

**Stem Cell Treatments of  
Mitral Valve Disease  
in Cavalier King Charles Spaniels**

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**PASS WITH MERIT**

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Mitral Valve Disease is the most prevalent cause of death in Cavalier King Charles Spaniels. The causes of the disease and the problems associated with it are well known within the veterinary profession as it is the most common heart disorder in older dogs of all breeds. However, the occurrence and commencement is typically much earlier in the life of Cavaliers. The health of the heart is of vital importance to the health of the dog, and a healthy heart can not only prolong the length of the dog's life, but also increase the quality of it. Mitral Valve Disease can lessen the life span of the dog and also cause much pain and discomfort. However, using medical advances into the world of stem cells, there could be a foreseeable future of repairing and replacing damaged heart muscle, and potentially treating, or even curing, Mitral Valve Disease.

Mitral Valve Disease (MVD) is a polygenetic acquired heart disease. The disorder, according to *CavalierHealth.org*, “afflicts over half of all Cavalier King Charles Spaniels by age 5 years and nearly all Cavaliers by age 10 years” and the percentage of Cavaliers which develop MVD murmurs “increases at a rate of about 10% per year.” MVD is a degeneration and fibrosis of the heart's mitral valve. The mitral valve is designed to prevent backflow. The disease causes the valve to allow some blood to flow backwards through to the atrium from the ventricle, as it no longer totally closes after each pumping action.

As Mitral Valve Disease progresses, more blood seeps from the left ventricle through the mitral valve and into the left atrium of the heart. The atrium gradually begins to swell and expand. This diminishes the ability of the ventricle to provide the adequate amount of blood to meet the requirements of the rest of the body. The heart, therefore, has to pump more intensely and more rapidly to meet those demands. As there is an increasing lack of blood pumping through the body, non-critical blood vessels are shut down and the flow of blood to the kidneys and the skin is decreased to conserve blood flow for vital organs, e.g. the brain. The enlarged size of the heart fills the chest cavity and causes pressure on the main airway - the left main bronchus. As MVD is a degenerative disease, the heart muscle around the mitral valve is damaged and weakened and stem cells could be used to repair and regenerate the heart muscles.

"Current stem cell research has suggested virtually unlimited potential for the treatment and cure of many presently untreatable diseases and disorders. However, it is only recently that the full extent of their capabilities has been realised. Stem cells are unspecialised cells that can renew themselves for long periods of time through cell division and can give rise to specialised cells, including heart muscle cells, blood cells or nerve cells. They can be induced by certain factors and triggers to become cells with special functions, such as the insulin-producing cells of the pancreas. When the cells replicate many times over, it is called proliferation. If, like the parent stem cells, the resulting cells from proliferation continue to be unspecialised, the cells are said to be capable of long-term self-renewal. When unspecialised cells give rise to specialised cells, the process is called differentiation.

Differentiation of stem cells is triggered by signals inside and outside of the cell. The internal signals are controlled by the cell's genes, and the external signals include chemicals secreted by other cells, physical contact with neighbouring cells, and certain molecules in the microenvironment. As Dr

Oyama, a specialist in the stem cell area of science, has been quoted, “They’re really quite plastic, or flexible, in what they grew up to be. It’s all about their environment, nature versus nurture.” (1)

Found among differentiated cells in a tissue or an organ are undifferentiated cells called adult stem cells (or somatic cells) which can go on to develop into specialised cells. The number of cell types that a given adult stem cell can become is increased by the plasticity of stem cells. Adult stem cells exist primarily to maintain and repair the tissue in which they are found. The use of adult stem cells would mean that the patient’s own cells could potentially be expanded in culture and then reintroduced into the patient. The use of autologous cells is a significant advantage as the cells would not be rejected by the immune system, there will not be shortages of the cells or of donors, and there are no ethical dilemmas that are involved in embryonic stem cells or donors.

In late 2007, a reprogramming technique that can cause adult stem cells to have many of the same properties and much of the potential that embryonic stem cells have was proven in principle. (2) Previous research has shown that adult stem cells can be fused with cells from other parts of the body, but because the fused cells contain twice the number of chromosomes, the cells have to be pre-programmed to know how many chromosomes they have. This enables the cells to reduce their chromosome number should they have too many, preventing trouble in dividing and ensuring that replenishing damaged tissue is a relatively easier task.

There are currently still several limitations to using adult stem cells. Although many different kinds of the multipotent stem cells have been identified in nerve cells, blood cells, muscle cells, skin cells, bone cells, etc., they are often present in only minute quantities, and hence can be very difficult to extract, isolate and purify. Also, adult stem cells may contain more DNA abnormalities, caused by toxins, sunlight, and errors in making more DNA copies during the course of a lifetime. However, adult stem cells already have a record of relatively effective and safe therapy, especially when cells from the patients themselves are used.

