic endothelial cells [18–20]. *MCP-1* has also been found to be expressed during migration in other cell lines such as tubular epithelial cells, oviduct epithelial cells, and fibroblasts [21–23]. While the expression of these genes has been investigated in different cell lines, the expression of the genes in the presence of dexamethasone has not yet been investigated in a wound healing assay.

The Roles of Pro-Migratory Genes and Tight Junction Proteins

In order for the retinal pigmented epithelium to be regenerated after cell death at the location of a tear or damage, the cells need to migrate towards each other. Involved in this process are numerous pro-migratory genes that aid this growth within the barrier of the retinal pigmented epithelium [24]. There are several pro-migratory genes that have been investigated in the retina including *MMP-2*, *MCP-1*, and *CXCR4* [24–26].

One of the most common primary ocular tumors among children is known to be retinoblastoma. Retinoblastoma arises from a mutation on the tumor suppressor gene *RB1* affecting amacrine cells located in the inner nuclear part of the retina [27]. If not diagnosed early enough, this type of tumor invades numerous parts of the eye including the choroid, sclera, and optic nerve. In retinoblastoma, there were two targets to block the metastasis of the disease. One of these targets was matrix metalloproteinases-2 (*MMP-2*) [24]. This gene is known to play a large role in cancer metastasis as well the migration of cells. In a recent study, *MMP-2* was inhibited in a retinoblastoma cell line to investigate the role of *MMP-2* in migration, angiogenesis, and production of cytokines [24]. The inhibition of *MMP-2* significantly reduced

migration in the retinoblastoma cell line showing that *MMP-2* is vital to the migration of these cells [24]. While retinoblastoma can be localized to just the amacrine cells, retinoblastoma has been found to affect retinal pigmented epithelial cells as well. Retinoblastoma can cause aberrations in the retinal pigmented epithelium as well as excess proliferation of the retinal pigmented epithelial cells that can lead to congenital hypertrophy [28]. Although it is extremely rare, malignant neoplasia and retinal pigmented epithelium adenocarcinomas have also been observed in patients recovering from retinoblastoma[28].

Proliferative vitreoretinopathy (PVR) is a common complication of RRD [10]. An *in vitro* study was performed to investigate the migratory and proliferative effects of monocyte chemotactic protein-1 (*MCP-1*) in human retinal pigmented epithelial cells [25]. A wound healing assay was performed on the confluent layer of cells before the cells were incubated with *MCP-1*, *IL-1 β* , and *TNF- α* . *MCP-1* stimulated the migration of the human retinal pigmented epithelial cells, however, *MCP-1* did not show a significant effect on the cell proliferation [25]. It was suggested that *MCP-1* regulates the development of PVR [25].

In response to proinflammatory cytokines, retinal pigmented epithelial cells have been shown to produce various chemokines [26]. A study used RT-PCR to determine that C-X-C chemokine receptor type 4 (*CXCR4*) was predominantly expressed by retinal pigmented epithelial cells [26]. When the cells were exposed to stromal cell-derived factor 1 α (SDF-1 α), the retinal pigmented epithelial cells migrated. Furthermore, *CXCR4* in combination with SDF-1 α regulates chronic inflammation as well as the subretinal neovascularization in the blood-retina barrier, specifically at the site of the retinal pigmented epithelial layer [26].

In particular retinal diseases, such as age-related macular degeneration, the retinal pigmented epithelium undergoes cell death causing vision loss. It has been shown through *in vivo* studies that retinal pigmented epithelium has the ability to regenerate the area undergoing cell death due to disease [29]. Glucocorticoids, specifically dexamethasone, have been shown to slow migration in cell lines, like the

human corneal epithelial cells. However, it has also been demonstrated that glucocorticoids have been an aid in improving the organization of the tight junctions within cells, developing a stronger epithelial barrier [17]. Understanding how the wound healing process works in retinal pigmented epithelial cells and how they are affected under different concentrations of glucocorticoids could help to improve our understanding of retinal pigmented epithelial cellular processes.

Tight junctions are vital in the functions of the retina. Tight junctions are complex structures known to regulate cell proliferation and paracellular diffusion [30,31]. Within the retinal pigmented epithelium, the tight junctions allow a barrier to be formed by creating cell-to-cell connections as well as regulating the diffusion of nutrients, ions, and gasses through the paracellular spaces within the barrier [30,31]. These neighboring cell connections allow for the development of the retinal pigmented epithelium and provide mechanical support for the blood-retina barrier [30]. While the functions of tight junctions vary among different types of epithelial cells, the epithelial barrier located in the retina (retinal pigmented epithelium) is known to interact with the neural retina containing photoreceptors, bipolar cells, and ganglion cells [31]. The secretion from these cells located in the neural retina is known to regulate the assembly and maturation of these tight junctions [31].

Specifically in retinal pigmented epithelium, the tight junction protein zonula occludens (ZO)-1 is known to regulate proliferation as well as gene expression of cell cycle genes such as *cyclin D1* and *PCNA* (Proliferating Cell Nuclear Antigen) [32]. ZO-1 regulates these actions by the inhibition of the Y-box transcription factor ZONAB (ZO-1-associated nucleic-acid-binding protein). In an *in vivo* study, the role of ZO-1 and its pathway was investigated in retinal pigmented epithelium by overexpressing ZONAB and knocking down its cellular inhibitor of ZO-1 [32]. It was found the overexpression of ZONAB, or the knockdown of ZO-1 resulted in increased proliferation in retinal pigmented epithelium [32]. These findings are critical to the understanding that ZO-1 and ZONAB are necessary in maintaining the homeostasis of the retinal pigmented epithelium layer. It also suggests that ZO-1 and ZONAB might be associated with

retinal diseases, proliferative vitreoretinopathy, and age-related macular degeneration, however this would have to be further investigated [32]. Although it is known that ZO-1 is a critical component in the retinal pigmented epithelium, it is unknown whether glucocorticoids affect the development of the tight junction protein, ZO-1.

Research investigating the effects of glucocorticoids on different parts of the eye including the cornea, trabecular network, lens, and optic nerve has been conducted. However, the retina, macula and retinal pigmented epithelium have not been examined [16]. While it is clear that glucocorticoids play a significant role in migratory genes, it is unclear which genes are being regulated in retinal pigmented epithelial cells, the epithelium that is being affected in patients diagnosed with diseases such as diabetic retinopathy, age related macular degeneration, and Stargardt disease [12]. This research investigating the effects of dexamethasone on retinal pigmented epithelial cells can show the potential of glucocorticoids as a treatment option for retinal detachment involving the retinal pigmented epithelium. By using glucocorticoids, such as dexamethasone, to treat retinal detachment diseases, this study can provide insights on the use of glucocorticoids and the development of fewer side effects. In this study, the effects of synthetic glucocorticoids, specifically dexamethasone, on migration and tight junctions are investigated in retinal pigmented epithelial cells.

