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Eye Tissue Regeneration: SEAM as a Cure For Prevalent Eye Diseases

Introduction

Embryonic stem cells were not harvested successfully from human embryos until 1998 (Yu J and Thomson JA 2016). For a scientific discovery that is relatively new, the use of stem cells, particularly embryonic stem cells, has caused its fair share of intense, world-wide controversy. It seems as if every other year, the United States government is changing its stance on stem cell research. Funding will be redacted for years, and then low and behold more funding will be given than ever before. For the short time that stem cells have been researched, they have caused a whirlwind of political events and have been on a rollercoaster of scientific breakthroughs and hiatuses due to the government's wavering stance on their research. The debate on the ethics of stem cells is climbing its way up to the top of some of the world's most controversial topics like gay rights and abortion. While the war on stem cells continues to rage on, some of the most promising disease cure options are being discovered through the use of stem cells. So while some might argue that the use of stem cells can cause great harm, science is also finding uses for them that will enhance the greater good. An area of disease that stem cell therapy shows promise in is eye diseases. In 2016, biologists from the University of Japan discovered the use of induced pluripotent stem cells (IPSCs) to regenerate eye tissue by making a self-formed ectodermal autonomous multizone, which is commonly abbreviated to SEAM (Hayashi et al. 2016). The SEAM of ocular cells mimics the

development of a human eye due to the fact that the cells form different zones that make up the whole eye as an entire organ (Hayashi et al. 2016). The SEAM of ocular cells that is still being researched and tested on in animal models is revealing itself to be a one day, promising cure for many of the eye diseases people of the world face today. Some of the most promising applications of eye tissue regeneration through a SEAM are for uses in people with diseases like corneal blindness, macular degeneration, and limbal stem cell deficiency. The SEAM that is being researched would not be possible without stem cells and all of the research that has been conducted to further their scientific advancement over the years. This review will discuss the history of stem cells, due to the SEAM being reliant on them, making their histories intertwined. Applications of the SEAM as a cure for the eye diseases aforementioned will also be discussed. Finally, the mechanisms behind the SEAM will be evaluated in order to determine if it is a viable solution to the outlined eye diseases.

History of Stem Cells: Scientific

SEAM was first discussed in a 2016 study published by *Nature*. The study described the use of induced pluripotent adult stem cells in making a SEAM of regenerative eye tissue (Hayashi et al. 2016). While using stem cells for regenerating tissues might seem like old news, the history of stem cells does not actually date back to that long ago. IPSCs, the cells of current interest while producing a SEAM, were not produced until 2007, a mere nine years before the discovery of SEAM.

While IPSCs were discovered relatively recently, the use of stem cells for human cell and tissue therapy was first done in the 1950s. Bone marrow transplants were the first type of human adult stem cell transplants to be performed. While the first transplants were done between identical twins, later on using the knowledge of human leukocyte antigen matching in the 1970s, transplants were able to be done between non-related donors and recipients (Glicksman 2018). Human leukocyte antigens are proteins that reside on cell surfaces and have to be matched during tissue transplants so that the recipient does not reject the donor (Scaradavou 2013). The adult stem cells used for marrow transplants do not have pluripotency, so they do have limitations in regards to what tissues they can generate into. This is why the harvesting of human embryonic cells in 1998 was a great step for regenerative medicine: embryonic stem cells are pluripotent and have the ability to differentiate into the 3 germ layers of the body, giving them the potential to give rise to nearly all cell types of a human (Dabrowska and Skopinski 2017). While embryonic stem cells have many practical applications, they have caused a great deal of controversy, due to the fact that obtaining them leads to the destruction of human embryos.

History of Stem Cells: Political

The controversy behind stem cells is a major component of their history, as well as the history of any technology produced via stem cells in the years after their

discovery. This review will examine the political history of stem cells in the United States, taking into account that stem cell policies differ around the world, attributable to controversial nature. Much of the political debate in the United States regarding stem cells is caused by research on embryos; however, induced pluripotent stem cells, the stem cell of interest in this review, has caused some political strife as well.