Perhaps the best-known stem cell therapy used to date is the bone marrow transplant which is used to treat leukaemia and other types of cancer, as well as various blood disorders. Usually, before any transplantation of the bone marrow takes place, the patient receives high doses of chemotherapy and/or radiation to eliminate any diseased bone marrow and to make room for the new marrow. The new stem cells are implanted into the patient by means of a blood transfusion. The stem cells then navigate their way to the bone and start to divide and multiply to produce more cells. Cells from bone and skeletal muscles have very good regenerative properties, and bone marrow stem cells can differentiate into nervous tissue, bone cells or even heart muscle.

Adult stem cells might provide medical solutions that avoid the ethical and legal problems of embryonic stem cell use.

In November 1998, James Thomson (US) successfully removed stem cells from spare embryos at a fertility clinic. Since its beginnings, embryonic stem cell research has been very controversial. As the stem cells are derived from embryos, there was, and still is, much ethical debate as to whether it is morally acceptable to use embryos in the centre of science. However, the stem cells from embryos are

totipotent and can develop into any type of cell, e.g. blood cell, heart cell, brain cell, etc. They can also divide without a limit to produce more stem cells. This discovery was a massive breakthrough in science in comparison to the adult stem cells found some twenty years previously, which originally appeared to be much less flexible and very limited in what they could develop into.

Using the findings of current research, treating and possibly curing MVD could be the future of stem cell medicine. As has been researched, there are two potential strategies – harvesting and growing new cells to implant, or the augmentation of what is already there. If the decision is made to harvest and grow new cells to implant into the patient, the main assessment is which type of stem cell to use. In the past, embryonic stem cells were thought of as the more promising type of stem cell to use as they were seen to be more flexible in their uses. However, there have always been issues with the use of embryos for harvesting the cells.

Embryonic stem cell research has become one of the biggest debates dividing the scientific and religious communities around the world. At the centre of the issue is one key question: When does life begin? To acquire stem cells, scientists either have to use an embryo that has already been conceived, or else clone an embryo using a cell from a patient's body and a donated egg. Either way, to harvest an embryo's stem cells, scientists must destroy it. Although that embryo may only contain four or five cells, some religious leaders say that destroying it is the equivalent of taking human life. Many governments have placed tight restrictions on stem cell research or have tightly limited funding for it. To calm the debate, scientists are exploring less controversial avenues of research, using adult stem cells that have been modified to act like embryonic stem cells, instead of creating a new embryo.

Scientists in the UK are only allowed to use human embryos for a restricted range of research, specified by Parliament. In comparison, Barack Obama has been a long-term supporter of greater stem cell research. His decision on 9th March 2009 to “vigorously support” new research opposed the views of former President George Bush, who was particularly critical of stem cell research. Obama introduced legislation that specifically permitted embryonic stem cell research in Illinois. This decision has been met with appreciation from stem cell researchers, but also with criticism from opponents and social conservatives. (3)

On the basis of current research, either embryonic or adult stem cells could potentially be used for the treatment of Mitral Valve Disease. However, as adult stem cells have very few, if any, ethical issues surrounding them, they would be the ideal type of stem cell to use - particularly if the autologous cells were used. As recent research has suggested that cells originating outside of the heart could be involved in its repair, there seems to be great promise in the future of regenerating damaged heart tissue. In male recipients of heart transplants from female donors, biopsies have shown the presence of male cells in the cardiomyocytes of the female heart, indicating that these cells originated outside of the heart and from the recipient's body. (4) The bone marrow is a likely source of such cells, raising the possibility that bone marrow transplantation could be used to supply stem cells for the diseased heart affected by Mitral Valve Disease.

Bone marrow contains two kinds of stem cells: hematopoietic and stromal. Hematopoietic stem cells form all the types of blood cells in the body, whereas the stromal cells are a mixed cell population that generates bone, cartilage, fat, and fibrous connective tissue. The cells can be stimulated by specific factors so that they develop into certain cells. By extracting the stem cells from the patient, subjecting the cells to hormones which trigger differentiation into the required type of cell and then re-implanting the cells into the patient, the stem cells will begin to divide and multiply in the targeted region to repair and regenerate the damaged area.