Materials and Methods

Cell Culture

A human retinal pigmented epithelial cell line (ARPE-19) was used throughout the study. ARPE-19 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Scientific, MA) containing 10% fetal bovine serum (VWR, PA), and 1% antibiotic and antimycotic (Thermo Scientific, MA) treatment at 37°C in the presence of 5% carbon dioxide. In a flask, cells were incubated in complete DMEM for approximately 24 hours before treatment of Dexamethasone or vehicle. To account for technical errors and reproducibility of data, each experimental approach was repeated at least two or three times.

In Vitro Wound Healing Assay

ARPE-19 cells were counted using a hemocytometer and 400,000 cells/well were plated into a 6-well plate. A day after plating the cells, they were treated with water as a vehicle or varying concentrations of Dexamethasone (10nM, 100nM and 1000nM) for 24 hours. A singular scratch was made down the middle of the well using a sterile p200 pipette tip. The cells were then washed with phosphate-buffered saline (PBS) to remove any dead or floating cells and replaced with fresh medium containing the vehicle or different concentrations of dexamethasone. In order to evaluate the scratch in the monolayer, cells were imaged at 10x magnification with a Leica upright microscope. Each experiment had three replicates of each condition and there were three independent experiments completed. Three images were taken of each condition in each well at 0 hours, 8 hours, and 24 hours. The area of the scratch was determined using ImageJ software at each of these time points. Images were taken only in X and Y planes. A t-test was used to determine significance.

The area of the wound closed in pixels/image was measured using the following formulas:

$$\begin{aligned}\text{Area Closed at 8 hours} &= (\text{area of wound at 0hr}) - (\text{area of wound at 8hr}) \\ \text{Area Closed at 24 hours} &= (\text{area of wound at 0hr}) - (\text{area of wound at 24hr})\end{aligned}$$

The percentage of wound area closed was measured using the following formula:

$$\% \text{ of wound closed} = [(\text{area of wound at 0hr} - \text{area of wound at 24hr}) / (\text{area of wound at 0hr})] * 100$$

Immunocytochemistry

ARPE-19 cells were counted and plated with 50,000 cells/chamber into a 4-well chamber slide (Lab-Tek, NY; catalog #154526). After 24 hours, the cells were treated with water as a vehicle or a 1000nM concentration of dexamethasone for 24 hours. Once treated, a sterile 200uL pipette tip was used to make a singular scratch in the middle of each well. The cells were then washed with PBS to remove any dead or floating cells and replaced with fresh medium containing the vehicle or 1000nM of dexamethasone. After 24 hours, the cells were fixed with 4% paraformaldehyde (PFA) for 10 minutes and then incubated with primary antibodies rabbit anti-GR (Cell Signaling, MA; catalog #12041, 1:500) and mouse anti-ZO-1 (Invitrogen, MA; catalog #339100, 1:500). After an overnight incubation, cells were then incubated with the corresponding secondary antibodies: goat anti-mouse Alexa 594 (Jackson Laboratories, PA; catalog #157633, 1:500) and goat anti-rabbit Alexa 488 (Jackson Laboratories, PA; catalog #159082, 1:500) along with Hoechst (Thermo Scientific, MA; catalog #H3570) to stain nuclei. Slides were then mounted with a cover slip using Fluoro-Gel mounting medium (Electron Microscopy Sciences, PA; catalog #17985-11). Images were taken using a Leica fluorescent microscope to determine changes in the pattern of protein expression. Different channels of the images were merged using the ImageJ software. The images were then blindly scored by Kadmiel lab members on a 1-3 scale (1-very little ZO-1, 3-very high activity of ZO-1) in order to analyze the protein activity of ZO-1 in the vehicle and dexamethasone conditions.

RNA Isolation and Quantitative RT-PCR

ARPE-19 were counted and 400,000 cells/well were plated into a 6-well plate. Cells were treated with water as a vehicle or a 1000nM concentration of Dexamethasone for 24 hours. A singular scratch was made down the middle of the well using a sterile p200 pipette tip. The cells were then washed with PBS to remove any dead or floating cells and replaced with fresh medium containing the vehicle or different concentrations of dexamethasone. PBS was added to the well and the cells along the leading edge of the scratch were collected using a cell scraper. Once removed, more PBS was added to the well

and the cells along the outer edge of the well were collected using a cell scraper. RNA was isolated using a Trizol-Chloroform extraction method. The concentration and purity of the RNA was measured and recorded using the Nanodrop. Using Taqman one-step qRT-PCR, individual mRNAs were determined. Taqman primers used in this experiment were *GILZ* (Hs00608272_m1, Mm00726417_s1), *PPIB* (Hs00168719_m1, Mm00478295_m1) as well as pro-migratory genes *MMP2*, *MCP-1*, and *CXCR4*. *GILZ* was used as an internal control for dexamethasone treatment because it is already known to be upregulated by dexamethasone. A SYBR Green 2-step qRT-PCR was also used to determine individual mRNAs. *PPIB* (HsPPIB-F1, 5'-AACGCAGGCAAAGACACCAACG-3'; HsPPIB-R1, 5'-TCTGTCTTGGTGCTCTCCACCT-3') and *GILZ* (HsGILZ_F1, 5'-ATCTGCAACCGCAACATCGACC-3'; HsGILZ_R1, 5'-GCATACATCAGATGATTCTTCACC-3') were used in this experiment. The main focus of target gene expression was the pro-migratory genes. These genes were then compared to the housekeeping, control gene, *PPIB* by analyzing the Ct values recorded from qRT-PCR.

SDS-PAGE and Western Blot

ARPE-19 cells were counted and 400,000 cells/well were plated into a 6-well plate. Cells were treated with water as a vehicle or a 1000nM concentration of Dexamethasone for 24 hours. Once treated, a scratch was made down the middle of the well using a p200 pipette tip. The cells were then washed with PBS to remove any dead or floating cells and replaced with fresh medium containing the vehicle or 1000nM concentration of dexamethasone. After 24 hours, the cells were washed with PBS and all cells throughout the plate were collected using a cell scraper. The cells were then lysed in a Tris-Glycine sodium dodecyl sulfate (SDS) sample buffer (Life Technologies, CA; catalog #1631915) with 5% beta-mercaptoethanol. Cells were passed through an insulin syringe several times before being heated (98°C) for five minutes. The protein lysates, along with a SeeBlue Pre-Stained standard (Invitrogen, MA; catalog #LC5925) with proteins of pre-determined molecular weights (in kilodaltons), was then loaded into a 4-20% Tris Mini Protean TGX gel (Bio-Rad, CA; catalog #4561093) and ran using electrophoresis. The gel was

transferred to a nitrocellulose membrane using a Turbo Transfer apparatus (Bio-Rad, CA, Standard SD transfer for 30 minutes) and was then blocked for one hour at room temperature in EveryBlot blocking buffer (Bio-Rad, CA; catalog #12010020). The membrane was incubated with primary antibodies rabbit anti-GR (Cell Signaling, MA; catalog #12041, 1:1000), mouse anti-ZO-1 (Invitrogen, MA; catalog #339100, 1:250), and mouse anti-ACTIN (Invitrogen, CA; catalog #MA5-11869, 1:10,000) overnight at 4°C shaking gently. All primary antibodies were diluted in Tris-Buffered Saline (TBS) (1X concentration). Blots were then washed with 1X TBS with 0.1% Tween (TBST) before being incubated with corresponding antibodies: goat anti-rabbit Alexa 680 secondary antibody (Jackson, PA; catalog #156734, 1:10,000) and/or goat anti-mouse IRDye800-conjugated secondary antibody (LICOR, NE; catalog #926-32210, 1:10,000) for one hour at room temperature. Amersham Typhoon Imaging was used to image the blot. Rabbit anti-GR antibody was imaged at 710nm to 730nm and mouse anti-ACTIN antibody as well as mouse anti-ZO-1 were imaged at 810nm to 840nm. The integrated density was measured through ImageJ software. ZO-1 was compared to actin using the following equation:

$$\text{Integrated density} = (\text{integrated density of ZO-1}) / (\text{integrated density of } \textit{actin})$$