Examining political controversy chronologically, a major point in stem cell history was the 2001 ban of federal funding on embryonic stem cell research outside of an existing 60 stem cells lines, enacted by former president George W. Bush (Timeline... 2015). This executive order, as to be expected, halted many applications of stem cell therapy in regenerative medicine on account of lack of research. In both 2005 and 2007, congress attempted to pass a Stem Cell Research Enhancement Act, allowing for research to continue on embryos made for, but not used in in vitro fertilization. While these acts were strongly favored by the House and Senate, both acts were vetoed by Bush (Timeline... 2015). It was not until 2009 that former president Obama reversed the 2001 ban on embryonic research, and federal funding was given back to embryonic stem cell research (Stem Cells… 2018). Stem cell research laws and funding still remain similar to 2009 in the year this review is being written, but as it can be seen from stem cells political history, laws are always subject to change (Tarne 2018).

Seeing as embryonic stem cell use has brought up a lot of debate in the past, many scientists try to use adult stem cells when making therapies and treatments that

they hope one day will be available to the public (Tarne 2018). IPSCs have been a topic of focus in the years since they were first discovered in 2007. IPSCs are derived from adult somatic cells, commonly skin fibroblasts, and are reprogrammed to pluripotency so that they are able to proliferate unlimitedly, and be differentiated into cells from the three germ layers of the human body (Glicksman 2018). IPSCs however, have not been able to escape from political controversy unscathed, although many consider them a better alternative to the use of embryonic cells.

Many advocates against embryonic stem cell research are also not in favor of the use of IPSCs due to something coined “gameteless reproduction” (Hoberg 2013). It is projected that eventually stem cell researchers will be able to derive an embryo from IPSCs by differentiating adult cells into egg and sperm (Hoberg 2013). This is why the word “gameteless reproduction” is being used; because gametes would never be used from a person, but instead made through the use of stem cells ex vivo. Critics of IPSCs claim that “gameteless reproduction” will only contribute to the current debate over human cloning, as well as bring up new moral and legal issues that the world has had yet to face (Hoberg 2013). While the controversy regarding stem cells continues, so does the potential of said cells for the cure of many common and debilitating eye disease.

Anatomy and Physiology of the Human Eye

Since this review will discuss several diseases and infections that affect different areas of the eye, general eye anatomy and physiology of structures pertaining to the diseases are important to include for clarity. Every part of the eye from the eyelashes to the optic nerve are essential in providing humans with adequate vision. Starting with anterior structures and moving posteriorly, the conjunctiva is the outermost layer of the eye and it contains the sclera, which is the white portion of the eyeball. The conjunctiva acts to protect the eye from getting bacteria and debris behind it, and the sclera acts to give the eye structure and shape. Still in the outer layer of the eye, the cornea acts as a focus tool for light as it enters the eye. A healthy cornea is clear so that light can properly funnel through it. Located just behind the cornea is the iris, the colored part of the eye. This structure contracts and expands depending on the amount of light coming into the pupil located at the center of it. Behind the pupil and iris is the lens, which works together along with the cornea to focus light onto the retina. Located in the very back of the eye is the retina. The retina contains cells called rods and cones which are receptors for light and color respectively. The rods and cones act to create a visible image by sending signals to the optic nerve, which carries these signals to the brain to be turned into images. The macula is the part of the central retina that has a high concentration of cones, which enables sharp, central vision (Anatomy… 2018).

