ELIXIR OF YOUTH:
THE APPLICATIONS OF STEM CELLS IN THE TREATMENT
OF MACULAR DEGENERATION AND OSTEOPOROSIS

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Abstract

In this paper we aim to show the potential uses for stem cells in the treatment of age related conditions, focusing on macular degeneration and osteoporosis. We will discuss the possible advancements, which could be made by injecting cultured embryonic stem cells into the retina of people with macular degeneration and focusing on tissue repair and regeneration of cells, as a possible treatment for people with osteoporosis. Using stem cells as possible treatments for these conditions could improve the lives of an aging population.

Introduction

Steeped in controversy, stem cells are a hot topic right now. So what is causing this divide in opinion between scientists and the general public? The answer is embryonic stem cells. These stem cells are derived from a four or five day old human embryo that is in the blastocyst phase of development [1]. They are unspecialised and can differentiate into all cell types in an organism, as well as having the ability to self-replicate. These properties of embryonic stem cells have caused interest and excitement in the scientific community ever since their discovery and their use has brought some moral qualms to the forefront our society.

However, stem cells can not only be derived from embryos. They are also found amongst differentiated cells in certain organs or tissues (stem cell niche) of our body and have the ability to self-renew. The main role of these somatic stem cells is to maintain and repair the tissue in which they are found. They do this by differentiating to produce a limited number of specialised cell types in its specific tissue [2]. Therefore, they do not boast as many uses as embryonic stem cells. However in 2007, scientists discovered that they can induce pluripotency [3] [4] in these somatic cells allowing them to differentiate into all cells from the three germ layers (endoderm, ectoderm, mesoderm [5]). Induced pluripotent stem cells have been genetically reprogrammed so that they return to an embryonic stem cell like state. This significant advance has relieved some of the ethical concerns of the public, due to the use of embryos in this area of science, creating hope that somatic stem cells will be manipulated in order to replace embryonic stem cells in the testing and development of new treatments.

Another problem to overcome when using somatic stem cells is the difficulty of culturing them. Somatic stem cells are harder to culture than embryonic stem cells because larger numbers of them are needed and there are normally very small numbers of stem cells in each type of tissue. Once somatic stem cells are removed from the body, their ability to divide is also reduced. However, some scientists are trying to grow large quantities of adult stem cells in cell cultures; manipulating them to try to generate specific cells for treating injuries or diseases.
A third, less-known type of stem cells is foetal stem cells, found in the umbilical cord blood or the placenta, which can be donated after birth. Lots of the stem cells obtained from this are differentiated, but some are still unspecialised. These stem cells are relatively easy to obtain, having a far lower rejection rate than bone marrow transplants if used and could be used to treat many different diseases [6].

In 1998 James Thompson (University of Wisconsin) was the first to isolate cells from blastocysts of early embryos and developed the first embryonic stem cell lines [7]. Stem cell lines are stem cells that have been allowed to divide into more stem cells for a long period of time without having any abnormalities. To create a stem cell line, scientists remove cells from the inner region of a blastocyst. These pluripotent cells are then plated in culture dishes containing growth mediums and nutrients. When these cells multiply and divide, an embryonic stem cell line has been created. By adding different growth factors, it is possible to induce these cells to develop and differentiate into specific cell types [8].

Since the 1960’s stem cells have been used in medicine during bone marrow transplants. Adult hematopoietic stem cells in blood and bone marrow have been proven effective in treating diseases such as leukaemia and sickle cell anaemia. However, extracting bone marrow from donors is an invasive procedure and some scientists have found hematopoietic stem cells in umbilical cord blood. Scientists have recently been experimenting with different uses of these cells for a variety of conditions. These include vascular disease, organ and tissue regeneration, Alzheimer's, strokes and heart failure and also other age related diseases [9].

We understand that stem cells are the reason for our being. Every single one of our cells has differentiated from stem cells. If stem cells can create an organism, can they also stop it from ageing? Our project looks at osteoporosis and age-related macular degeneration and how stem cells can be utilized in slowing down or curing these illnesses, which not only affects those suffering from these illnesses but also the national economic state.