Statistical analysis

Results are represented based on the mean +/- the standard error of the mean. To determine statistical significance of the results, a student t-test was performed (Excel). ** $P < 0.01$ and * $P < 0.05$ were used to represent significant results.

Results

The Effect of Dexamethasone on Wound Healing in ARPE-19 cells.

The effect of dexamethasone on migration was investigated in a wound healing assay using retinal pigmented epithelial cells. ARPE-19 cells were plated in a 6-well plate and a wound healing assay was completed after 24 hours of dexamethasone treatment of varying concentrations (0nM, 10nM, 100nM, and 1000nM). Three independent experiments were conducted and the representative images of the area of the wound at 0 hours, 8 hours and 24 hours are shown in Fig. 4A. An average of all three independent experiments is shown in Fig. 4B demonstrating that dexamethasone delays wound healing in retinal pigmented epithelial cells.

In the first experiment, each of the conditions showed an increase in the amount of area closed after 8 hours of incubation (Fig. 4B). The 0nM condition showed the largest increase with about 37% of the wound area closed. The 10nM and 100nM conditions also showed increases with about 35% and 30%, respectively, of the wound area closed. The 1000nM condition showed a statistically significant decrease in wound area closed compared to the 0nM condition with about 31% of the area closed ($P<0.05$) (Fig. 5A). After 24 hours of incubation, each of the conditions continued to increase in the amount of wound area closed. The 0nM condition continued to show the largest increase with about 81% of the wound area closed. The 1000nM condition continued to show significantly less migration with only 66% of the wound area closed after 24 hours ($P<0.05$). The 10nM and 100nM conditions also showed less migration with about 78% and 68%, respectively, of wound area closed (Fig. 5A).

In the second experiment, each of the conditions showed an increase in the amount of wound area closed after 8 hours of incubation. The 100nM condition showed the largest increase with about 35% of the wound area closed. The 1000nM condition also showed a large increase with about 33% of the wound area closed after 8 hours. The 0nM and 10nM conditions showed similar results with about 25% and 27%, respectively, of wound area closed. Dexamethasone did not show any statistically significant

effect in any of the conditions after 8 hours of incubation (Fig. 5B). After 24 hours, the wound area continued to close in each of the conditions. The 1000nM condition showed the largest increase with about 83% of the wound area closed. The 0nM and 100nM condition showed very similar results with 78.9% and 79.4%, respectively, of wound area closed after 24 hours. The 10nM condition showed the least amount of migration with about 70% of wound area closed (Fig. 5B). Dexamethasone did not show any statistically significant effect on migration in any of the conditions after 24 hours.

In the third experiment, each of the conditions showed an increase in the amount of wound area closed after 8 hours of incubation. Similarly, to the first experiment, the 0nM condition showed the largest increase with about 35% of the wound area closed. The 100nM condition showed an increase with about 31% of wound area closed after 8 hours. The 10nM and 1000nM conditions showed similar results with about 27% and 26%, respectively, of wound area closed (Fig. 5C). Dexamethasone did not show any statistically significant effect on migration in any of the conditions after 8 hours. After 24 hours, each of the conditions continued to close the wound area. The 0nM condition continued to show the largest increase with about 77% of wound area closed. The 10nM and 100nM condition also showed increases with about 64% and 68%, respectively, of wound area closed after 24 hours. The 1000nM condition showed statistically significant delayed wound healing with about 60% of wound area closed ($P<0.05$) (Fig. 5C).

The results from all three independent experiments were averaged and combined. After 8 hours of treatment, each of the conditions showed an increase in the amount of area closed (Fig. 4B). However, dexamethasone had no statistically significant effect in any of the conditions at the 8-hour time point. The 0nM condition showed the largest increase with about 32% of the wound area closed over an 8-hour period. The 10nM and 1000nM conditions had similar increases with 29.7% and 29.6%, respectively, of the area closed after 8 hours (Fig. 5D). After 24 hours, the wound area continued to close in each of the conditions (Fig. 4B). The 0nM condition continued to have the largest increase in the amount of wound

area closed after 24 hours with about 78.8% of the wound area closed. However, at the 24-hour time point, the 1000nM condition showed a significant change ($P<0.05$) with the least amount of area closed with about 69.7% of the wound area closed. The 10nM and 100nM conditions also showed an increase over 24 hours with 70.7% and 71.8%, respectively, of the wound area closed. The 10nM dexamethasone treatment showed a significant decrease ($P<0.05$). The 100nM condition did not show any significant effect (Fig. 5D). The results demonstrate that Dexamethasone is delaying wound healing in retinal pigmented epithelial cells (Figs. 4,5).

