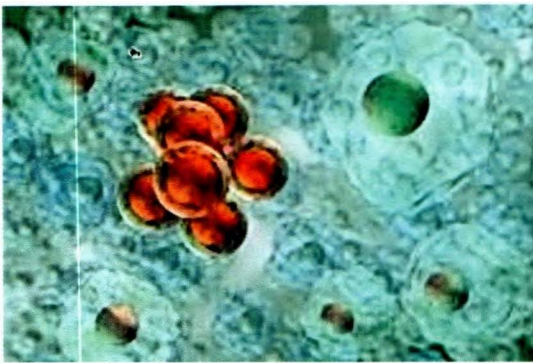


*This article was written and dictated by my esteemed colleague, Dr. Michael Brown, M.D. DC. Dr. Brown was a wonderful teacher and superb doctor who passed away too young. The information dictated by Dr. Brown still remains pertinent today and I corrected spelling and grammar to make it more easily readable and I find it useful to provide to my patients. I hope you like it.*

*Charles F Mahl M.D.*



# Introduction to Basic STEM CELL Therapies in Orthopedic Medicine

## **SVF (Stromal Vascular Fraction), Bone Marrow Stomal Cell (BMSC) & Other Stem Cell Technologies: An historical review of these techniques utilized in orthopedic medicine.**

### **INTRODUCTION:**

The concept of stem cell use in the practice of medicine has captured the attention of both the public and physicians throughout the world over the last 2 decades. Most people who think of stem cell therapy think of tissue engineering of new organs, new body parts, and the possible concept of life extension. Embryonic stem cell research sparked this initial "craze". Interest was sufficient enough for substantial funding for stem cell research. For example, in California 100's of millions of dollars was set aside to progress this field. When those funds became available almost all of funding was used to fund basic science research. Very little of the money was given to physicians/clinicians for actual clinical research to help patients with real disease! Many of the stem cell technologies that are receiving the most funding are actually not the most "hopeful" and can involve induction of a stem cell state in adult cells that have risks of tumor formation for example which will restrict its use for years before it will be a viable treatment option.

While basic scientists have continued to work on the laboratory research utilizing stem cells, physicians and surgeons have been advancing the field of clinical application. The "baby boomer" population is aging and demanding new methods to restore function and improve pain. As a regenerative medicine specialist in the field of physical medicine & rehabilitation / pain medicine, with a focused attention in musculoskeletal diseases and orthopedics, I have been working with regenerative technology for my entire career. With my interest in helping musculoskeletal diseases and orthopedic degenerative diseases and injury, I first turned to the use of prolotherapy. Later I began to use cellular therapies such as using platelets (Platelet Rich Plasma, platelet lysates, etc.) to induce healing. Over the last decade, physicians and surgeons have been pioneering orthopedics with the use of various stem cell technologies. Most importantly has been the use of autologous

stem cell techniques, which means utilizing cells obtained from your own body. By doing so, there is a genetic match and no risk of communicable disease. In addition, autologous stem cells do not have the same risk for certain types of tumor formation that is often associated with embryonic stem cell technologies.

Although we will cover both bone marrow and adipose tissue (fat) derived stem cell as a means of education we have abandoned the use of adipose tissue derived stem cell techniques for the most part within our practice which will be described later. Our initial exploration of stem cell therapies, began with our utilization of bone marrow blood as the source of cells. This process involves the aspiration of bone marrow blood and specific laboratory processing to isolate a specific stem cell note as “mesenchymal stem cells”. We utilized the stem cell procedures to improve healing of orthopedic and spine conditions.

Bone marrow was one of the first areas of interest to extract and use stem cells which was done as early as 1963.<sup>1</sup> We have personally used autologous stem cells from bone marrow to enhance spinal surgical procedures several years ago with astounding success. Later we used these same cells to enhance healing of soft tissues and musculoskeletal injury and degenerative conditions. The next trend in stem cell techniques involve the use of adipose tissue as a source of stem cell. The discipline of plastic surgery had been advancing the field of using "adipose tissue" or fat from the body for cosmetic surgery for decades. It is through their pioneering efforts and continual advancements in this field that SVF (stromal vascular fraction) techniques were developed. At the time this advanced stem cell therapies beyond the use of bone marrow. Another discipline advanced the use of adipose tissue derived stem cell injections to improve healing the ligaments, tendons and arthritic joints were veterinarians!<sup>2-3</sup> We begin utilizing adipose tissue derived stem cell early on and enjoyed a great deal of success using these techniques. We continue to use these techniques until 2014 when we abandoned the use of adipose tissue as the source of stem cell. We did not abandon these techniques because they did not work but rather abandoned them until which time FDA regulatory issues can be worked out within the US. Fortunately, we believe we have a more effective technology that remains compliant with FDA regulations. We use adipose cells as a source of fat grafting to give us scaffolds to hold stem cells and promote regeneration.