A SEAM of Ocular Cells Formed by IPSCs

The areas of the eye mentioned in the above section are all areas of the eye that can be produced by forming a SEAM. In the 2016 study published by *Nature,* researchers published their findings on SEAM; including how the SEAM was made, what areas of the eye it was representative of, and animal trials that were conducted using the tissue generated by SEAM. A SEAM is produced by cultivating tissues from IPSCs that represent 4 different concentric zones of the eye (Hayashi et al. 2016). The zones have characteristics of the ocular surface, the lens, the neural retina, and the retinal pigment epithelium (Fuest and Mehta 2016). Zone 1 of the SEAM represents the neuroectoderm, from which the retina develops. Zone 2 of the SEAM represents the retinal pigment epithelium, neural crest, and neuro-retina. The iris and the stroma of the cornea both have origins in the neural crest, representative of zone 2. Zone 3 of the SEAM represents the ocular surface epithelium, where corneal epithelial cells are located. Zone 3 was shown to have characteristics of corneal, limbal, and conjunctival epithelial cells. Finally, zone 4 represents general surface ectodermal cells, which are likely to differentiate into epidermal keratinocytes. These keratinocytes are representative of the cell type that is found in the epidermis of any skin on the body (Hayashi et al. 2016).

In the original study that described SEAM, the researchers stated that their goal was not to form retinal tissue. Production of retinal tissue through IPSCs had been done

before; therefore, their goal was to generate corneal epithelial tissue that could be transplanted after being produced *ex vivo*. Although the goal of the experiment was not to form retinal tissue, SEAM represents the formation of an entire eye from the posterior retinal to the anterior ocular surface. Zone 3 is the major area of interest in this study, and the data of the study collectively showed that zone 3 had characteristics that could be used to produce ocular epithelial tissue that have potential be used in human surgical situations in the future (Hayashi et al. 2016). Another unique feature of the SEAM that was created compared to other studies attempting to produce corneal tissue, was that feeder cells were not used. Feeder cells are normally used to support the growth of stem cells like the IPSCs being used in the study, however they were not used in the production of a SEAM, giving the Hayashi et al. study an advantage. The use of feeder cells can lead to the impurity of the cells that are trying to be grown. In previous studies, IPSCs were induced to be corneal epithelial-like, but since feeder cells were used, they were never able to fully differentiate and develop into completely pure corneal epithelial cells. Moreover, the corneal epithelial stem cells that are produced by the SEAM are purified through the use of antibodies. These characteristics of the methods used to generate a SEAM give them an advantage in future clinical trials because the corneal cells derived from the SEAM will not have impurities that could have come from feeder cells (Hayashi et al. 2017).

In the Hayashi et al. study, rabbits were used to demonstrate the applications of transplanting eye tissue made from the production of a SEAM. Female rabbits were used in this experiment, and they were induced to have corneal stem cell deficiencies in order to blind them. Creating the epithelial-limbal stem cell deficiency was done through a corneal and limbal lamellar keratectomy, which removed the rabbits' ocular epithelial tissue. The rabbits were then given corneal grafts from the corneal epithelial stem cells created through making a SEAM. The corneal sheets that were transplanted onto the damaged rabbit eyes were able to regain function and restore sight to the rabbits. The study stresses the point that this specific type of ocular surface tissue regeneration is a novel idea, because previous studies have only used somatic cells to regain corneal function with the long-term outcomes not having much promise (Hayashi et al. 2016). The experiment performed by Hayashi et al. has been discussed in several review and editorial articles all praising the groundbreaking science, as well as outlining some of the potential complications with the advanced eye research.

Limitations and Potential Solutions

In a 2018 review article by Sasamoto et al., some limitations of using SEAM for eye regeneration are discussed. Although human trials using SEAM have not yet been conducted, high cost of the technology is already being considered as a potential drawback. Producing a SEAM is reliant upon the use of IPSCs. In order to properly obtain the IPSCs, appropriate techniques must be followed to acquire viable cells. Most

research involving stem cells is costly due to the advanced nature of it. Additionally, after the corneal sheets are transplanted into the eyes of the recipient, strict eye drop regimens must be followed and time must be allowed for the sheets to differentiate into new corneal epithelial cells. Therefore, another drawback of SEAM that is considered is the amount of time it takes to produce IPSCs, as well as the time it takes to produce a SEAM. According to another editorial article, the transplanted epithelial cells take about 13-18 weeks to differentiate to their mature potential as corneal epithelial cells (Fuest and Mehta 2016). Another risk potential that has been cited is the potential for tumorigenicity of cells that have developed from IPSCs. This higher risk for tumorigenicity comes from uncontrolled growth of the IPSCs (Sasamoto et al. 2016). Like many experiments in the early stages of trials, there are still areas of SEAM that need to be improved to gain full benefits from it in the future.