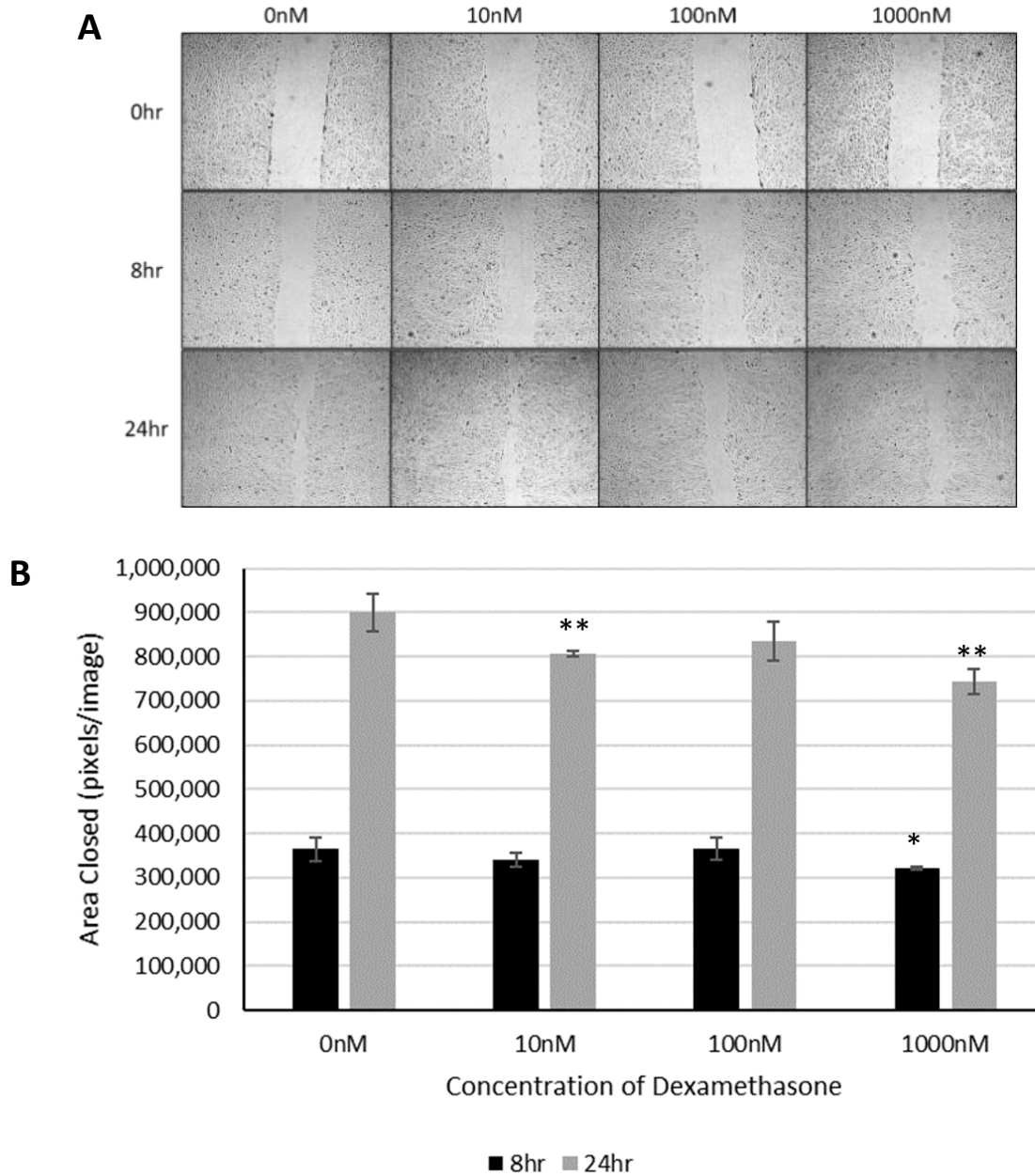


Fig. 4 Dexamethasone treatment on ARPE-19 cells in a wound healing assay. (A) The scratches in different concentrations of Dexamethasone (0nM, 10nM, 100nM, and 1000nM) were imaged at 0hr, 8hr, and 24hr using a Leica upright microscope (10x dry objective). (B) Using FIJI software, the area of the scratch was outlined and measured in pixels/image. The area closed was compared to the control by subtracting the area open at 8 hours or 24 hours from the area open at 0 hours. Each experiment had 3 replicates of each condition and there were 3 independent experiments completed resulting in n=9 for each condition. Error bars represent the mean +/- the standard error of the mean. (* $P < 0.05$, ** $P < 0.01$)

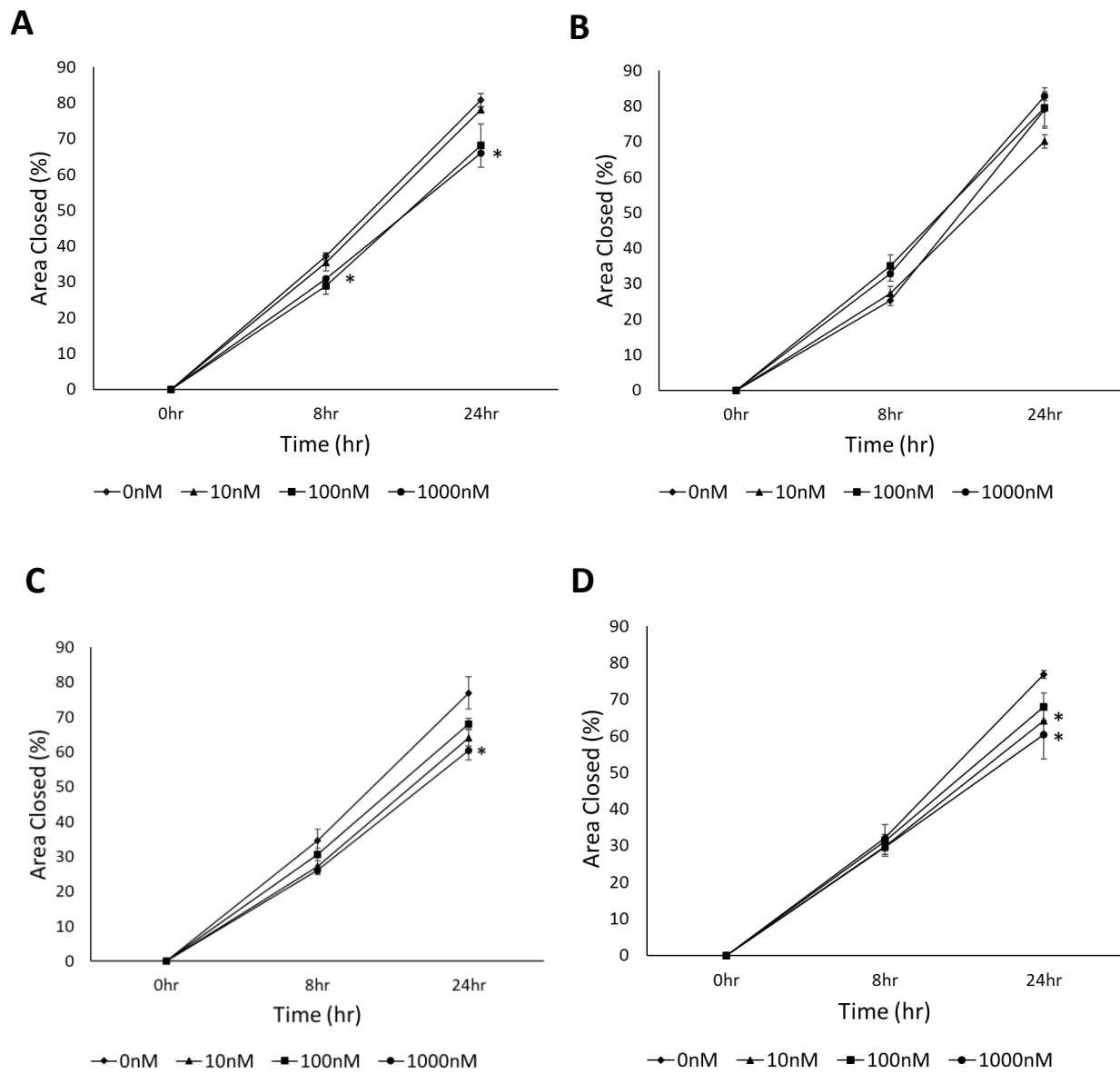


Fig. 5 percentage of area closed over 24 hours of vehicle or dexamethasone treatment. (A) Experiment 1. (B) Experiment 2. (C) Experiment 3 (D) The averaged percent of area closed from all 3 independent experiments. Each experiment had 3 replicates of each condition and there were 3 experiments completed resulting in n=9 for each condition. Error bars represent the mean $\pm$ the standard error of the mean. (* $P < 0.05$).