#### THE TAKE-HOME MESSAGE:

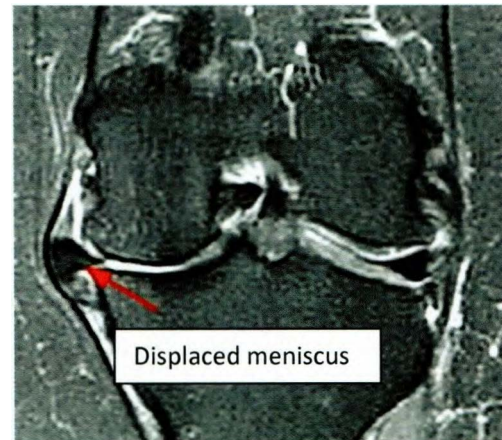
I am going to present the take home message of this article now rather than place it at the end of the article. The reason for this is many individuals interested in regenerative medicine just want to know the big picture and have little interest in the science. For those of you who have more interest in the science of regenerative medicine I have included a lot of the science behind some of the regenerative medicine techniques currently being utilized or utilized in the most recent past.

The take-home message is this ... **we have moved beyond stem cell therapy!** There is a misconception in the public about stem cells. Many individuals in the public have traveled many miles and to many countries paying huge amounts of money for the hopes of a cure for their disease. Many of these patients have been taken advantage of because they do not understand the science of stem cell therapy. The patient with an arthritic knee joint for example may have the misconception that if we placed stem cells inside the joint we are going to somehow magically restore them back to normal and grow all their cartilage back. Such is not the case. Yes, there may be some demonstration of articular cartilage regeneration utilizing current stem cell technologies. And yes, an individual with significant arthritis and advanced degenerative joint disease may experience improvement of symptoms



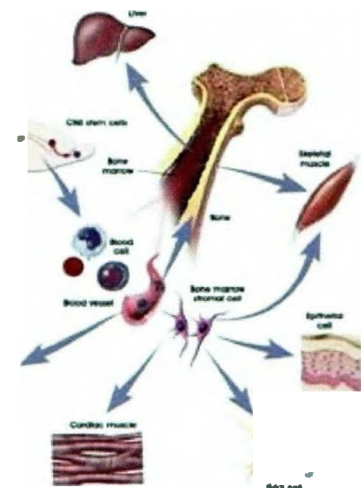
following these injections, but it is important to realize that the relief of pain and improved function may not be just simply because there was a regeneration of cartilage. Such is typically not the case.

For example, the individual on the right was someone who had significant knee pain secondary to a torn meniscus and advanced degenerative joint disease. The red arrow points to the meniscus that has extruded out from the joint. An individual with such a condition may undergo various types of regenerative injection techniques and even stem cell therapy and could feel some improvement. But the likelihood of regenerating the cartilage and especially doing anything for the misaligned meniscus is highly unlikely. The reason why these individuals may experience some improvement is that the regenerative injection procedures have the ability to take over control of the inflammatory and biochemical environment of the knee joint. By changing inflammation in the expression of inflammation the patient feels much better.



In addition to injecting the cells into the joint to hopefully create some regenerative changes we also dramatically affect the inflammatory response which can last for a period of months or years. Physicians who are highly trained in regenerative therapy do not only rely on joint injections but also we use diagnostic imaging such as ultrasonography and other techniques to direct injections into the ligaments, tendons and structures around the joint as well to further support the regenerative process. For example many patients with arthritic knees especially those with had previous surgeries on their cartilage may have a misaligned meniscus. This misaligned or subluxated meniscus is part of the problem. Some skilled regenerative medicine physicians perform more advanced procedures such as injecting fat tissue and fat grafts with stem cell behind or around the misaligned meniscus. The concept is to try to stabilize the meniscus, provide a connective tissue buttress and place regenerative cells near the perimeter of the meniscus. We have used this technique on multiple patients with this problem. Often, the reason why they may experience improvement is because of the effect of regenerative cells on the chemical environment of the knee as described above.

