

Using Stem Cell Therapy For Natural Secretion of  
Mosquito repellents In Animals

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**PASS WITH DISTINCTION**

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## **Abstract**

Embryonic Stem Cells can hold the key to cures for many diseases that have previously been thought of as untreatable, such as spinal cord damage and Alzheimer's. Research into them is fairly recent, the last 10 years; however it is now being looked into using adult stem cells also. There are many controversial issues involving the use of embryos for stem cells, however using adult stem cells, such as from hair follicles, does not raise these issues. In this paper we are going to discuss how stem cells from hair follicles can be altered to make them a natural repellent of mosquitoes in animals.

## **Introduction**

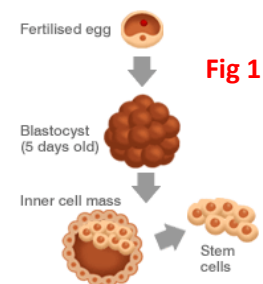
### **(I) The Beginning of Stem Cells**

Stem cells are described in the Biological Sciences Review, (volume 21, number 3. Pg. 26) as having two essential features. The first being that they are a self-renewing population, when one divides at least one of the new cells is also a stem cell. Secondly, they give rise to more specialised types of cell, which means they can become a stem cell, or another cell e.g. a brain cell. An embryonic stem cell is an unspecialised cell; they have the ability to become any cell in the body. Research is going into embryonic stem cells and their uses in tissue repair in adults. The work on embryonic stem cells first started in 1998 when James Thompson successfully removed cells from spare embryos at fertility clinics and established the first human embryonic stem cell line.

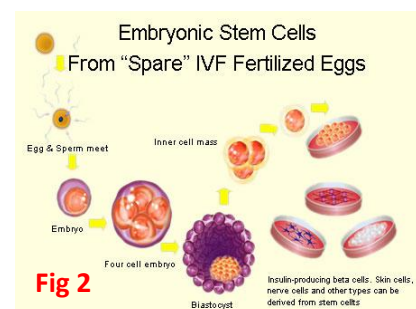
### **(II) Extraction of Stem Cells**

An embryonic stem cell is taken from a 'spare' embryo from IVF (In Vitro Fertilisation) which have been donated by the parents. The cells are taken when the embryos are 5 days old and the embryo is a blastocyst, which is a hollow, microscopic ball of cells. There is a layer of cells surrounding the blastocyst called the trophoblast and within the blastocyst there is a small mass of approximately 30 cells within blastocoels (see Fig 1 and 2). This small mass of cells is where the stem cells are taken from, which have the ability to become any type of specialised cell in the body, such as a brain cell, or a blood cell.

When stem cells are taken, they are cultivated in a culture dish in a research laboratory, containing a nutrient broth known as culture medium. The inner surface of this dish contains a layer of mouse embryonic stem cells which are treated so they won't divide by mitosis. This is called the feeder layer, which enables the dividing stem cells to stick. The cells are then left to divide with the feeder cells, which also release nutrients into the culture medium. The cell culture could last 6 months or more, and from the inner cell mass, there could be millions of undifferentiated embryonic stem cells which are used in several applications.



**Fig 1**



**Fig 2**

### **(III) Applications and Future Uses of Stem Cell Research**

Many believe that the use of embryonic stem cells is the future of medicine, as they can be used to potentially cure diseases, which at the moment are thought to be incurable such as spinal cord damage, heart disease and diabetes.

Currently, embryonic stem cells are being used in the treatment of cancer. The blood from the umbilical cord is one of the richest sources of stem cells in the body. Normally this blood is inaccessible because the cord is discarded immediately after birth. However it is becoming more and more popular for parents to keep or donate umbilical cords from a newborn child into a cord blood bank, in case these cells are required in the future. The cord blood stem cells can be used to treat many diseases such as sickle cell anaemia, leukaemia and Hunter syndrome. In treatment of leukaemia, stem cells are transplanted via a bone marrow transplant, which means the need for chemotherapy is abolished and the side effects are unlikely to be experienced. The stem cells grow to replace the damaged or destroyed white blood cells. However the disadvantage to this treatment is that large numbers of the stem cells are needed, otherwise the treatment would be ineffective. One in every 100,000 bone marrow cell is a stem cell which means large quantities of the stem cells are required. According to the BBC News website 'Doctors would prefer to be able to culture large numbers of blood stem cells in the lab before transplantation. But this has so far proved impossible - until now.'