The Effect of Dexamethasone on Pro-Migratory Genes in ARPE-19 cells

The effect of dexamethasone on pro-migratory genes was investigated in a wound healing assay by comparing the leading edge of the scratch to the outer edge of the scratch in retinal pigmented epithelial cells. ARPE-19 cells were plated in a 6-well plate and a wound healing assay was completed after 24 hours of treatment of either water as a vehicle or 1000nM of dexamethasone. Cells were collected from the leading edge of the scratch as well as the outer edge of the well. RNA was then extracted from the cells for qRT-PCR. All samples yielded sufficient amounts of RNA (ng/ μ L) to be used in qRT-PCR, excluding “DEX 3 LEADING EDGE” and “DEX 3 OUTER EDGE” from the second experiment [Table 1]. Multiple experiments of qRT-PCR were completed using the 1-Step Taqman Master Mix as well as 2-Step SYBR Master Mix for a known dexamethasone-regulated gene *IGFBP5* and the housekeeping gene *PP1B*; however, due to technical difficulties, amplification of genes was not detected.

Table 1: RNA concentration measurements

A

SAMPLE	260/280	260/230	NANOGRAMS/MICROLITER
VEH 1 LEADING EDGE	1.71	0.30	22.6
VEH 1 OUTER EDGE	1.90	1.14	151.3
VEH 2 LEADING EDGE	1.94	1.65	230.4
VEH 2 OUTER EDGE	1.90	1.08	164.5
VEH 3 LEADING EDGE	1.90	2.05	156.0
VEH 3 OUTER EDGE	1.85	0.87	150.6
DEX 1 LEADING EDGE	1.89	1.10	146.7
DEX 1 OUTER EDGE	1.84	1.40	56.7
DEX 2 LEADING EDGE	1.91	0.83	99.9
DEX 2 OUTER EDGE	1.92	1.26	188.6
DEX 3 LEADING EDGE	1.90	1.15	146.8
DEX 3 OUTER EDGE	1.94	1.34	188.6

B

SAMPLE	260/280	260/230	NANOGRAMS/MICROLITER
VEH 1 LEADING EDGE	1.33	0.40	27.1
VEH 1 OUTER EDGE	1.29	0.36	19.5
VEH 2 LEADING EDGE	1.33	0.42	64.0
VEH 2 OUTER EDGE	1.22	0.83	50.9
VEH 3 LEADING EDGE	1.30	0.38	25.0
VEH 3 OUTER EDGE	1.26	0.74	46.0
DEX 1 LEADING EDGE	1.30	0.43	28.8
DEX 1 OUTER EDGE	1.31	0.43	63.5
DEX 2 LEADING EDGE	1.32	0.37	31.6
DEX 2 OUTER EDGE	1.30	0.40	21.3
DEX 3 LEADING EDGE	1.34	0.48	89.7
DEX 3 OUTER EDGE	1.23	0.40	5.8

C

SAMPLE	260/280	260/230	NANOGRAMS/MICROLITER
VEH 1 LEADING EDGE	1.28	0.30	17.7
VEH 1 OUTER EDGE	1.30	0.36	41.8
VEH 2 LEADING EDGE	1.31	0.39	36.3
VEH 2 OUTER EDGE	1.32	0.40	72.7
VEH 3 LEADING EDGE	1.32	0.35	15.2
VEH 3 OUTER EDGE	1.33	0.39	76.7
DEX 1 LEADING EDGE	1.34	0.27	62.3
DEX 1 OUTER EDGE	1.33	0.26	49.7
DEX 2 LEADING EDGE	1.34	0.34	61.5
DEX 2 OUTER EDGE	1.34	0.41	78.7
DEX 3 LEADING EDGE	1.31	0.37	60.5
DEX 3 OUTER EDGE	1.36	0.43	100.5

Table 1: RNA concentrations from three independent wound healing experiments. (A) Experiment 1 (B) Experiment 2 (C) Experiment 3. RNA was collected from the leading edge of the scratch as well as the outer edge of the well from the vehicle and 1000nM dexamethasone treated cells. Each table contains 260/280 and 260/230 ratios and RNA concentrations obtained from the Nanodrop.

The Effect of Dexamethasone on Tight Junctions in ARPE-19 cells

The effects of dexamethasone on tight junction proteins, specifically ZO-1, were investigated using immunocytochemistry in a wound healing assay in retinal pigmented epithelial cells. ARPE-19 cells were cultured and plated in a 4-well chamber slide with 0nM and 1000nM treatment of dexamethasone and a wound healing assay as described previously was completed in each well. Cells were stained with the primary antibodies of anti-ZO-1 and anti-GR. Cells were then stained with secondary antibodies and Hoechst to identify the nucleus. Results showed that the glucocorticoid receptor (GR) is present in the retinal pigmented epithelial cells without any dexamethasone. More specifically, the GR is more localized in the nucleus when dexamethasone is present indicating ligand-mediated nuclear translocation of GR (Fig. 6A and 6B). As a control, one chamber in each experiment did not receive any primary antibodies, however it received secondary antibodies along with Hoechst. This was done to ensure that the secondary antibodies were not binding to non-specific signals. Without the primary antibodies, ZO-1 and GR channels showed no signal, while Hoechst showed nuclear staining which was expected (Fig. 6C). The blinded scores of the levels of ZO-1 only differed by 5.6% between the average scores. There was no statistically significant difference in the levels of ZO-1 between the cells treated with dexamethasone and the cells not treated with dexamethasone (Fig. 6D).

