The Subretinal Space of the Eye

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The subretinal space is located between the retinal pigment epithelium (RPE) and the photoreceptive cells. The majority of the retina is a delicate matrix of photoreceptive cells and their support network which are responsible for human vision. These cells are separated from the cornea by a layer of pigment epithelium. The RPE has tight junctions, effectively insulating the inside of the retina from systemic circulation; the contents of the retina can then be controlled by transcellular transport.

Keywords: anatomy ; subretinal ; drug delivery

1. Anatomy and Key Cells

There are many causes of blindness; however, some of the most common are those conditions that affect the retina. The retina contains the photoreceptive cells of the eye and can be degraded or damaged by numerous conditions or events. Whether the cause for vision loss is genetic or the result of another conditionsuch as diabetes, one of the golden spots for treatment is the subretinal space [1]. The subretinal space is located next to the photoreceptors that these conditions degrade, making it ideal for quick drug delivery [1].

The subretinal space is located between the retinal pigment epithelium (RPE) and the photoreceptive cells [1]. The majority of the retina is a delicate matrix of photoreceptive cells and their support network which are responsible for human vision. These cells are separated from the cornea by a layer of pigment epithelium. The RPE has tight junctions, effectively insulating the inside of the retina from systemic circulation; the contents of the retina can then be controlled by transcellular transport [2]. This barrier works in tandem with the retinal vascular endothelium to turn the subretinal space of the eye into an immune-privileged space. Due to this, the RPE can exert some control over the immune system by secreting immune-modulatory factors such as interleukin-8 (IL-8), complement factor H (CFH), and monocyte chemotactic protein-1 (MCP1) to activate and deactivate it in response to the disease state of the eye [3][4]. In addition to its immune secretions, the RPE has MHC receptors and toll-like receptors allowing it to respond to signals from the immune system [2]. Besides immune interactions, the RPE routinely secretes growth factors and signaling molecules into the subretinal space and the choroid which are important for ensuring the stability of the photoreceptors and the choroid [2].

The other side of the subretinal space is the photoreceptive cells which are responsible for light detection and are the first layer of the neuroretina [5]. These cells are not actually attached to the RPE; instead, they rest near it, leaving a spot for a subretinal space [6]. This space is a consequence of how the neuroretina forms during embryonic development [6]. The photoreceptors are connected to interneurons, a set of cells that process the raw signal; these connect to ganglion cells that carry visual signals to the brain. The final part of the neuroretina is the glial cells, consisting of Müller cells, astrocytes, microglia, and oligodendrocytes [6]. Müller cells are the most prevalent glial cells in the eye and are found throughout the neuroretina. Their proximal and distal extensions form the inner limiting membrane and the outer limiting membrane, respectively. They are important to maintaining the internal environment of the retina [6]. Astrocytes come from stem cells in the optic nerve and are found in the superficial layers of the neuroretina. Microglia enter the retina from circulation; they are phagocytic cells that are part of the immune system and can migrate throughout the retina [6]. Finally, oligodendrocytes form the myelin sheath of neurons.

The retinal vascular endothelium makes up the other side of the blood–retina barrier via the tight junctions of the retinal blood vessels that prevent fluid leakage [3]. These vessels are responsible for supplying nutrients to the inner eye, where the ganglion and bipolar cells are located [3]. The blood–retina barrier grants the eye immune privilege which severely isolates the subretinal space. However, this isolation can come at a price when immune privilege is compromised. When trauma, infection, or degradation cause antigens to leave the retina, the immune system will not recognize them and autoreactive T cells can be activated [3]. It is also important to mention that immune-privileged does not mean that the subretinal space is completely separated from the immune system. In reality, it mostly protects the retinal tissue from the immune system when it is healthy [3]. During infection, systemic signals and chemokines are released, and activated T

cells can enter the subretinal space as easily as any other tissue [7]. Once T cells have entered the retina, they start to accumulate and attract macrophages [3]. The macrophages cause inflammation, which can cause damage to the retina [3].

2. Interface

2.1. Subretinal Injections

There are many approaches currently under investigation for how to deliver drugs and other treatments into the subretinal space. The isolated nature of the retina means that its natural defenses must be breached for any therapeutics to gain entry. Currently, the approaches for administering subretinal injections fall into three categories: (1) a transcorneal route through the pupil and passing the lens, vitreous, and retina [8][9]; (2) a transscleral route entering the pars plana or limbus areas and crossing through the vitreous to enter through the subretina through the opposite side of retina [10][11][12]; and (3) a transscleral route through the choroid and Bruch's membrane that bypasses the retina [1][13][14][15]. These routes are effective and appropriate for the delivery of viruses, viral particles, liposomes, plasmids, drugs, and formulations and can be used as collection points to measure the contents of the subretinal space [1][16]. However, they have the drawback of causing retinal injury and permanent detachment after several uses [5]. Current research efforts explore the potential of drug delivery systems that would release the drug over a period of time; however, these are all still in their clinical study phase [5]. There are also some concerns over the feasibility of mass-producing these delivery systems due to their fragility and complexity [5].