The Macular Disease Society has estimated that up to 40,000 people develop wet age-related macular degeneration (wet AMD) and 44,000 people dry age-related macular degeneration (dry AMD) each year [10]. Ageing in the skeletal system is also on the rise and is the main cause of pain and disability in the elderly. Around 230,000 people every year suffer an osteoporotic fracture, with an estimated cost to the NHS of £1.8billion a year. The number of AMD sufferers and osteoporotic fractures is estimated to increase rapidly over the next few decades due to the ageing population, which will result in a larger economic burden on the NHS, therefore it is an issue needing an urgent and affordable solution.

**Discussion**

We have decided to discuss two different ways of using stem cells as a treatment. In conducting our research, we have delved into methods of tissue repair and transplantation
of stem cells. In addition, we have researched ways of manipulating stem cells in the body itself in parallel with the body’s own mechanisms and healing processes, often referred to as regeneration. To look closely at these two different approaches to stem cells, we will concentrate on its possible uses in Macular Degeneration and Osteoporosis, both of which relate to ageing.

**Macular Degeneration**

Macular Degeneration is a progressive blindness that does not render the patient completely blind, rather reducing their central vision. The degeneration of central vision occurs when the macula at the centre of the retina, which is responsible for central vision, does not function as it used to. This is commonly seen in patients over the age of sixty-five.

As we age, our central vision could deteriorate due to damage to the retinal pigment epithelial cells resulting in one third of adults over the age of seventy-five affected by age-related macular degeneration (AMD). AMD can be described as ‘wet’ or ‘dry’, with ‘dry’ being the less severe form of the disease whereby the retina gradually deteriorates, rather than the abnormal growth of blood vessels within the retina that is associated as being ‘wet’. Another form of the disease is Stargardt’s macular dystrophy which is a form of inherited macular degeneration in juveniles. Inherited through recessive genes, it develops in late childhood, resulting in legal blindness (11). The deterioration of the retina can be induced and increased by a number of factors from a high fat diet, low in nutrients and antioxidants, to prolonged sun exposure (12).

Currently, there isn’t any treatment for dry AMD (13), however replacing the damaged retinal pigment epithelial cells (RPE cells) is suggested to have the ability to improve the central vision and cure dry AMD. The work of Idelson [2009] (14) has determined that human embryonic stem cells (hESCs) can be stimulated to differentiate into retinal pigment epithelial cells in the presence of nicotinamide and TGF – β factors such as Activin A.
To promote this type of differentiation, embryonic stem cells will need to be cultured on a serum, which does not contain any differentiation or growth factors \(^{[15]}\). Therefore scientists can control the addition of factors such as nicotinamide and Activin A, allowing them to manipulate the outcomes and products of the differentiation. This serum will help improve cell adhesion and enhance differentiation.

Once the hESCs have differentiated into the RPE cells, they will need to be injected into the choroid (sub-retinal space) \(^{[16]}\), which is shown in Figure 2 as being just above the pigment epithelial layer. From here, the RPE cells will randomly spread out across the retina therefore it will take time for sufficient RPE cells to reach the macula, where the damaged RPE cells are situated, and improve the central vision.

Wet AMD cannot only be explained by damage to the RPE cells. It is also caused by abnormal growth of blood vessels around the macula due to the damage to these RPE cells. Not only is the damage limited to these cells, but the photoreceptor cells (rod and cone cells) also undergo damage \(^{[17]}\) caused by the swelling and the scar tissue as a result of the abnormal blood vessel growth. Currently, there is a treatment called anti-vascular endothelium growth factor (anti-VEGF) which can prevent further degeneration to the macula by stopping additional growth of these blood vessels \(^{[18]}\). However, this treatment can cause discomfort to the patient, needs administering over multiple sessions and will not reverse any damage caused or repair the vision.

We propose the use of RPE cells, derived from hESCs, in combination with the anti-VEGF treatment therefore abnormal growth of blood vessels is prevented and damage to the old RPE cells is reversed. Research by Lanza R at the Advanced Cell Technology (2004) shows that hESCs can also be stimulated to differentiate into photoreceptors cells, which when transplanted into the retina of young mice, they will move into the photoreceptor layer of the retina \(^{[19]}\). We suggest that a combination of these three treatments can be used as prophylactic treatment to slow down or prevent further loss in central vision of those who are suffering from wet AMD in order to stabilise the deterioration of the macula.