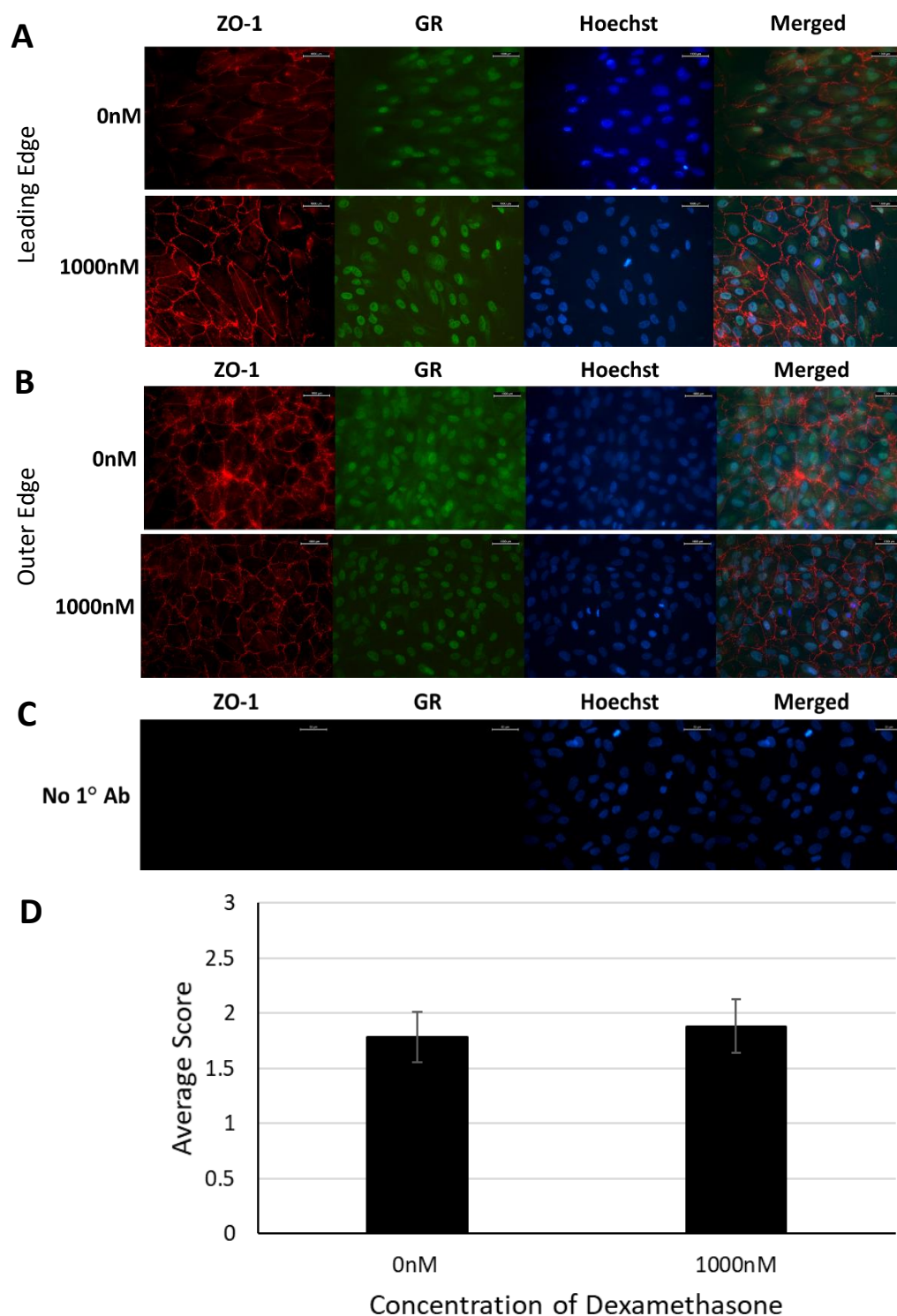


Fig. 6 Immunocytochemistry of ARPE-19 cells in response to dexamethasone treatment (A) Representative images showing ARPE-19 cells along the leading edge of the scratch. (B) Representative images showing ARPE-19 cells along the outer edge of the well. (C) No primary antibody control. (D) The average of scores of intensities of ZO-1 staining scored by students blinded from the conditions and treatments. Each experiment produced 2 replicates of each condition and there were 2 experiments completed. Three images were taken of each condition in each well resulting in n=12 for each condition. The scale bar represents 1000μM. “Merged” images represent ZO-1, GR, and Hoechst in a single image. All images were imaged at 40x dry subjective using a Leica Fluorescent microscope. Error bars represent the mean +/- the standard error of the mean.

The Effect of Dexamethasone on Tight Junctions in ARPE-19 Cells

The effect of dexamethasone on tight junction proteins, specifically ZO-1 in retinal pigmented epithelial cells was investigated through immunoblotting. Western blot was also performed to confirm that glucocorticoid receptors are expressed in retinal pigmented epithelial cells. ARPE-19 cells were plated in a 6-well plate with 0nM and 1000nM treatment of dexamethasone and a wound healing assay was completed in each well. All the cells in each well were then collected and a Western Blot was performed targeting ZO-1 and GR. Results show that GR is present and robustly expressed in retinal pigmented epithelial cells, exhibiting a well characterized ligand-mediated downregulation GR protein in cells treated with dexamethasone (Fig. 7A). The levels of integrated density of ZO-1 were then measured using ImageJ software. Retinal pigmented epithelial cells treated with dexamethasone had a lower integrated density while untreated cells had a higher integrated density of ZO-1, suggesting a decrease in total ZO-1 protein in cells treated with dexamethasone (Fig. 7B and 7C). The 1000nM concentration of dexamethasone showed a statistically significant decrease in comparison with the 0nM concentration ($P < 0.01$) (Fig. 7C). Immunoblotting demonstrated that dexamethasone significantly decreases the levels of tight junction protein, ZO-1 in retinal pigmented epithelial cells.