Potential solutions to the drawbacks mentioned above are already being formulated in order to perfect the use of IPSCs in making a SEAM. In regards to the lengthy process of obtaining IPSCs and making a SEAM, as well as the high costs of both of these processes, a solution that has been suggested is the creation of human leukocyte antigen (HLA)-typed IPSC banks (Sasamoto et al.). Mentioned earlier in this article, human leukocyte antigens are cell surface proteins that can be matched in donor-recipient situations. While taking cells directly from the patients to make IPSCs for them that can be made into a SEAM has benefits like low chance of rejection, using

HLA banks to stem cell match could be more financially and time feasible (Taylor 2012). As for the increased chance of tumorigenicity, suggestions have been made to use direct reprogramming of cells as opposed to IPSCs. In the article by Sasamoto et al., some cell types are discussed that would good candidates for reprogramming. Cells that are being considered as having promising potential to be reprogrammed include: bone marrow mesenchymal cells, hair follicle stem cells, skin epithelial stem cells, dental pulp stem cell sheets, and nasal mucosal epithelial cell sheets (Sasamoto et al. 2016). Although there are still modifications that need to be made to SEAM to make it more financially and medically dependable, researchers have high hopes for its future and are preparing for the next stages of starting human trials after their successful trials on the rabbits (Fuest and Mehta 2018).

SEAM applications in Limbal Stem Cell Deficiency

Cause of Limbal Stem Cell Deficiency

In the *Nature* study described above, limbal stem cell deficiency was induced in rabbits in order to test the effectiveness of corneal epithelial sheets produced by SEAM; however limbal stem cell deficiency a condition that affects humans too. The outer layer of the corneal epithelium is essential for vision, and it needs to constantly renewed with corneal stem cells. This is due to the need of the cornea to stay transparent, for effective vision to be achieved. The stem cells that are crucial for keeping the cornea functioning properly are located in cornea-conjunctival transition zone, commonly referred to as the limbus. When there is damage to the limbal area or cornea, as

demonstrated on the rabbits in the Hayashi et al. experiment, this can result in severe vision loss or even total blindness. The vision loss is a consequence of the formation of conjunctival epithelium (Selver et al. 2017). The conjunctival tissue replaces the normally transparent corneal surface with hazy tissue. Limbal stem cell deficiencies can arise from several factors and can affect anyone in the world. Limbal stem cell deficiency can be acquired by having burns or trauma to the eye, inflammatory diseases, and genetic diseases, and even the overuse of contact lenses (Sasamoto et al. 2018) . While limbal stem cell deficiency is an extremely painful and enervating disease, treatment of limbal stem cell deficiency is currently hard to come by, and does not offer the most promising of outcomes (Ahmad 2012).

Current Treatments for Limbal Stem Cell Deficiency

Before limbal stem cell deficiency can even begin to be treated, it first has to be diagnosed. Often times, limbal stem cell deficiency is not properly diagnosed and a corneal transplant is given as treatment, and this does not result in desired outcomes. If the disease does happen to be properly diagnosed, tribulations are still faced in order to try and alleviate its symptoms. Limbal cell transplantation is one of the current treatments for this disease. This involves taking tissue from a donor eye and transplanting it into the affected individual. However, there is quite a lot of risk to the donor during this procedure, so only a small amount of tissue can be obtained. Even if tissue is successfully taken from a donor eye, the donor can be left with complications like scarring, chronic inflammation, discomfort, as well as infection (Fuest and Mehta 2016). Donor shortage is also a significant issue when it comes to limbal stem cell deficiency (Research… 2016). In the miniscule amount of tissue that can actually be

taken from the donor, an even smaller percentage of this tissue actually contains limbal stem cells (Selver et al. 2017). Cells donated from cadavers have also been considered as a treatment option, but these have shortcomings as well. In the case of transplantation from either living or dead donors, recipients often need long-term immunosuppression so that they do not reject the donated tissue (Fuest and Mehta). While improvements in the damaged eye are sometimes seen short term, current treatments often are not shown to last over the long term (Ahmad 2012).