The effects of subretinal injections on eye tissue are not fully understood. It is known that injections into the subretinal space will result in the formation of a bleb, a temporary detachment of the photoreceptors from the RPE. This separation is necessary for drugs to reach the cells within the retina; however, it also damages the outer retina [17]. Studies have demonstrated that the force from the temporary retinal detachment by injections can alter the photoreceptive and RPE cells. During the detachment, photoreceptors are swollen and fragmented while the RPE cells are damaged, ultimately negatively affecting the ability of the subretina to reattach [18]. There is currently ongoing research in animals to limit the trauma-caused retinal bleb formation; however, there are no standard procedures established to date [18].

Despite their side effects, subretinal injections are used to treat numerous conditions and are seen as a potential method of treatment for many more. Clinically, subretinal injections are used to treat retinal degenerative diseases such as age-related macular degeneration, retinitis pigmentosa, Leber's congenital amaurosis, and Stargardt disease [1]. There is currently ongoing research for the treatments of these diseases with the use of subretinal injections of viral vector delivery for gene therapy and stem cell delivery for cell therapy [1].

2.2. Subretinal Transplants

Subretinal transplants have been performed using RPE and photoreceptive cells, as well as some stem cells [3]. Subretinal transplants are one of the potential ways to treat damaged or degrading retinas; however, they have had limited success [3]. The immune privilege of the retina does not extend to the grafted tissue, so the patient will require the use of immunosuppressants which tend to target the adaptive immune system [3][19]. Despite this, many grafts still have issues with cell survival, as neutrophils and macrophages target and engulf the cells as part of an innate immune response [19]. It is notable that recent phase 1 and 2 trials of subretinal transplants in humans have been more promising. Trials evaluating the success of subretinal injection of pluripotent stem cells showed that the cells were tolerated by the body and a portion of the patients experienced visual improvement for the duration of the trial [20]. Other research has shown that with proper immune suppression photoreceptors could be successfully transplanted and integrated into the subretina [21]. While these results are optimistic, there is still much research to be done in this area.

2.3. Retina Prostheses

A retinal prosthesis, a type of bionic eye, is an implantable electronic device designed to stimulate the sensation of vision in the eyes of individuals with significant retinal diseases and is relatively new to the market in both the United States and Europe. This is in part due to new nanofabrication techniques that have allowed for the production of smaller and less invasive devices [22]. While many devices are fixed onto the surface of the retina, some are placed into the subretinal space, which removes the need for device fixation. The perceived advantage of subretinal implants is that the device is implanted where the degenerated photoreceptors are, allowing the system to take advantage of the natural retinal structures and to have greater similarity to physiological systems [22]. However, the photoreceptor systems that these devices are trying to take advantage of are often damaged by disease or through device implantation [22]. It is also notable that devices are challenging for surgeons to implant due to their location and the underlying degeneration that results in unwanted adhesion to the retina and retinal pigment epithelium [22].

There are several subretinal implants currently on the market or in testing. The earliest was the Boston Retinal Implant Project, which used single electrode stimulation to treat retinitis pigmentosa and orbital cancer. While this device does not produce functionally useful vision, its developer does have a more advanced device in clinical trials [22]. This device notably needed an external power source to function. Another device is the Artificial Silicon Retina which is supposed to stimulate the retina in response to ambient light by converting it into an electrical signal using the ambient light as its power source [22]. This device is a silicon retina array with 5000 micro-photodiodes and iridium-tipped microelectrodes and is implanted into the superior retina. In the trial study of this implant, four of six patients could detect phosphenes, which are light spots in the visual field of the implant. It is also notable that some patients experience visual enhancement outside of the field of implant, suggesting that this device can influence nerve growth in the retina [22]. This device was ultimately concluded to not produce sufficient photocurrent to stimulate neurons from ambient light alone. [22].

The Alpha IMS and AMS are the only subretinal devices approved for sale in Europe. Like the Artificial Silicon Retina, the Alpha IMS uses a photovoltaic array consisting of a microchip with 1500 photodiode-amplified electrodes [22]. However, this device also uses an external power source to amplify the signal it produces from light, giving it an advantage over the Artificial Silicon Retina [22]. This external power reaches the implant by a silicon cable linked to a fixation pad in the orbit. Some users of this device were able to sense motion, while approximately 20% could see letters or objects and about 30% saw no visual improvement; the rest were able to perceive light and had improved ability to localize it. It is notable that the object recognition only improved for about 3 months but eventually fell significantly [22]. The Alpha AMS is a 1600 photodiode system that has improved longevity over the original [22].