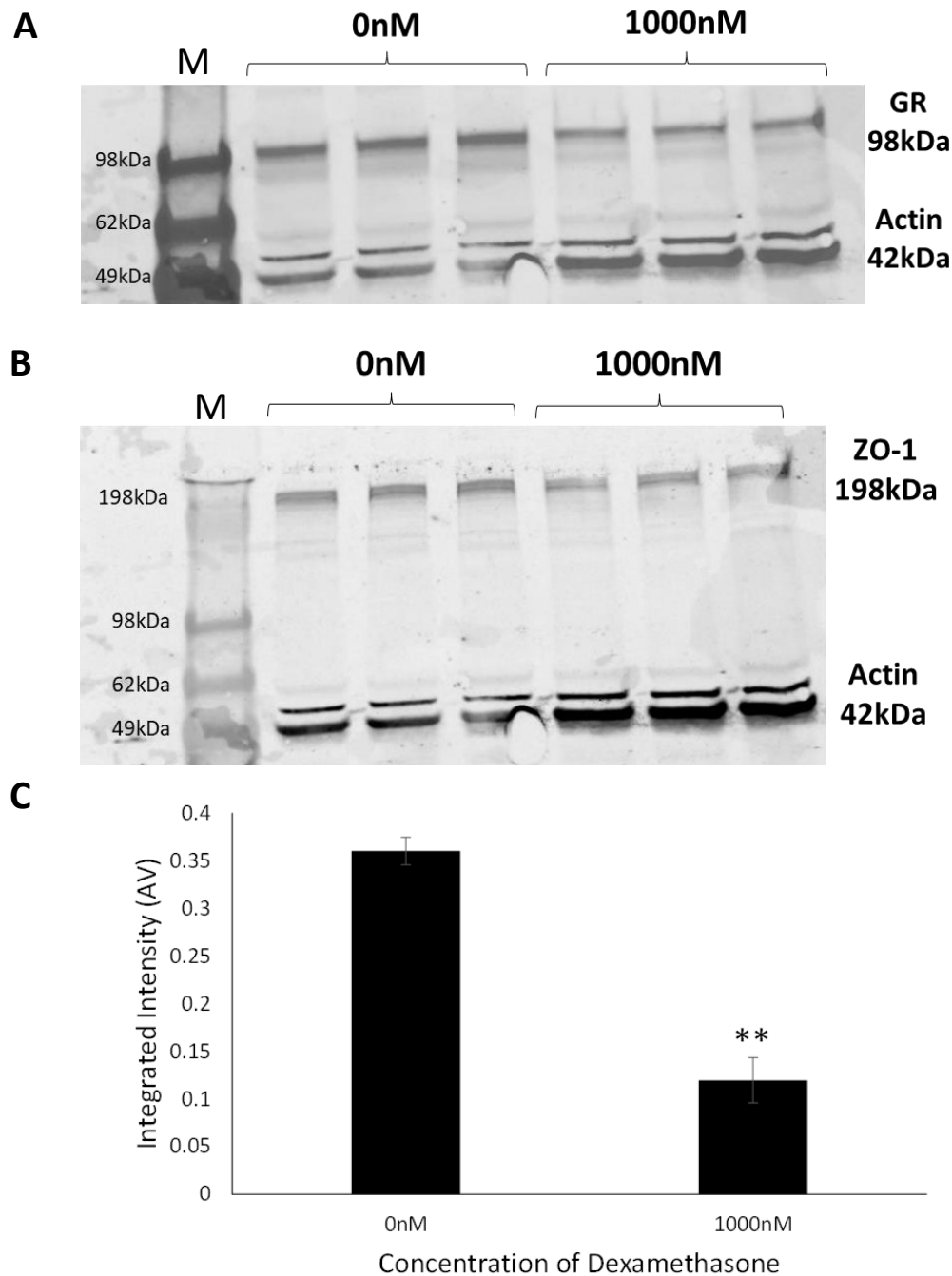


Fig. 7 Dexamethasone treatment on ARPE-19 cells by immunoblotting. (A) GR protein level expressed in retinal pigmented epithelial cells. (B) ZO-1 protein level in retinal pigmented epithelial cells treated with 0nM or 1000nM concentrations of dexamethasone. (C) The integrated intensity of ZO-1 in relation to actin in retinal pigmented epithelial cells that were treated with 0nM or 1000nM concentrations of dexamethasone. M represents the protein standard. Error bars represent the mean $\pm$ the standard error of the mean. (** $P < 0.01$)

Discussion

Synthetic glucocorticoids are widely used to treat inflammatory and autoimmune conditions due to their anti-inflammatory and anti-angiogenic roles. In this study, the effects of varying concentrations of dexamethasone were determined in retinal pigmented epithelial cells in an *in vitro* wound healing assay. The wound healing assay results show that there is a significant difference in the 1000nM dexamethasone condition compared to the vehicle condition. Overall, there was an increase in the wound area closed in the vehicle condition and there was a decrease in the closed wound area when the cells were treated with dexamethasone. This suggests that dexamethasone was delaying the migration and/or proliferation of the retinal pigmented epithelial cells (Fig. 8). Protein analysis showed that there was no significant difference in tight junction distribution along the leading edge of the wound between the 1000nM treated cells and the vehicle condition. Immunoblotting showed that there was a decrease in tight junction protein expression under the presence of dexamethasone (Fig. 8).

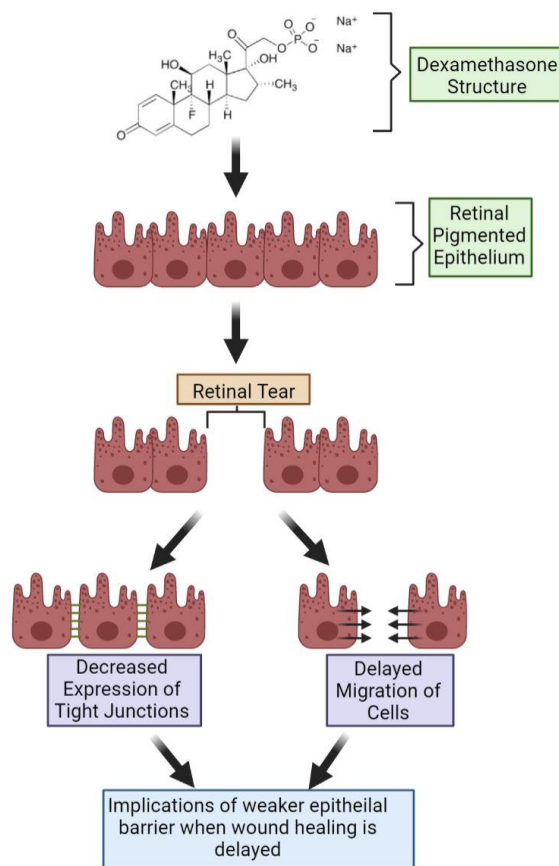


Fig. 8 Summary Figure. Retinal pigmented epithelial cells were treated with varying concentrations of dexamethasone. Wound healing assay showed delayed migration when dexamethasone was present compared to when dexamethasone was absent. When dexamethasone is present, tight junction proteins (colored in green) were less prevalent and potentially form a weaker epithelial barrier. When wound healing is delayed, there is an implication of a weaker epithelial barrier when retinal pigmented cells are treated with dexamethasone. Created with BioRender.com.

The effect of dexamethasone on retinal pigmented epithelial cells in a wound healing assay showed a decrease in each condition when the data from three independent experiments were combined and averaged. More specifically, there was a significant decrease in the 10nM and 1000nM conditions compared to the 0nM, vehicle condition. In other cell lines, a similar trend has been shown in the effect of glucocorticoids on migration and proliferation. For example, a study conducted by McBeth et. al., investigated the effects of dexamethasone on the transitional uroepithelial cancer cell line T24. Cells were treated with 100nM of dexamethasone 30 minutes prior to the wound healing assay. Dexamethasone significantly decreased migration while also decreasing the expression of different genes [33]. A similar trend was seen in corneal epithelial cells. Corneal epithelial cells were treated with a 1000nM of

dexamethasone overnight before a wound healing assay was performed [17]. In the corneal epithelial cells, there was a significant decrease in the wound area closed in the dexamethasone treated condition [17]. There was a similar trend shown adipose tissue-derived mesenchymal stem cells treated with a 100nM of dexamethasone for 24 hours before an *in vivo* wound healing assay was performed. Dexamethasone treated cells had reduced wound healing compared to the non-treated cells [34].

Although the 10nM and 1000nM conditions showed a significant difference in this study, there was no significant effect shown in the 100nM condition in this study. One reason for the variance in the 100nM condition may be due to the second experiment completed. While two out of three experiments showed similar trends, one experiment showed that the 100nM and 1000nM conditions had a larger increase in the wound area closed than the 0nM condition. This could be due to unequal number of cells being plated or that the plates may not have been fully confluent when the wound healing assay was performed. In a study conducted by Han, et. al, mitomycin C was used to inhibit proliferation 2 hours before the wound healing assay was performed. Because mitomycin C inhibits proliferation, the migration alone of retinal pigmented epithelial cells can be further investigated [25]. In future studies, it would be important to repeat the wound healing assay, especially the 100nM condition. Based on previous studies, the clinical dose is estimated to be 10-150mM of dexamethasone which is several magnitudes higher than the doses used in this *in vitro* study [38]. Overall, the decrease in wound area closed in the 10nM condition and 1000nM condition shows a potential mechanism of how dexamethasone delays migration and proliferation in retinal pigmented epithelial cells.