The US Proceedings of the National Academy of Science from the University of Washington had tested stem cells from mice. The stem cells were cultivated in large numbers and kept alive for four months. These stem cells were then injected into mice, which no longer had stem cells, as they had previously been destroyed. These cells created a whole new blood system for the mice. Results found that the mice were coping perfectly well with the stem cells and were showing no signs of harmful effects. This is controversial as this experiment violated animal rights, but it produced good results and hopefully it would benefit to future research.

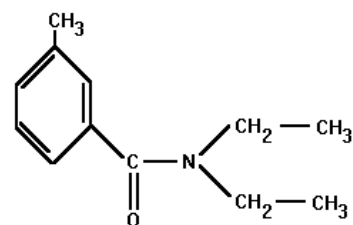
There are endless possibilities for stem cell research in the future such as treating spinal cord damage by using stem cells to repair tissue damage in the body, or using adult stem cells to partially restore sight lost resulting from retinal damage. Doctors had done this particular test to animals and they had found that sight had been partially restored. Perhaps with further research in the future, stem cells can completely restore eyesight.

### **Our Theory of Potential Treatment**

Domestic pets suffering from ticks, mites or mosquito bites when travelling abroad are the most common problems that vets come across everyday. These parasitic pests spread very quickly from animal to animal and it can possibly infect the owners too. These parasitic pests can lead to distressing situations, such as hair loss and several infectious diseases like malaria from mosquitoes.

#### **(I) Existing Treatment**

The current diagnosis for these parasites is to use different types of ticks and mites repellent sprays, such as Mycodex, or shampoo which are applied directly on the skin, however the method of applying these repellents can be very problematic for pet owners. In some of the insect repellents used, DEET (diethyl-meta-toluamide) is the active ingredient.



**DEET Molecular Structure**

DEET was artificially made by the United States Army and was put to use in military action in 1946 for insect infested areas. Studies such as one conducted by Katherine L. Margo (published on the Journal of Family Practice in October 2002), had shown the effectiveness of DEET by comparing various repellents containing different concentration of DEET and other substances like citronella. Results had shown that higher concentration of DEET repelled more mosquitoes, which concludes that 'DEET is the most effective mosquito

repellent.' The reason why DEET is so effective against mosquitoes is because the odour it produces is unpleasant for them and it's thought that DEET blocks the receptors for lactic acid and other substances which are produced from our bodies; thus the insects are unable to reach the host to feed. Although DEET has shown to be very effective, there are many side effects which follow after using this substance in repellents. One effect is skin irritation and cases of neuro-toxicity in cat and dogs, so applying it would probably make matters worse, perhaps having more serious effects than the original cause, i.e. the parasitic bite. So, another alternative insect repellent is in order.

Studies conducted by Mike Tyler and Craig Williams (2006), had shown that the certain types of Australian frogs, particularly the Australian Green Tree Frog, *Litoria caerulea*, secrete chemicals from their skin which naturally repel female mosquitoes, *C. annulirostris*. The gene for that secretion of chemicals are present in all of the cells, therefore we can extract these genes. This involves taking a sample of white blood cells from the frog as they contain a nucleus, whereas red blood cells' nucleus is absent. The next stage is to spin the blood sample at high speed which allows the blood cells to separate from the blood fluid. With the blood cells, a solution is mixed in which causes the cells to burst, so that their content are released. The content of that cell is spun again to separate the nuclei, which contains the majority of its genetic material. Using enzymes which deteriorate proteins, the DNA is released from the nucleus.