So before you consider a stem cell therapy for a joint injury, tendon injury, soft tissue injury or degenerative joint condition it is important that you understand both the potential for benefit as well as the limitations of the procedure that you are committing to. In our practice, we set aside significant time to consult and evaluate our patients and the specific needs and expectations of the proposed treatment and procedures. Every individual presents with a unique case that needs to be taken into consideration. We will spend the time to carefully analyze the problem, obtain a precision diagnosis and discuss potential regenerative medicine procedures that may have a definitive treatment for your condition. We have learned only by the painful process of experience that stem cells alone are often inadequate and that we must combine the use of multiple advanced regenerative procedures that could involve growth factors, extracellular matrix and scaffolding, and other techniques to provide a successful outcome.



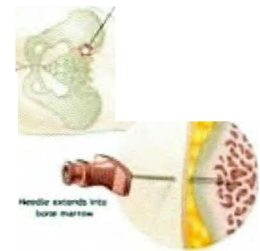
## THE CONCEPT OF AUTOLOGOUS TISSUES IN REGENERATIVE MEDICINE:

Before we begin this important discussion I want you to understand that when we use cells and tissues rich in growth factors and stem cells these are “your” cells. The term “autologous” means that we obtain the tissues and cells from your body and transplant them to other locations to exert their effect. This means they are an exact DNA match. More importantly when using popper closed techniques which we will describe later there is no way of contracting communicable disease such as HIV, Hepatitis B or C because it is your blood with no contact with another patient’s blood.

## BONE MARROW AS A SOURCE OF REGENERATIVE POTENTIAL:

Bone marrow contains many cellular elements and is the manufacturing plant for your blood which includes red blood cells, platelets and white blood cells.

These blood cells and the method in which they replicate themselves in the bone marrow is a highly complex system which we will not cover in this article. Bone marrow also contains mesenchymal stem cells which provide a means of becoming other types of cells which we call a multi-potent cell. The cells can become osteoblasts (that form bone), chondrocytes (that form cartilage), myelocytes (that form muscles) and other cells when they are placed in the environment in which the cells exist. Apart from this there are also endothelial stem cells within bone marrow that can form blood vessels which we utilize in certain vascular diseases today as a means of regenerative therapy.



Many patients become concerned about the potential pain of tapping the bone marrow and extracting or aspirating bone marrow blood. They may have heard of patients with cancer undergoing bone marrow biopsies having a painful experience. Such does not have to be the case. We use several methods to make this experience as painless as possible.



The first is a specific local anesthetic that has the ability to anesthetize the periosteum of bone which is the connective tissue covering the bone. Because bone marrow inside the bone is richly innervated by nerve endings sensitive to pain most of the discomfort affiliated with bone marrow aspiration of blood actually comes from simply drawing blood out of the marrow and the stimulation of these pain fibers. Therefore, another simple trick that we use is to inject a local anesthetic (ropivacaine) in the bone marrow to anesthetize these fibers before drawing the blood. This makes for a much more comfortable experience. Rather than using standard techniques used in the past for bone marrow aspiration we now use a small drill (seen to the right) with a very special tipped needle. This is designed to access the bone marrow with a smaller needle than typically used. This drill makes access of the bone marrow much easier and less painful.

There are numerous laboratory methods now available to process bone marrow blood to access the mesenchymal stem cell population that exists in bone marrow blood. It turns out that the mesenchymal stem cells obtained from bone marrow have inherent chondrogenic (cartilage forming) properties which may be ideally suited for therapeutic use in cartilage regeneration.<sup>4-5</sup>



### STEM CELLS FROM A BOTTLE:

There are a number of companies, who have been able to accomplish FDA approval for various stem cell products. Regardless of how they obtained FDA approval they claim when approaching physicians to be a stem cell product in a vial. These types of stem cells are called allogeneic stem cells which means they are obtained from a donor. Autologous means when cells are obtained from your body. There is a trend where many physicians “jumping in” to the stem cell business is advertising stem cell therapy, only charging a patient \$7000-\$8000 for simple injection of the stem cells obtained from a vial. The truth of the matter is that although there are multiple growth factors that may be contained within some of the tissues obtained from the bottle our institution basic scientists have not been able to culture any stem cells from these products. We have found no “colony forming units” (CFUs) obtained from these products and therefore we personally consider them a dead vial of cells. These injections do elicit some beneficial effects which I have been exposed to personally but we want the consumer to know that they should be aware of what they are really getting in these products. We find the product to be extremely expensive to purchase and the therapeutic value too variable. And most of all, our institution does not believe the value of this product is from actual stem cell but probably from growth factors which we can find alternatives for that are much less expensive.