Data collected from gene expression through qRT-PCR would have shed light on the mechanism of dexamethasone delaying wound healing. RNA was extracted from 36 samples of retinal pigmented epithelial cells; 18 samples were collected from the leading edge of the scratch and 18 samples were collected from the outer edge of the well. One-step Taqman qRT-PCR was repeated twice and 2-step SYBR Green qRT-PCR was performed one time. There was no amplification in any of these experiments. It was

speculated that the qPCR machine available to us is unable to detect the signal of either Taqman or SYBR Green fluorescent dyes under the conditions we have used because multiple users from our group experienced similar technical difficulties. Further troubleshooting of the PCR conditions and the reagents may result in PCR amplification. A decrease in pro-migratory gene expression as well as an increase in tight junction gene expression in samples collected from the leading edge was predicted to be seen with dexamethasone treatment when compared to the vehicle treatment. In other cell lines, it has been found that dexamethasone decreases migration. For example, transitional uroepithelial cancer cells treated with 100nM of dexamethasone significantly decreased migration while also decreasing the expression of the genes *miR144*, *miR181a*, and *miR181c* [33]. Human corneal epithelial cells treated with dexamethasone for 6 hours before being collected for RNA extraction altered corneal epithelial expression, where hundreds of genes, including *IL-6* and *MCP-1* were downregulated and upregulated, respectively, by activation of the glucocorticoid receptor [17]. These genes are expressed through the inflammatory response as well as migration of the cells. It has not been researched how migration at the gene level is affected by dexamethasone in retinal pigmented epithelial cells. In the future, the RNA samples already collected from this study can be used to investigate the effects of dexamethasone on pro-migratory genes such as *MMP2*, *MCP-1*, and *CXCR4* in retinal pigmented epithelial cells. In future studies, it would be beneficial to investigate other migratory genes, and possibly inflammatory genes that may be potentially affected by dexamethasone in these cells. This can be done by investigating genes regulating various cellular functions associated with cellular movement. These results can show a potential mechanism of how dexamethasone affects migration at the gene level at the leading edge of a wound compared to cells at the outer edge where the cells are not involved in migration in retinal pigmented epithelial cells.

Immunocytochemistry was used to investigate the effects of dexamethasone on tight junction proteins in retinal pigmented epithelial cells. There were no significant results found in the immunocytochemistry data collected. The dexamethasone treated cells did not show any significant

difference in the arrangement of the tight junctions compared to the vehicle condition. The blinded scoring of the images showed that the average score of the 1000nM dexamethasone condition only differed from the average score of the vehicle condition by 5.6%. In human corneal epithelial cells, it has been shown that glucocorticoids, specifically dexamethasone, impact the arrangement of tight junctions. Cells were treated with a 100nM concentration of dexamethasone for 24 hours. Corneal epithelial cells treated with dexamethasone had a greater distribution of ZO-1 along the plasma membrane compared to the untreated cells [17]. An increase in the patterns of ZO-1 along the plasma membrane was expected in the retinal pigmented epithelial cells along the leading edge of the scratch. However, there could be multiple factors as to why these results showed no difference between the dexamethasone treated cells and untreated cells. One explanation might be that it was not a long enough treatment time. By potentially increasing the dexamethasone treatment time to 48 hours, there could be a longer opportunity for the expression to change at the protein level. In future studies, it would be important to repeat the study and to potentially investigate different concentrations of dexamethasone as well as other types of synthetic glucocorticoids. For example, a study conducted by Zhaeentan, et. al., investigated the effects of hydrocortisone, another type of synthetic glucocorticoid, on tight junctions in human fallopian epithelial cells. Results from this study showed an increase in expression of genes involved in tight junctions suggesting that glucocorticoids increase the expression of these tight junctions [35]. However, in this study investigating dexamethasone on tight junction proteins, the results of the immunocytochemistry showed no difference in the dexamethasone treated cells compared to the untreated cells. While it has been shown through the wound healing assay that there is a delay in wound healing, there does not seem to be a change in the expression of ZO-1. This might suggest that ZO-1 is not required for delaying migration, however it may help with wound healing later on. By investigating tight junctions like ZO-1 over a longer time period, the role of tight junctions in wound healing can be better understood. These results

can lead to further investigation of the effect of dexamethasone on tight junctions in retinal pigmented epithelial cells.

Specifically in retinal pigmented epithelium, ZO-1 is known to regulate proliferation and gene expression of cell cycle genes such as *cyclin D1* and *PCNA* [32]. A western blot investigated the effect of dexamethasone on tight junction proteins along the leading edge of a wound in retinal pigmented epithelial cells. There was a significant decrease in the expression of the tight junction protein, ZO-1, in retinal pigmented epithelial cells treated with dexamethasone compared to untreated cells. In a study conducted by Singer et. al, the effect of dexamethasone on ZO-1 protein levels was investigated in cultured 31EG4 mammary epithelial cells. Western blot technique results showed that dexamethasone treated cells had increased ZO-1 protein levels compared to the untreated cells [36]. The results from Singer et. al, contradict the results that were found in this study. One reason for this could be that the experiment was only completed once. Further investigation and experimental replicates can confirm or deny the results that were found in this study. It would also benefit to culture retinal pigmented epithelial cells in a petri dish in order to collect more cells along the leading edge of the scratch. In future studies, it would be beneficial to investigate the effect on dexamethasone on other tight junction proteins such as E-cadherin or claudin. The effect of hydrocortisone on tight junctions in human fallopian epithelial cells showed an increased expression of *Claudin-3*, *Claudin-4*, *E-cadherin*, *Zona occludin-1* and *Desmoglein-1* genes [35]. Overall, these results show a potential mechanism of how dexamethasone can create a tighter barrier at the leading edge of the wound in retinal pigmented epithelial cells.

Retinal detachment affects about 1 in 10,000 people per year and is more prevalent in people aged 60 to 70 years old [10]. One of the most crucial functions of the retinal pigmented epithelium is maintaining a strong and protective barrier, however in retinal detachment diseases, this barrier is weak and often tears. In the clinical setting, dexamethasone and other synthetic glucocorticoids are used to treat numerous auto-immune diseases as well as inflammatory diseases, including many ocular diseases

[15]. By investigating synthetic glucocorticoids, the mechanism in how these drugs affect our bodies can be further investigated and then can be applied to patient care. More specifically, understanding the effects of dexamethasone on migration and proliferation in retinal pigmented epithelial cells can potentially create treatment options for those diagnosed with retinal detachment or other retinal diseases that impact the retinal pigmented epithelium. Currently, a formulation of intravitreal steroids is being used for the prevention of PVR formation after vitreoretinal surgery [37]. In future studies, investigating and comparing other glucocorticoids in addition to dexamethasone may be beneficial in understanding how different synthetic glucocorticoids affect migration and tight junctions in retinal pigmented epithelial cells.

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