A clinical study was conducted recently by the University of California and Advanced Cell Technology, a biotechnology company, which looked at the possibility and ultimately the possible dangers involved in the transplantation of human embryonic stem cells into the retina to cure Stargardt’s macular dystrophy and dry AMD. The study aimed to test this theory on one patient with the age-related AMD and one with Stargardt’s macular dystrophy. This clinical trial involved injecting the stem cells which were developed into specialised retinal cells, into areas of the eye that surrounded the retina in the hope that the retina would be restored to a healthy state that can support light-sensitive cells required for sight. The patients were monitored for four months and within this time no problems such as rejection of the cells, abnormal cell growth or tumours were experienced. However, this
trial, though successful, cannot be completely relied upon as currently, only two patients have undergone the trial and thus, the success is questionable \(^{(20)}\).

With trials only having been carried out on two patients, scientists need to look into expanding the number of patients that are tried and tested in order to gain a full understanding of the reliability and success of the use of stem cells on humans to treat an otherwise incurable disease. Also, the patients, though monitored for a period of four months, need to be under continued analysis to endure that no side effects are suffered at a later date. Thus, now the possibility of this particular use of stem cells has proved to have advantageous effects, the time period of testing needs to be increased and the number of patients used in the trials also needs to be increased to further broaden the scale of successful cases before this can be recognised as a valid treatment for macular degeneration. These findings are pointing to the suggestion that patients suffering from loss of central vision can have their sight restored, increasing their quality of life and in the long term, decreasing the dependency on NHS resources after initial successful treatment.

**Osteoporosis**

Unlike the discussion on AMD, we will be looking closely at regeneration in order to come up with a solution to osteoporosis. This involves using the body’s healing mechanisms and manipulating it in order to reach the desired outcome. To understand osteoporosis we need to look closely at the balance between bone formation and bone resorption. Bone formation is carried out by osteoblasts and resorption is carried out by osteoclasts \(^{(21)}\). This is all monitored by the osteocytes in the bone matrix. Osteoporosis can be described as a net loss in bone mass. Bone formation exceeds resorption until around 20 years of age when peak bone mass is achieved however during old age, resorption exceeds formation. This is what leads to a net loss in bone mass which increases the risk of fractures, especially in the hip, spine and forearm.

So far, there has been a lot of research on anti-resorptive methods for slowing down the effects of osteoporosis. However, research into stem cells offers new, possible cures for osteoporosis by increasing the number of mature osteoblasts. A solution that we have studied falls under the category of regenerative medicine, where mesenchymal stem cells can be stimulated into differentiating into osteoblasts. This would increase the rate of bone formation leading to an overall increase in the bone mineral density (BMD) and consequently, fewer incidences of osteoporotic fractures.

Another prospective way of using stem cells is for tissue repair. 1 in 3 women and 1 in 5 men will experience osteoporotic fractures which could disable them and cause them severe pain. However, stem cells can also be useful in speeding up the recovery time and increasing the strength of the bone in the fractured area.
To use stem cells in both ways, we will have to understand how to stimulate differentiation. In our body, the Wnt pathway coupled with RUNX-2 (runt related transcription factor 2) and Osx (osterix) stimulate the differentiation of mesenchymal stem cells into pre-osteoblasts \cite{22, 23} and therefore osteoblasts. However the DKK1 pathway inhibits this differentiation but is essential in the later stages of maturation in osteoblasts. There are also many other factors which can be used in addition to these conditions to increase osteoblastic differentiation.

Extensive research has gone into bone morphogenic proteins (BMP) which can induce osteoblastic differentiation from mesenchymal stem cells. More specifically, the publications of Shiozawa and Laflamme prove that BMP2, BMP6 and BMP7 have these properties \cite{23, 24}. The mechanism is not entirely known but the work of Yamaguchi (2000)\cite{25} shows that BMPs, along with vitamin D3, increase core-binding factor alpha 1 (Cbfa1) expression and that mice lacking this transcription factor did not show signs of bone formation. Multiple studies have shown that cbfa1 is not only a major stimulator of osteoblastic differentiation but is also essential in the maturation and functioning of osteoblasts and can be referred to as the ‘molecular switch in osteoblast biology’ \cite{26} Cbfa1 also leads to the upregulation of expression of osteocalcin which is well correlated with increase in bone formation and therefore BMD. Consequently, increase in certain BMPs would result in a higher proportion of osteoblasts compared to osteoclasts. In theory, this should speed up bone formation and slow down osteoporosis. However, there the work of Vukicevic (2009)\cite{24} concludes that BMP6 is released by the mesenchymal