### FAT AS A SOURCE OF REGENERATION POTENTIAL:

Adipose Tissue Complex (ATC) is the fat cells, stroma cells (connective tissue and supportive cells), blood vessels and all the cells and tissues found in fat. These cells and tissues are involved in energy homostasis, acting as a large scale endocrine organ capable of secreting many regulatory proteins and hormone ( cytokines, chemokines, adipokines) which regulate many functions in your body which include immunity, metabolism, reproduction, and inflammation. Although a simple fat cell seen to the right may make up the majority of what you call fat there are a host of other cells in your fat as well. Zuk, and his colleagues in 2001 and 2002 were the first to identify the extent and nature of “undifferentiated nucleated cell populations found in fat.”<sup>6-7</sup>



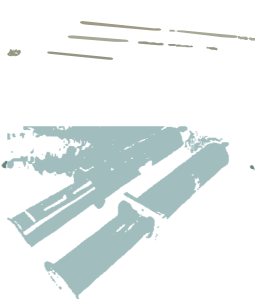
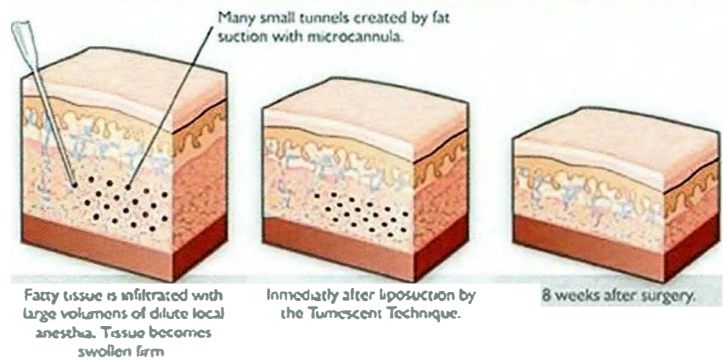
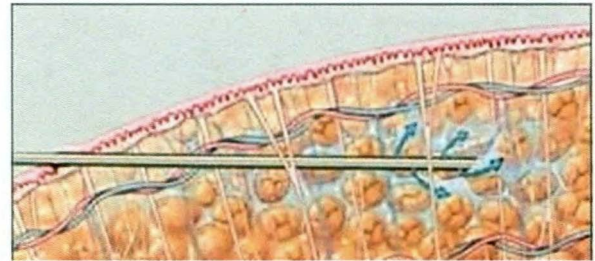
Yes, your fat or adipose tissue is rich in stem cells! It is rich in growth factors and a host of proteins and substances that can be used therapeutically. These cells have the same lineage and thus the same genetic “program” as bone, muscle, ligament and cartilage. Thus, we refer to them as “mesenchymal stem cells”. With a rather simple aspiration of fat cells and the other surrounding tissues also found in fat, we can gain access to these stem cells and growth factors. In fact vast quantities of stem cells can be extracted this way. These discoveries sparked an extensive clinical and research effort to provide us with an understanding of the autologous fat grafting (AFG) techniques that we use today. It is not the fat cells we are interested in. It is these other cells found with them that is our focus. Interesting enough

normal fat cells will die when transplanted but that is exactly what we want. The death of the fat cell stimulates its own stem cells and progenitor cells to become even more active!

## METHODS OF LIPOASPIRATION TO OBTAIN AUTOLOGOUS FAT FOR STEM CELL THERAPY:

The method of obtaining autologous fat graft for purpose of making the stromal vascular fraction used for stem cell therapy is rather simple. One could consider this a micro-liposuction. It is obtained from the fat layer below your skin. Local anesthetic is initially injected under skin for purpose of numbing the skin and then a needle is gently inserted into the

fat layer and local anesthetic and normal saline is infiltrated into the fat. This mixes fluid and fat together in a process called tumescent liposuction. In this process you are floating fluid and fat together so that the fat cells and other supportive tissues are loosened up so that they can be aspirated into a special syringe.



