

**WILL AMNIOTIC ADVANCEMENTS STEM THE FEUDS OF  
EMBRYONIC EXPERIMENTATION?**

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**PASS WITH DISTINCTION**

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## Abstract

Stem Cell treatments have been at the centre of medical advancement for a number of decades, displaying exciting prospects into a future of regenerative medicine. Embryonic stem cells have, for years, been at the forefront of this research but the ethical issues surrounding it have made it hard to progress further. This paper discusses the possible solution for this problem in the advancement of amniotic fluid stem cells as an ethically friendly yet competitively effective method of treating previously fatal diseases. The ways in which this new type can be implemented into current medicine are investigated as well as discussing the practicality and viability of this seemingly revolutionary research and whether it really could replace current methods and solve the ethical issues surrounding embryonic stem cell research.

## Introduction

The discovery of stem cells has been a relatively recent yet revolutionary medical advancement. Since the discovery of the utilisation of this process for potential growth of new tissue initiated by Canadian scientists Ernest McCulloch and James Till who discovered their unique properties of self-renewal and differentiation, winning them the Lasker prize for basic medical research in the 1960s<sup>1</sup>, stem cells' organ forming, regenerative and reparative tendencies have opened up thousands of possibilities for organ transplantation, tissue repair and replacement of defective tissue in all fields of medicine<sup>2</sup>. Their unique property of being unspecialized cells with the potential to self-replicate indefinitely and differentiate into any number of specialized adult cells has meant that researchers have performed extensive experimentation to discover possible uses.

Much of the research carried out uses embryonic stem cells. These are classed as Omnipotent since they can differentiate into any type of cell. This has huge implications as it means that potentially any part of the body can be reformed, but this process has also created a storm of controversy. Embryonic stem cells are extracted from 'spare' embryos from 'in vitro' treatment with the consent of the donor and the inner mass is grown in a culture, its environment monitored to control its differentiation<sup>4</sup>. In doing this, the initial embryo itself dies and for many people this raises serious ethical issues due to the diverse views held in society on the moral and legal status of an early embryo. Religious opinions hold particular importance with important figures such as the Pope whose declaration stated that an embryo has the "dignity proper to a person." (Dignitas Personae 2008) and condemned the use of embryonic stem cell research entirely<sup>5</sup>. This presents difficulties for researchers who wish to respect these opinions as well as providing the most modern and effectual treatment to save the maximum number of lives. Part of a doctor's job is to 'respect the hard-won scientific gains of those physicians in whose steps I walk, and gladly share such knowledge as is mine with those who are to follow' (Hippocratic Oath *Louis Lasagne 1964*) which seems to imply that if embryonic research has been discovered, it is a doctor's oath to use this amazing discovery to advance medicine further, however they must also respect the moral grounds that society depends on and this is the issue that present day scientists are trying to overcome.

In recent years therefore, there has been a shift from the development of embryonic cells to discovering alternative stem cell sources in the body and to try to utilise these cells in the same way that has proven so successful with embryonic stem cells. The factors many scientists have had to consider are; diversity of the cell (omnipotency, pluripotency, multipotency); availability; ease of extraction; and finally moral and social implications of the usage of the cell. Many different cell varieties have been discovered including adult stem cells, cord blood cells and most recently IPSs (Induced Pluripotent Stem Cells)<sup>3</sup>. However, upon researching each alternative perhaps one of the most promising alternatives is the development of amniotic fluid stem cells. These cells float freely in the amniotic fluid of pregnant women and have been found to carry almost exactly the same traits as embryonic stem cells without the issue of destroying a potential life. This exciting

prospect and just how viable this alternative is in terms of competing with the use of embryonic and other stem cells are the foci of this paper.

### **Why Amniotic Fluid Stem Cells (AFSCs)?**

There are many methods of obtaining different types of stem cells, all with their advantages and disadvantages, so why are amniotic fluid stem cells so special? As the name suggests, these stem cells originate in the amniotic fluid which surrounds the baby in the womb providing protection and cushioning to the baby<sup>6</sup>. However it also plays an important role in the development of internal organs thus suggesting the presence of stem cells. One of the advantages is the large availability of these cells as they are already commonly obtained through a test known as an amniocentesis.<sup>7</sup> This test is performed on certain risk groups of pregnant women, usually between their 15<sup>th</sup> and 20<sup>th</sup> weeks, to diagnose chromosomal disorders and neural defects such as Spinal Bifida. A fine needle is injected into the amniotic sac of the woman and a small sample of her amniotic fluid is extracted. However at the moment after the tests have taken place, the fluid is simply discarded. This easy and already established method of maintaining these stem cells could be harnessed to provide a routine donation of stem cells in an ethical and renewable way<sup>3</sup>. Another important benefit of these cells is that due to the tissue being an exact match to the baby there is no immuno-rejection risk; another advantage they have over embryonic stem cells which would not come from the patient and therefore would present a risk of being rejected<sup>8</sup>.