## **(II) Method**

### **(a) Gene Probe**

From the DNA that is extracted from the frog's white blood cell, we can study the DNA in more detail by using a gene probe, a radioactive single strand of DNA. The probe can be found by looking in into a 'gene library,' or we can artificially synthesise it. The DNA's double helix is 'unzipped' to make one single strand. This can be done by using a technique called gel electrophoresis, which involves mixing the DNA with agarose (jelly-like substance) and apply an electrical current through it. This 'sieves' the DNA into smaller fragments, as DNA is separated by size. Once we have the single stand of DNA, we can line them up to make a specific sequence of primary structures of proteins. This allows us to find the complementary base sequence to those primary structures of proteins. Once the base sequence is formed, we place these into a 'gene machine' which organises the DNA into the correct bases. Radioactive phosphorus is also added to allow the gene probe to be radioactive.

### **(b) Southern Blotting**

Once the radioactive gene probe is made, the DNA from the frog's nucleus is chopped up with a special protein called restriction enzyme. There are many different varieties of restriction enzymes which cut at specific sequences; therefore we can cut the DNA in sequences which are complementary to the gene probe. When the DNA is in smaller pieces, the technique, gel electrophoresis, is used again to separate the little DNA pieces into fragments by size. The staining appears to be smears down each line, which indicates the different fragments that range in sizes.

Next, the southern blotting technique is carried out. The stains are placed on to a sponge which acts as a transfer buffer. A nylon membrane is placed on top, but before that the agarose gel is treated on to the DNA smears to break the double strands into single strands. A pile of absorbent paper towels are placed with a heavy weight on top. This is left for a period time, and during that time the paper towel absorbs the buffer, which allows the DNA to be transferred on to the nylon membrane. The gene probe, which is also single

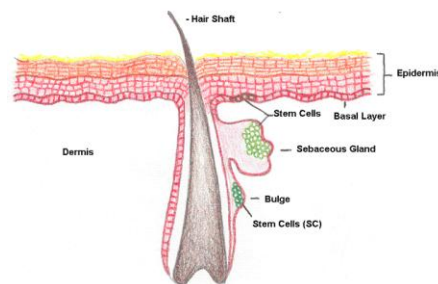
stranded, is then incubated onto the nylon membrane. Because the DNA and the probe is single stranded, and they have complementary bases to each other, the two strands are matched and joined together to produce a double helix. This process is called hybridization. After this process, the extra probes are washed away from the nylon membrane, so that only the double stranded DNA are left. Using an autoradiography, the radioactive gene probe can be detected and the location of the two single strands bound together can be seen.

### **(c) Generating DNA using Polymerase Chain Reaction (PCR)**

Polymerase chain reaction (PCR) is one of the processes used to amplify DNA, which only require a few known sequence bases. PCR relies on the natural ability of DNA to replicate involving mitosis and using a unique type of DNA polymerase. This enzyme copies a short section of the DNA, called primers and the bases. The sample of DNA then generates to make many copies of itself by mitosis. The reaction itself requires a heat-resistant type of polymerase enzyme. The contribution of Mullis and other colleagues developed the heat resistance properties of the enzymes because as from before, due to enzymes denaturing at hot temperatures, fresh enzymes had to added in intervals, which proved to be quite tedious. This is sufficient as the high temperature unzips the DNA into two single strands, which allows the polymerase to make more copies. Once the entire DNA has been generated, we can use the southern blotting procedure again to combine the 2 complementary based probes and the DNA. Also this time round, due to PCR the end result would be an increasing yield of DNA with the natural insect-repellent secretion gene.

### **(d) Inserting DNA in Hair Follicles**

Hair is a source of protection, warmth, camouflage and other advantages. A hair follicle is made up of 3 components, the dermal papillia, hair fibre and the root sheaths. The main adult stem cells are found in the bulge under the epidermis. Hair follicles are the ultimate source of cells that regenerate the skin and the growth of new hairs. There are 3 main parts which contain stem cells, bulge, sebaceous gland and in the basal layer of the epidermis. With the DNA that we have extracted from the Australian Green Tree frog, the DNA is inserted into stem cell which is found in the sebaceous gland. The modified stem cell would differentiate and generate glands that contain the mosquito repellent gene, thus allowing the animal to secrete natural insect-repellent similar to the Australian Green Tree frog.