Current areas of investigation are the triggers for cell differentiation and increasing the numbers of stem cells. Researchers have found that giving mice a hormone known for building bones increased their production of blood stem cells. Some of the mice received injections of parathyroid hormone while others, a control group, did not. All of the mice that received the treatment with the parathyroid hormone survived. Only 27 percent of the control group survived. The hormone involved in the study is currently approved for use in humans as a treatment for osteoporosis. This existing approval allows researchers to skip the extensive safety trials needed when new drugs are used in humans and move straight into testing for effectiveness. (5)

Scientists have demonstrated that they can grow human stem cells in culture by blocking an enzyme, aldehyde dehydrogenase (ALDH), which naturally triggers stem cells to mature and differentiate, increasing the number of stem cells by 3.4 times. This discovery may enable scientists to grow stem cells more rapidly for transplantation into patients. More recently, a newly found and tested stimulating factor for stem cells is hyperbaric oxygen treatment (HBOT). This involves breathing pure oxygen to higher than atmospheric pressure in an enclosed chamber. The results of the use of this treatment showed eight times the number of stem cells circulating in the patients' bodies. (6) The increase in oxygen causes the body to create more red blood cells to cope with the abundance of oxygen. More red blood cells mean that there are more hematopoietic stem cells distributed throughout the body.

The foundations of the research into stem cell treatments for Mitral Valve Disease could also be used to aid treatment for other diseases and disorders, for example, lameness in livestock, arthritis in Golden Retrievers and retinal dysplasia in Labrador Retrievers. Retinal dysplasia is an eye disease affecting the retina of animals, and more rarely, humans. However, Labradors are particularly susceptible to the disorder. It is a non-progressive disease caused by genetic defects, and animals are born with as severe a condition as they will ever get, although it can also be caused by viral infections or drugs.

Retinal dysplasia is a type of retinal malformation. The word "dysplasia" means "a defective development of an organ or structure". The disease occurs when the two layers of the retina do not form together properly. Mild dysplasia shows as folds in the inner retinal layer, called retinal folds. In severe forms of dysplasia, the two retinal layers do not come together at all, and retinal detachment occurs. In Labradors, a combination of retinal dysplasia and skeletal defects has been described. This condition is known as oculoskeletal dysplasia. Retinal folds rarely cause vision problems for the individual dog. They represent small blind spots which are probably not even noticed by the dog. However, large amounts of

dysplasia (geographic dysplasia) may lead to large deficits in the visual field, and dogs with retinal detachments (severe dysplasia) are completely blind.

The knowledge of how stem cells can help treat MVD could be applied to the treatment of retinal dysplasia. As the disease is caused by the retinal layers not forming together properly, it could be possible that stem cells may be able to differentiate to produce retinal stem cells which would repair and regenerate the missing cells between the two layers. This treatment has two possible outcomes. Either the layers come together and the retinal dysplasia is completely treated, or the layers come partly together and the blind spots affecting the vision of the dog are reduced.

Lameness in livestock can cause the end of a horse's competitive life, the end of a cow's working life, or the end of a sheep's life. Tendon, ligament and cartilage injuries can range from minor inflammations to complete ruptures, which can result in permanent lameness. The risk of re-injury after damaging a tendon or ligament is very high. However, stem cells have the potential capability to overturn this damage. Stem cells taken from the animal's bone marrow can be differentiated in culture to develop into tendon, ligament, cartilage, or bone cells. These cells can be transplanted into the animal to replace the damaged tissue, thereby treating lameness. This revolution in livestock medicine could save livestock owners everywhere millions of pounds that are presently spent on attempting to ensure that their animals are in their best shape.

As Mitral Valve Disease in Cavalier King Charles Spaniels is a degenerative disease, it could be possible to use stem cells to regenerate the affected mitral valve in the heart. From the research previously conducted, it is shown that bone marrow stromal cells can be differentiated and specialised under specific conditions into different types of cell, and can therefore be developed into cardiac muscle. These cells have been induced by external factors, such as introduction to hormones, and will become cells with the specific function of beating cells in the heart. The stem cells will divide and multiply to produce more cardiac muscle and therefore regenerate and repair the mitral valve. As the muscle is repairing, it is suggested in other reports that more stem cells from the bone marrow will naturally migrate to the heart to aid the process of reparation. In spite of this, Mitral Valve Disease is polygenetic, hence stem cells will not be able to prevent the disease from affecting the dogs, as only DNA modification can stop the passing on of the alleles which cause the disease. Stem cells will, however, enable the treatment of the disorder and therefore prevent any further suffering and discomfort to the Cavalier King Charles Spaniels.

Hypothetically, it is potentially possible to take stem cells from any body tissue and, under suitable conditions, induce them to become specialised cells which can repair and regenerate damaged muscle. New data suggests that stem cells may exist in the heart and can repair damage, preventing scar tissue from permanently replacing the functioning heart muscle. (7) By injecting bone marrow stromal cells into the diseased heart, there is hope in the future that the stem cells, with the right stimulus, could potentially regenerate the heart muscle.

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