By floating the fat cells and tissues, local anesthetic and normal saline it provides a means of being able to poke small holes into the tissue with a specialized cannula. The fat cells and supportive tissues then can be aspirated into the specialized cannula that is attached to a syringe. As shown in the picture to the left, the specialized cannula does not have to be that large. Remember, this is not liposuction! In the process of liposuction large quantities of fat are aspirated from your body in order to contour your body by plastic surgeons. This is a process where we take small quantities of fat. Because the fat tissue is suspended in fluid it is much easier to poke small

holes into the fat and aspirate it. After the procedure the fluid then is reabsorbed and 8 weeks later all the fat is filled back in and the tissue returns to normal as shown to the right.

The process is done under a local anesthetic. You can experience some soreness in the region where the aspiration took place. Most individuals will state that it is a mild soreness that goes away within a few days. Although the procedure can also be done under conscious sedation at our surgery center this is typically not necessary.

## WHY DO PEOPLE FLY TO FOREIGN COUNTRIES FOR STEM CELL THERAPIES RATHER THAN REMAINING IN THE US?



Currently, the FDA in the United States has placed significant restrictions on physicians utilizing this technology. These regulations are being questioned and the amount of control over medical companies, hospitals and physicians the FDA will have in the future and the future regulations that will govern this new era of medicine is being decided in the courts in the next few years. There has been a “great debate” over whether or not the manner in which we process fat to obtain stem cells falls within the “minimal manipulation” rule of cells that are used for stem cell transplant procedures. In 2006 the FDA made a single word change in the regulation the changes their ability to regulate stem cell procedures that were previously considered a surgical transplant and thus the practice of medicine. The FDA made to change of one word. The changed “another” to “a” person. That means cells transferred from an individual to another individual was previously regulated such as blood banking where blood transfusions are given to cells transferred from a patient to the same patient. That means that in the future we may need to literally license your cells in your own body as a drug! This debate lead us to eventually discontinue the use of adipose derived stem cell therapy.

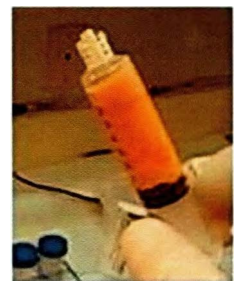


Because physicians in the US are unable to take your stem cells and culture them and expand their numbers into the 100s of millions or billions as can be accomplished with culture and expansion it has lead to some individuals seeking care outside the US borders and thus participating in “medical tourism.” That is US citizens are traveling abroad to stem cell facilities where they can have both bone marrow blood and fat extracted and expanded to improved stem cell numbers. Many of the outcomes described in the literature utilizing bone marrow and fat are actually studies carried out utilizing expanded cells and therefore there may be some issues comparing data from simple autologous transplant to expanded cell transplants.

Despite the fact that expanded cell practices can be available in other countries many individuals who travel abroad for stem cell therapies are actually individuals who are being taken great advantage of. Most of these facilities are not utilizing expanded cells. Most of these individuals are being charged huge prices to take advantage of US citizen spending capacity. Therefore, we asked those considering medical tourism to receive stem cell therapy to be very careful about their decisions and who they are selecting to perform these procedures.

## UNDERSTANDING ADIPOSE-DERIVED STROMAL VASCULAR FRACTION (AD-SVF):

Our first introduction to these techniques were from plastic surgeons working overseas that were developing methods to improve the fat graft survival utilized in cosmetic surgery to treat wrinkles in the face. They discovered that adipose tissue or fat injected subcutaneously under wrinkles survive better if they were combined with stem cell. These same stem cells were later determined to improve healing and also noted to induce a number of autoimmune regulation functions that became affective and treatment and various disease states.



### **Why did AD-SVF become so popular?**

The stromal vascular fraction taken from adipose tissues from one area of your body and transplanting them into another area represents a simple surgical procedure. Something again that plastic surgeons have been doing for years. In an arthritic joint for example a knee joint chronic inflammation dramatically affects and induces your own stem cells in the area from being able to proliferate and heal. Chronic inflammation causes a poor environment for cartilage to replicate and repair. By pre-treating the joint and reducing inflammation and then transplanting non-manipulated adipose tissue cell concentrates coupled with high concentrations of platelet derived growth factors and signaling proteins the placement of the cells and growth factors within an arthritic joint changes the environment of the joint and regulates the expression of chronic inflammation. It is not simply a matter of regenerating cartilage but rather a change in chemical environment that produces the clinical effect experienced by patients. We will address this issue again shortly.