Finally, the Photovoltaic Retinal Implant (PRIMA) Bionic Vision System relies on pixels that receive near-IR light pulsed from a pair of glasses which is used to stimulate an electrode [22]. This electrode stimulates another electrode connected to iridium oxide coated photodiodes, which then stimulate the adjacent neural tissue [22]. This allows for improved spatial resolution and scalability without requiring additional wires. For PRIMA, animal testing results have been promising, and human clinical trials are underway [22].

3. Current Research

3.1. Gene Therapy

The use of viral vectors to edit the genome is being investigated as one of the ways of treating inherited retinal disease. These treatments would be placed into subretina as it would allow the vectors to target the photoreceptor or RPE cells while limiting the immune response and dosage needed [1]. Studies already completed in animals suggest that the adeno-associated virus (AAV) is a feasible method for longer-term gene expression in the retina; continued work on this vector will allow it to be applied to more diseases and improve efficiency [23]. It is the most common method for delivering genetic material to the retina [1]. It has been used to successfully target both RPE and photoreceptor cells for the treatment of various degenerative conditions in animal models [1]. Other vectors, such as helper-dependent adenoviral vectors, have been used to improve AAV's abilities [1]. Lentiviral vectors have also been used for gene therapy of the retina [1]. While most of the work thus far has focused on genetic and degenerative conditions, some work has also been done on treating autoimmune uveoretinitis in animals [1]. For humans, AAV-based treatments are in clinical trials; these tests have shown that AAV treatments are not systemically toxic and there are no serious adverse events associated with their use, as well as showing promising improvements in patients' vision [1]. It is notable that these treatments are administered by subretinal injection [1].

3.2. Cell Therapy

Cell therapy is the placing of cells into the subretina, generally by subretinal injection and recently via a microcatheter, to treat retinal degenerative diseases [1][24]. These systems typically involve the injection of stem cells intended to integrate into the retinal layers and help restore function or support cell regeneration; however, sometimes other photoreceptive or RPE cells have also been used [1][21] While animal studies have suggested that this technique is safe and nontoxic, there are concerns over the high risk of complications. Currently, some phase 1 and 2 studies are ongoing [1]. Gandhi et al. at the Mayo Clinic recently demonstrated the safety and efficacy of degradable fibrin hydrogels for subretinal implantation to facilitate the precise and uninterrupted implantation of an RPE monolayer [25]. These promising hydrogels effectively degraded from the space in 8 weeks following delivery and represent the first fully degradable scaffold developed to treat macular degeneration and degenerative diseases of the retina [25].

3.3. Novel Delivery Methods

Due to the segregated nature of the subretina, there is much research interest in exploring how to successfully deliver drugs through minimally invasive means. While there is a large variety of methods for drug delivery under investigation, here will limit itself to the more common ones. One of the routes considered is the use of nanoparticles to assist in drug delivery. Nanoparticles could be used to protect the drug and transport it through the blood–retina barrier or to allow it to have sustained release. Cerium oxide nanoparticles have been used to scavenge reactive oxygen species in the eyes of mice, thus preventing oxidative stress and serving as a proof of concept for their ability to slow disease progression [26]. Nanoparticles can also be used to encapsulate DNA or RNA and aid in its uptake into retinal cells without the use of a viral vector [27]. This would allow for a different means of gene therapy for the cells in the eye [27]. In terms of increasing drug dosage and extending drug delivery time, research has shown that nanoparticle encapsulation can be used to deliver hydrophobic compounds to RPE cells over an extended period [28].

Liposomes are another potential way to deliver drugs to the subretina, as their hydrophobicity would allow them to cross the blood–retina barrier by means of diffusion. PEG molecules have been used to encapsulate drugs for delivery to the brain across the blood–brain barrier, a similar anatomical barrier [29]. Given the similarities between the blood–brain barrier and the blood–retina barrier, there is a good chance such a system would also be effective for drug delivery to the subretinal space [29].

Injectable hydrogels are a potential means of long-term drug delivery in the subretinal space. Hyaluronic acid hydrogels have previously been used to transplant retinal progenitor cells into the subretinal space [30]. The transplanted cells were distributed evenly within the subretinal space after three weeks and had shown characteristics indicative of maturation into photoreceptors [30]. Hydrogels could serve as a method for the long-term release of drugs and biologics into the subretinal space [31]. Their use would negate the need for repeated subretinal injections; however, it would still require the formation of a bleb and introduce trauma to the eye.

Another means of allowing drugs to enter the subretinal space would be modulating the blood–retina barrier. This would be accomplished by creating temporary openings in the membrane. After this, drugs can enter from the bloodstream, thus negating the need for repeated injections. This has previously been done by siRNA-mediated knockdown of a protein related to the function of the tight junctions between the cells of the barrier [32]. Targets for this are claudin-5, which is one of the three proteins that make up tight junctions [32], and occludin, another protein component of tight junctions that was targeted in the blood–brain barrier [33]. While this means of drug delivery would not be appropriate for chronic conditions, it could be used for one-time delivery, like those needed for gene therapy [5].

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