Research has revealed that the stem cells from amniotic fluid can differentiate into at least 6 cellular lineages from all three germ layers thereby classifying it as a pluripotent stem cell (De Coppi *et al.* 2007). This is fantastic news as it means that the diversity of diseases and illnesses that can be treated by this single cell type is huge therefore suppressing the need for any other type of stem cell extraction. Telomerase lines were maintained for 250 population doublings (De Coppi *et al.* 2007) exceeding the average of 50 pd meaning that the life cycle of these cells would be much greater than that of an average somatic stem cell which is telomerase negative<sup>9</sup>. These cells also seem to have no teratoma risk which is linked to embryonic cells, and divide at a rapid rate of 36 hours. All this research supports the use of amniotic cells as a perfect contender for the future of regenerative medicine.

### **What Research Is Occurring?**

Research on all stem cell types has been happening on a grand scale since their initial discovery. However, many limitations are placed upon stem cell research, in particular the halting of government funding by George Bush in February 2001 and the vetoing of the expansion of funding in early 2007<sup>10</sup> have limited the extent that the research can develop into. Nevertheless, Bush's call for support to be laid on alternative stem cell methods<sup>11</sup> has led to further advancement of amniotic stem cell research which has been vigorous, especially within the past few years and many fascinating discoveries have been uncovered.

Initially, scientists investigated the opportunity of these cells with regards to multi-potent properties, but more recently, researchers such as Dr Anthony Atala's team from the Wake Forest Institute for Regenerative Medicine<sup>12</sup> have made some fantastic new discoveries which are beginning to prove their pluripotency. In May 2007, after an extensive research programme, the publication of 'Isolation Of Amniotic Stem Cell Lines With Potential for Therapy'<sup>13</sup> was released in which scientists managed to isolate these cells from human amniocentesis specimens using magnetic microspheres and discovered the surface antigen c-Kit (receptor for stem cells). Differentiation into adipogenic, osteogenic, myogenic and other pathways was recorded by the presence of mRNAs specific to multiple lineages and markers on the cells confirmed that they

differentiated from a single cell. Many more experiments have been conducted on mice such as the injection of induced neural cells into the skulls of mice with diseased brains which successfully created new connections with healthy neurons. The amnio stem cells within male fetuses' amniotic fluid have also been found to contain Y chromosomes<sup>12</sup>, thereby proving that the cells came from the babies rather than the mother's DNA. Most recently, a 2008 study<sup>14</sup> found that injection of these cells into differently damaged lung tissues provided the desired result of differentiation into the relevant tissue type and repair. However although the amount of research into this exciting new discovery is increasing, especially at the Wake Forest Institute, much more can be done to open new doors into the field of regenerative therapy.

Certainly, the lack of international research being conducted towards amniotic stem cell research is a real barrier to its progress and this issue must be addressed. With key researcher Dr Paulo De Coppi working in Great Ormond Street Hospital in London<sup>15</sup>, research is being progressed extensively in the UK and the support of three other scientists constantly working on the project is promising. The new tissue engineering centre at Great Ormond Street being opened in May 2009 will open up a variety of employment opportunities for young scientists who could help push the programme further in the UK.

### **Research Prospects**

The next steps in the development of these cells will be the engineering of an entire organ. The creation of an organ from human amniotic fluid stem cells would be a huge step forward and have big implications for the future of these cell types. They have already been proven to spontaneously differentiate in certain conditions as in the lungs of mice using a microinjection<sup>14</sup>, so the potential for them to spontaneously differentiate into whole organs seems logical. If proven successful, the creation of a human organ seems only a short step away, basing the principles of the mouse's reactions as being similar to that of a human's. It seems likely that if the cells can differentiate when surrounded by cells within an organism, scientists can create those same conditions outside the body with only a small sample of the surrounding tissue to initiate division and differentiation of the stem cells. A sample of tissue from a human could be placed into a culture with many undifferentiated AFSCs and as in the experiment in the lungs of the mice whereby the AFSCs differentiated into the parts of the lung surrounding it, the same should occur with the cells in a human tissue sample, ideally eventually creating an entirely new organ. As they don't seem to show any sign of teratoma risks, the risks involved in experimentation on human tissue seems relatively low when conducted in the brand new environment of the tissue engineering centre. As described as having telomeres beyond 250 population doublings and remaining stable as well as exhibiting self renewal markers (de Coppi 2007), this research would not need huge numbers of samples in order to conduct extensive research and with a short doubling time of only 36 hours, growth rate over a month would be fantastic meaning the potential to create an organ in a short period of time is very suggestible.