SEAM as a Novel Treatment for Limbal Stem Cell Deficiency

The rabbits in the 2016 *Nature* study are a glimpse into the future for the cure of limbal stem deficiency in humans. By growing corneal sheets from cells made in the third zone of SEAM, sight was restored in the limbal stem cell deficient rabbits. The researchers behind this experiment concluded their article with the statement that they are working on preparing SEAM to be ready for the use on humans (Hayashi et al. 2016). One of the major benefits of using SEAM to replenish corneal stem cells is that IPSCs are behind the mechanism of tissue regeneration. As mentioned before, IPSCs are not always time efficient, but they are a much more viable option compared to the method of donated tissue that is currently being used. This is a result of the fact that IPSCs can be made from adult cells gathered from the person who has the deficiency to begin with. The stem cells that are taken from the individual affected with the deficiency can be induced to pluripotency, and then made into a SEAM, which eliminates the potential for immunological rejection. Not only is the issue of rejection solved by using epithelial cells created by the SEAM, but the need for a donor would also be taken off the table (Research… 2016). Hereby, using cells created by the SEAM is a solution to

the major issues, and associated long-term complications, that are currently involved in the treatment of limbal stem cell deficiency.

Use of a SEAM to regenerate corneal cells: Potential Cure of Corneal Blindness

Cause and Prevalence of Corneal Blindness

Limbal Stem Cell Deficiency is a leading cause of the greater, overarching issue that is corneal blindness, which affects over six-million people around the world (Selver 2016). The prevalence of corneal blindness differs vastly in different areas around the world due to factors that will be further discussed later in this review. Corneal blindness is one of the world's leading causes of blindness after cataracts, glaucoma, and macular degeneration (Wong et al. 2017). There are several causes of corneal blindness, but it most often occurs as a consequence of infection and inflammation. Blindness is brought on due to scarring on the cornea from the vast list of infections and diseases that lead to corneal blindness (Whitcher et al. 2001). This review will focus on trachoma as an example of an infection that ultimately causes corneal blindness, due to the large amount of individuals it affects, having visually impaired an estimated 2.2 million individuals worldwide, with 1.2 of these people left completely blinded (Boyd 2015).

Trachoma is an infection of both eyes caused the bacteria *Chlamydia trachomatis*. While trachoma is easily treatable in the developed world, people in developing countries who do not have proper access to medical attention are at a much higher risk for the infection to leave them completely blind. Furthermore, statistics have shown that mostly women and children make up the population of people with active trachoma infections. If trachoma is not treated properly, corneal scarring can occur because the eyelashes will turn into the eye and scratch and irritate the cornea. This will

eventually lead to enough scratching and irritation that the cornea clouds over, and is no longer viable to see out of (Boyd 2015).

Current Treatments for Corneal Blindness

Trachoma and several other eye diseases have left millions around the world without sight; and unfortunately, many of these people will stay blind forever due to the limited amount of treatment currently available to them. Corneal transplantation and corneal grafts are the current cure for corneal blindness, but there is an extreme shortage of donors, as well as limited resources to obtain and properly store the donated corneas. As opposed to the limbal stem cells, corneal donations do not usually have to be matched from donor to recipient to evade rejection. While this does make transplantations easier, in 2012 there was only about one donated cornea for every 70 people in need of one (Wong et al. 2017). Not only are there not enough corneas available, but there are also a shortage of corneal surgeons around the world who are trained to perform the transplantation surgeries (Oliva et al. 2012). Furthermore, access to eye banks, where donated corneas are stored also poses an issue. If corneas happen to get donated, they have to be stored properly and time sensitivity plays a key role in maintaining viable tissue. All around the world, there is a lack of notification systems to alert facilities that are capable of storing the donated tissues. If too much time has passed, the corneas will no longer be able to be transplanted into a donor, and they are wasted and have to be discarded (Wong et al. 2017).