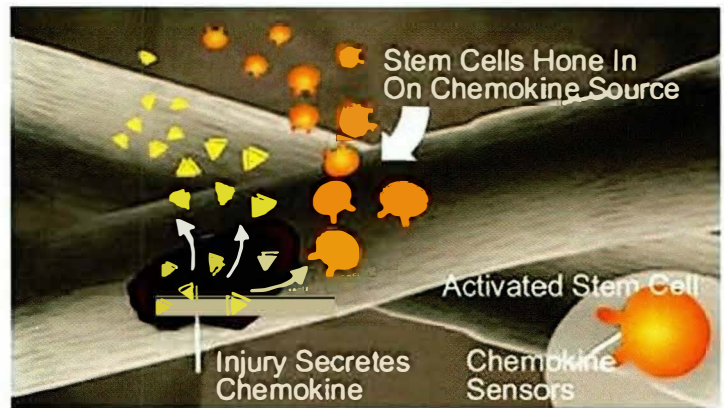
An aspiration of fat contains more cells than just fat tissue but also numerous other cells which include connective tissue cells, blood vessels, and the vast number of other nucleated cells that have regulatory function in fat and in your body. You must remember that fat acts like an endocrine gland and has regulatory function. Because adipose tissue is a combination of different tissues and cells we call the aspiration of fat tissue “adipose tissue complex” (ATC). Adipose tissue complex is involved in energy regulation as well as the secretion of a number of regulatory proteins called cytokines, chemokines, and adipokines which are involved in many tissue functions including inflammation, immunity, metabolism and reproduction of cells. Plastic surgeons began to utilize platelet concentrates obtained from the same patient’s blood and the growth factors contained in platelets to enhance tissue growth and tissue grafting success.<sup>8-10</sup>

When fat cells are transplanted they actually die off slowly and begin to secrete important proteins that stimulate progenitor cells to action and may be part of the success of stem cell therapies used in orthopedic application. Due to the high concentrations of mesenchymal stem cells found in the area of fat and its supportive tissues compared to bone marrow many researchers have begun to intensively examine the potential of fat that is a less invasive and readily available resource for stem cell therapies. In addition to the regulatory cells and stem cells within adipose tissue complex fat also provides a three-dimensional biological scaffolding that can encourage adhesion of cells to fill gaps in tissues that are torn or injured.

For example we often inject the connective tissue and fat cells within a torn section of tendon as a means of a tissue filler while the stem cells stimulate regeneration to fill in the gap. The cells can be deposited within tissue defects under ultrasound guidance.<sup>12-13</sup>

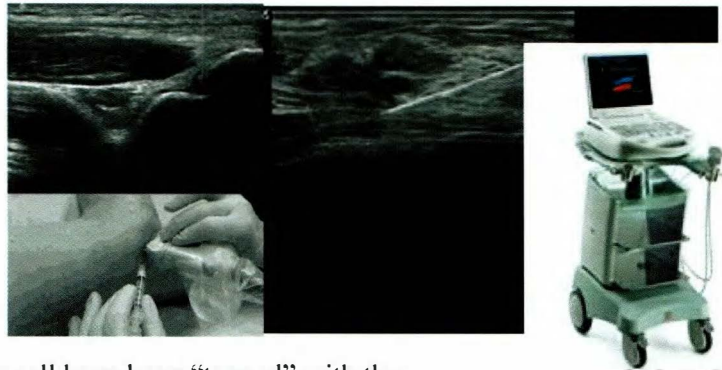


Fat and its supportive tissues also contain T-regulatory cells that may also promote regenerative properties as well as promote tolerance of the tissue transplantation. And may represent part of the reason stem cells from fat are becoming successful in numerous applications including heart disease, diabetes, and even liver failure. In addition the adipose derived or fat derived stromal vascular fraction offers important regulatory cells that alter inflammation which is part of the reason they work so well with arthritic and degenerative diseases.<sup>15</sup>



### HOW DOES A STEM CELL KNOW WHERE TO GO?

One of the most fascinating parts of moderate stem cell therapy is our understanding of cell “homing”. Activated stem cells express certain receptors on the surface of the cell that are sensitive to chemicals secreted by inflamed and injured tissues. These chemicals are called chemokines. There are special small proteins that are secreted in the area and circulate around your body that tell a stem cell where to go.