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\caption{Figure 3: http://www.cliffsnotes.com/study_guide/Recombinant-DNA-and-Biotechnology.topicArticleId-8524,articleId-8439.html}
\end{figure}
stem cells before osteoblastic differentiation and suggests that BMP6 would be more effective, in smaller amounts, than BMP7 at stimulating this type of differentiation. The work of Shiozawa (2010)\(^{27}\), supports these conclusions and even suggests that hematopoietic stem cells (HSCs), produce BMP2 and BMP6. HSCs when subjected to acute stress such as a single acute bleed were studied to be more effective in producing these proteins\(^{28}\).

Further research will be needed to determine the effectiveness of these proteins, in isolation or in combination.

Currently, BMP2 is FDA approved for treating spinal fusions. BMPs are only found in trace amounts in our bone therefore another method needs to be used to produce high amounts of BMPs\(^ {29}\). A possible method is the recombinant technique, which creates genetically engineered cells, which can produce high amounts of BMP. Figure 3 shows the DNA recombinant technique using a plasmid as the vector and a bacterium as the host cell. To do this, the segment of DNA (gene) which contains the information on producing BMPs is isolated using a restriction enzyme and inserted into a vector (viruses or plasmids are most commonly used)\(^ {30}\). Ligase enzymes bind the gene with the vector. This vector will be inserted into a host cell, which is usually bacterial (E-coli) and this concludes the transformation stage. Currently, Medtronic, a medical technology company, uses recombinant BMP2 to speed up bone formation in a spinal fusion. Using their technique\(^ {31}\), a collagen sponge soaked in the recombinant human BMP solution, could be attached to an area of the bone with low BMD (bone mineral density). The BMPs would be produced over a long period of time to gradually increase the BMD. This technique would be most effective in cases of osteoporotic fractures, where the collagen sponge could be placed inside a titanium device and placed at the fracture site. The titanium cage can stabilize the bone and hold it in position while the BMP product can speed up the recovery time by increasing the rate of bone formation.

Figure 4 shows the activation of osteoclasts by the interaction between RANK ligand on the osteoblasts and RANK on the osteoclasts. This suggests that increasing the number of mature osteoblasts, will not only speed up bone formation, but also lead to an increase in the number of mature osteoclasts responsible for bone resorption. This poses a major problem and without extensive testing, we cannot be sure when the rate of bone resorption will start to match the rate of bone formation. Therefore this solution may only be viable for a short treatment time, such as treating an osteoporotic fracture\(^ {31}\).
Conclusion

Concerning AMD, the use of stem cells may actively improve the damaged retinas, opening up the possibility of the regeneration of the individual’s sight or in the least, a more permanent method of slowing the progression of blindness. With the use of embryonic stem cells the chances of rejection of this invasive method is decreased as the cells are from the tissues of the patient and thus are of the same blood type and share the same DNA, consequently increasing the possibility of more successful surgery.

The discussion surrounding osteoporosis suggests that this technology could quickly heal and maybe even prevent osteoporotic fractures and therefore the possibility of relieving the economic stress on the NHS. If this treatment were successful, it would offer a new lease of life for those suffering from osteoporosis, as it could reduce the period of pain and disability from osteoporotic fractures by speeding up the recovery time. We have not yet considered the cost of this treatment. Creating the recombinant human BMP technology is very expensive and consequently this treatment, if developed, could only be administered locally to the area of the fracture and may not be viable in treating low bone mineral density, without a fracture. Another area to consider is side effects from this type of treatment. BMP2 has not been FDA approved for anterior cervical fusions as it has led to soft tissue swelling and even death in some cases.

Both these treatments will need to be more extensively tested and scrutinised before they are implemented on a larger scale. However, the future of stem cell research looks bright, with an endless array of new and exciting research advances, which we hope will be able to offer solution to these two crucial problems.
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