The mass culturing of these cells can take place in a very short amount of time; therefore availability of these cells for research must meet these demands. As mentioned before, many women undergo a simple test known as amniocentesis<sup>17</sup> during pregnancy whereby a thin needle extracts a small amount of fluid from the amniotic sac. This routine test which is used for diagnosing disorders during pregnancy extracts precisely the right tissue containing the stem cells needed to conduct the research. If this process was made routine for every pregnant woman at their 15<sup>th</sup>-20<sup>th</sup> week check up, there would most definitely be sufficient samples that could be used to culture many different specimens in different parts of the laboratory under different conditions to assess the best results possible. Research could then be developed into which factors such as

light, temperature and conditions cause the cells to culture at the fastest rate and even study what conditions trigger the differentiation of these cells into the different types.

### **Treatment Prospects**

Alongside this laboratory progress, once the development of research has progressed to the stage that scientists are able to confirm what conditions are ideal, the variety of differentiation possibilities and the ways in which the cells react in surrounding tissues, the possibility to move onto human treatment seems highly likely.

A development of AFSCs in the clinical world is the prospect of 'banking' the amniotic fluid of pregnant women who choose to opt for it and so provide the unborn child with an instant tissue match of stem cells for them to use at any time during their lives should they need it. Using an amniocentesis test to extract the cells, they could be banked and logged under the patient's medical notes for use in the future. Based on the proposed trials above being successful in proving differentiation when placed in any environment with the relevant cell type, this would eliminate the need for tissue matching for any transplants required. A tissue sample would simply need to be extracted from the patient from the area needed and when placed amongst the stem cells, would cause them to differentiate and culture into a new organ which could then be transplanted into the patient in a relatively short period of time.

However the most prominent suggestion of the advancement of AFSCs has to be the possibility that fetuses with defects identified in the prenatal screenings before birth can have an organ prepared and ready to be transplanted as soon as they are born. Currently, defects identified during pregnancy are often left until birth where there is then a big rush against time to find an organ or blood transplant suitable to correct the problem. With the prospect of AFSCs being able to create suitably matching tissue from amniotic fluid whilst the baby is still in the womb, the organ could then grow alongside the baby itself and be ready for transplantation upon birth, saving time and money spent in the stressful rush after the baby is born as is current procedure. This research has already advanced to the stage that large animal models have been successful in treating conditions including congenital diaphragmatic hernia, tracheal and chest wall defects, bladder extrophy, and cardiac anomalies.

As current research is not extensive enough to identify the triggers that activate the differentiation of these cells, it is not possible to create a specific organ without a current tissue sample from the patient. Research is developing to try and identify this mystery; however it seems possible to be able to cause differentiation of these cells without requiring this knowledge. When surrounded by cells of a certain type, the AFSCs differentiate into the required cell type to replace damaged cells. This knowledge could be applied to the defective cells of a fetus; if the type of cell required to cure the defective part could be extracted from the fetus without causing any kind of damage to the unborn child, the child could be provided with a brand new organ upon birth.

At present, Dr Dario Fauza's current research in Boston Children's Hospital<sup>18</sup> has been able to create tissues using collagen scaffolds by isolating the mesenchymal stem cells from the amniotic fluid and after sufficiently culturing them, grafting them onto the scaffold to grow into the correct shape. Therefore it seems that once a sample of the correct cell type has been obtained, differentiation of the stem cells can occur and be grown by means mentioned above into the correct organ for transplantation. Using this, scaffolds of various organs can be created for use once differentiation of the stem cells has begun to occur and provides the control of development for the growth of these new tissues to be made alongside the growth of the baby in the womb.