### **Conclusion**

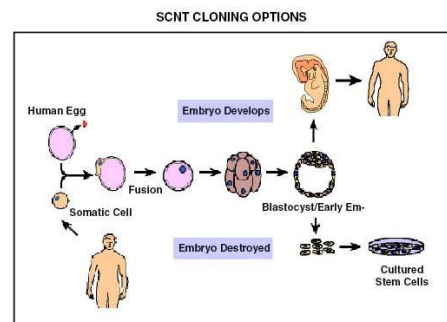
Using these methods, this process may be seen as highly ethical. There had been much bad press about the way stem cells are obtained and used for research projects. However, even though many disadvantages are of concern, there are also advantages which come along with it.

The conventional way of carrying out stem cell research is obtaining the stem cells from embryonic stem cells. This is because embryonic cells have the potential to differentiate into many different cells as they are more totipotent than adult stem cells. Conversely, adult stem cells have limited options e.g. the stem cells in the bone marrow can only differentiate into white or red blood cells. Our research, however, is based on adult

stem cells in hair follicles; therefore this eliminates a majority of concerns as we are not obtaining embryos for embryonic stem cells which would be used for research. However, if scientists are to conduct this theory practically, animal testing would potentially be involved, which may cause some ethical concerns. But, even more so, animal testing would be the only option to investigate whether the effects are successful or not as there are probably no alternatives to test this theory, apart from human volunteers. Even if human volunteers were used, our human sample would not represent our population, which are domestic animals whether the tests were successful or not.

### **(I) Ethical Issues**

A number of people believe that the uses of stem cells are not the way forward to conduct research. Some consider that embryos, which are used to obtain embryonic stem cells, have potential human life and if we take that away that from them, this is a form of murder. From a religious perspective, embryos never had a 'choice' as to whether they should live or die. Their 'human' right to life was thoughtlessly taken from them and they had no say in the matter. Therapeutic cloning can be used to extract stem cells which involve growing the embryo to be destroyed. The extracted stem cells would be cultivated into a new tissue, which is genetically identical to that of the adult (see fig 3). Because the embryo is destroyed during the process ethical issues arises as potential human life is wasted.



**Fig. 3**

Graphic courtesy National Institute of Health, U.S. Department of Health and Human Services (adapted).

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Others argue that stem cell research in the far future can lead to knowledge on how to clone humans, as therapeutic cloning was previously done on Dolly the Sheep. It is hard to say whether this is true, but we have seen devastating consequences of other research, even with good intentions, such as nuclear research. It can also be theoretically possible that human life can be formed from scratch, similar to the story of Frankenstein by Mary Shelley, but this would arouse many controversies. i.e. "We should not mess with human life." Fortunately for this thought experiment, we are using adult stem cells from hair follicles rather than embryonic stem cells. This would avoid ethical concerns as we are not losing potential life for this experiment and they do not require an embryo, which would be thrown away. Although moral principles are avoided, inserting the modified DNA in the stem cell may cause several other severe problems.

### **(II) Concerns with the Modified DNA**

From the research conducted by professor Mike Tyler and Dr Craig Williams, they had found they the secretion from the Australian Green Tree frog produced repulsive odour, stating that "The smell is just not very good... some smell of rotting flesh, some of nuts, some of thyme leaves." Inserting this gene into pets would cause much concerns with add possibilities of potential hair loss, skin irritation or perhaps affecting other components under the epidermis, such as gland cells in cats. Also with some specific breed of animals, these chemicals secreted may be toxic as they may not be tolerant to them. Excluding all those potential dangers, the use of problematic repellent sprays for pets are eliminated as the animal is already as natural insect secretion occurs which makes life easier for the owners.

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## **Images**

Extraction of stem cells from a blastocyst (**Fig 1**)

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Embryonic stem cells extracted from IVF fertilised eggs (**Fig 2**)

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Molecular structure of DEET

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Anatomical location of stem cells in hair follicles

<http://www.umdni.edu/gsbsnweb/stemcell/scofthemoth/EpSCsci.htm>

Therapeutic cloning diagram (**Fig 3**)

<http://www.mccl.org/view.image?id=459>