Corneal Tissue Obtained from a SEAM as a Cure for Corneal Blindness

As it can be inferred from the current treatments section above, many of the issues regarding corneal transplants revolve around lack of donations and how the donations are received. Using tissue produced by the third zone of the SEAM could take out many of the human factors that are inhibiting the current treatment models. Clear corneal sheets were produced from cells taken from the third zone (Herkewitz 2016). While corneas produced from a SEAM are still in their preliminary research phase, having only been experimented on animals thus far, they are a promising technique for the future. The use of a SEAM could solve the issue of time sensitivity because the corneas are purposefully being grown, so lack the lack of notification of donor tissue would not be applicable. Furthermore, as time progresses, and researchers perfect the craft of making a SEAM, corneas will not have to be donated. As of now, corneas are being donated by cadavers to people in need, but with the use of a SEAM, they can be grown for people in need using cells taken from their own body. While SEAM is not yet ready to provide the world with the corneas it needs yet, it is quite possible in the near future that it could be a revolutionary treatment for the millions suffering from corneal blindness.

Use of a SEAM to regenerate retinal cells: Potential Cure of Macular Degeneration

Cause of Macular Degeneration

Macular degeneration comes in two forms, wet and dry macular degeneration, and it is age related. Most people suffering from macular degeneration have dry macular degeneration. The underlying cause of dry macular degeneration is not known. However, it is known that deposits on the retina and behind the macula form and cause

the macula to deteriorate over time. Once a person has dry macular degeneration, it affects both eyes, but can progress at different rates in each eye. As for wet macular degeneration, around 10-15% with macular degeneration have the wet type. Although less people have wet macular degeneration, it is the type that causes more severe vision loss. Wet macular degeneration causes blood vessels under the retina to grow towards the macula. These blood vessels tend to break and bleed, causing damage to the macula which make the macula detach from its base. When the macula detaches, central vision is lost quickly and to a great extent. Unlike many other eye diseases, there are no current cures or treatments that exist at all for either types of macular degeneration (Mogk 2018). As a leading cause of vision loss around the world, a cure for macular degeneration desperately needs to be developed, and SEAM could be that cure.

SEAM as a Potential Cure for Macular Degeneration

While the researchers who originally produced the SEAM were focused on making corneal tissue, they also managed to construct cells that form into retinal tissue as well. As previously mentioned, the second zone of the SEAM consists of cells representative of the retinal pigment epithelium and neuroretina (Fuest and Mehta 2016). Therefore, SEAM has the potential to reconstruct retinal tissue that has been damaged through macular degeneration, which will allow for transplantation of retinal tissue that has been constructed ex vivo. Once again, the issue of rejection does not have to be considered with SEAM, because if need be, the IPSCs can be produced from the recipient's own cells. (Research 2016). Looking further into the future of SEAM,

the world is one step closer to growing an entire human eyeball in a lab, which will hold potential cures for even the incurable, like macular degeneration.

Conclusion

The research that has been presented in this review is still in its early stages of development; however, this does not take away from the fact that it needs to be taken into serious consideration by the scientific and medical communities. Producing a SEAM through the use of induced pluripotent stem cells is a potential cure for many eye diseases that rob millions of sight around the world today. The original researchers who first produced the SEAM published a follow-up article, again in *Nature*, which described how the production of a SEAM will add to a greater understanding of the human eye as it is currently known. The 2017 *Nature* article sheds light on the fact that while the SEAM is still in its days of infancy, it is lending a hand to understanding the origins of the different tissues that make up the human eye. Therefore, while its clinical applications are still in the works, a SEAM is acting as a tool for the study of more fundamental eye tissues. Curing diseases through structures produced by a SEAM can be expected in future years to come, but in the present, a SEAM will provide scientists with an expanded knowledge of human eye tissues in the earliest of developments (Hayashi et al. 2017). While funding might be scarcer than needed for stem cells and regenerative medicine, while there are health risks that need to be considered, and while time is always against the scientists devoting their lives to this research; SEAM is a worthwhile endeavor that has the potential to improve the lives of millions.

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