This opens up the possibility that a sample of the cell type needed, be extracted from the fetus using new technology. With nanotechnology now emerging, logic elements consisting of only a few cubic nanometers<sup>19</sup> could travel into the body of the fetus through the placenta of the mother and be directed to the relevant part of the body enclosing a sample of the cells. This sample could be used to differentiate the AFSCs in a culture outside of the body of the fetus and be transplanted at birth. At present the most likely source of this nanotechnology would be in the use of wireless microgrippers<sup>20</sup>. Each measuring only one tenth of a millimetre, this new development is a potential tool to extract the cells needed to initiate differentiation of the stem cells. As they are made from nickel-plated materials, magnets are used to direct these tiny biopsy machines around the body, in particular to the cells of the growing fetus, to collect a sample to be cultured outside of the body alongside the growing baby. Tests on animal fetuses must be conducted to ensure that the removal of a cell from the fetus does not affect its growth as well as the use of a foreign object in such a delicate area. The biopsy taken from the required region of the fetus needed to grow a relevant organ or tissue can then be introduced to the amniotic stem cells removed during the amniocentesis test or upon requirement to stimulate their differentiation into the rest of the tissue.

Through further development of these new nano-machines, the possibilities could be almost endless. The wireless microgrippers have been manoeuvred successfully around test tubes and the next step is to move onto humans using MRI or CT scans to guide the tools. They are stimulated to close upon certain temperature or bio-chemical changes and so could be programmed to clamp based on the conditions of that region in the body of the fetus needed.

In theory, the process taken place would be such that as soon as a problem is found with the child, for example a defective kidney, a sample of stem cells from the fetus' amniotic fluid is isolated and cultured. Meanwhile, a microgripper is directed with the use of MRI imaging and magnetic technology into the blood stream of the mother and through the umbilical cord into the blood stream of the fetus. There it would be programmed to clamp shut under the conditions typical in a human kidney such as the presence of erythropoietin<sup>21</sup> and then be once again directed out of the system where the living kidney cells are extracted and cultured within the stem cells to differentiate them. Built on a scaffold-like structure, a kidney will then be carefully grown under correct conditions whilst the baby is still developing and as soon as it is born, this newly formed organ can be transplanted straight into the child's body without the fear of rejection and strong immunosuppressant drugs.

### **Limitations**

AFSCs are a particularly exciting new discovery of a stem cell source due to the fact that they raise no ethical issues in their extraction like embryonic cells do at present with the destruction of the living embryo. However there are various limitations to take into account when considering the usage of these cells for treatment in the future.

Firstly, there is a very clear lack of replicated research into AFSCs that has been highlighted by various scientists criticizing their capabilities. Dr Atala's work in the Wake Field Hospital is extremely promising, but more reproducible evidence needs to be produced in order for people to consider these stem cells reliable enough to continue to fund and back the alternative therapy. As well as this, their true pluripotency has been questioned as in Atala's 2007 experimentations, only about 1% of samples tested had a surface marker that is a hallmark of embryonic stem cells implying pluripotency<sup>22</sup>. This very small proportion questions the availability of enough samples to fulfil research and clinical usages.

Funding for new programmes is very tight and the introduction of a new way of treating patients would put a lot of stress on the NHS as well as private hospitals. Therefore, politically, there needs to be further action to persuade the British government to act as President Obama has in granting further funding into stem cell research, if not embryonic, then especially amniotic fluid advancements. The research required is still very vast and it will take years for these kinds of ideas to become regular practice in NHS hospitals throughout the country. Therefore the suggestion that they will replace embryonic stem cells is unreasonable at present. Embryonic stem cells are still proven to provide more variety in their omnipotency and although presenting possible tumour risks and ethical issues for many people, they are still far more advanced in their research and so would give scientists a more realistic solution at present whilst other more ethically pleasing methods are being developed. Scientists such as George Daley<sup>23</sup> argue that although they are a fantastic discovery, they are by no means a replacement for embryonic stem cells as embryonic stem cells also provide an amazing insight into early human development which could lead to being vital research towards possibly solving genetic disorders before birth even occurs.

### **Conclusion**

It is clear that Amniotic Fluid Stem Cells hold fantastic potential to provide the world of regenerative medicine with a new, ethically friendly alternative to embryonic or adult stem cells currently being developed and can potentially create a whole new field of medicine in which nanotechnology combines with parallel tissue culturing alongside the growth of the fetus to ensure that organs are ready to be transplanted upon birth. As well as this, it holds the potential to provide every person with a supply of an exact tissue match to their own which can later be used during reparative treatment to extend the powers of potential medicinal treatment. This would eliminate the need for immuno-suppressant drugs and organ donation waiting lists, potentially saving millions of lives currently lost.

It is clear that currently, the lack of research into this relatively new aspect of stem cell therapy ensures that embryonic and adult stem cells will most definitely continue to be needed to treat more conditions at present and if the research suggested above is conducted successfully, then in the future, the need for these less satisfactory methods of regenerative medicine will be significantly decreased.

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