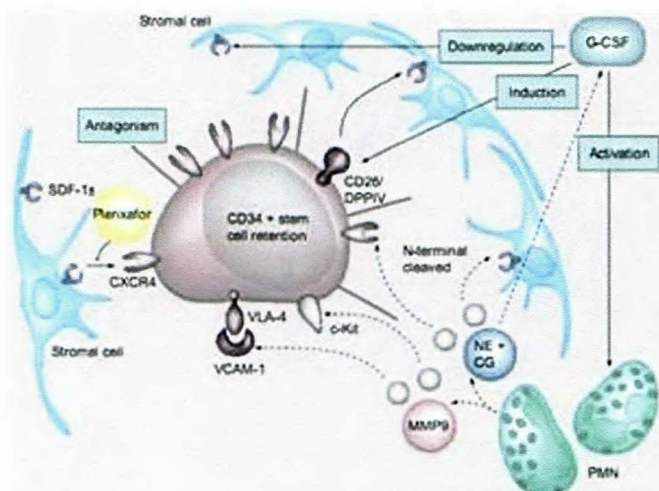


Studies have been done where the stem cell have been “tagged” with the radioisotope so that it could be tracked like a homing beacon. When stem cells are injected via IV one can see that they accumulate in the area of a focal inflammation. This is the process we call “homing”. Typically stem cells are injected and at the focal site of local injury but can also be given IV.

In our practice because we focused more on orthopedic conditions we use ultrasonography to place stem cells in a precise location of tissue injury.

### THE BIOCHEMISTRY OF STEM CELL CONTROL OF INFLAMMATION AND HEALING:

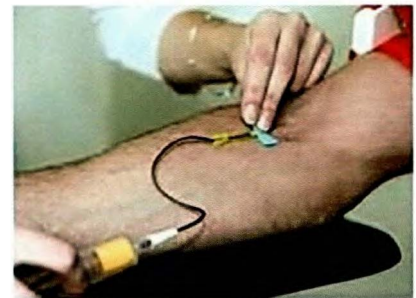
The biochemical and complex regulation of cell to cell signaling that transpires when stem cells, platelets, growth factors, and other substances are injected with stem cells is a vast subject. We can only cover a small amount of the science and biochemistry involved. Scientist are still sorting out this complex process.



Where there is tissue injury, joint or soft tissue inflammation there is expression of specific pro-inflammatory cytokines and other substances that perpetuate the inflammation and pain. As I have stated earlier the manner in which modern autologous or adult derived stem cell therapies work is not just by rebuilding tissues but by controlling the inflammatory environment. For example adipose derived or fat derived stromal cells taken from your body express a receptor for CD34. This is a binding site for certain chemical signals. Initially the expression of CD34 by these stem cells was thought to be a method by which they differentiate and replace damage tissues. Researchers now believe that there are countless signals by a host of different substances secreted that signal the repair by these cells. These are altered or enhanced by growth factors such as VEGF, HGF, IGF-1, and FGF-2.

Because growth factors have such an important role in stimulating stem cells to do their job we obtain a sample of your blood before the procedure and separate your platelets out of your blood to extract growth factors to enhance the ability of your own stem cells to do their job at a local site where we injected them.

Traktuev and his colleagues investigating stem cell biochemistry research reported an active and robust mitogenic response when these cells were exposed to basic-FGF, EGF, PDGF-bb. These growth factors are found around the blood vessels and in the fat we extract from your body. When we add a high density platelet rich plasma that we manufacture from your blood it provides a means of providing these cell growth factors and cell stimulating to promote healing and replacement of damage tissues when the cells are transplanted. This is exactly why we prepare a number of platelet preparations from your own blood at the same time we do these injections. We have covered the subject of PRP and how I use PRP to induce regeneration and tissue healing in previous articles on this website. The same PRP and other processes whereby we extract the growth factors from the platelets we obtained from your blood are used in modern stem cell therapies to improve the healing potential of the cells with transplant into joints and soft tissues.



#### STROMAL VASCULAR FRACTION & STEM CELLS CONTROL INFLAMMATION:

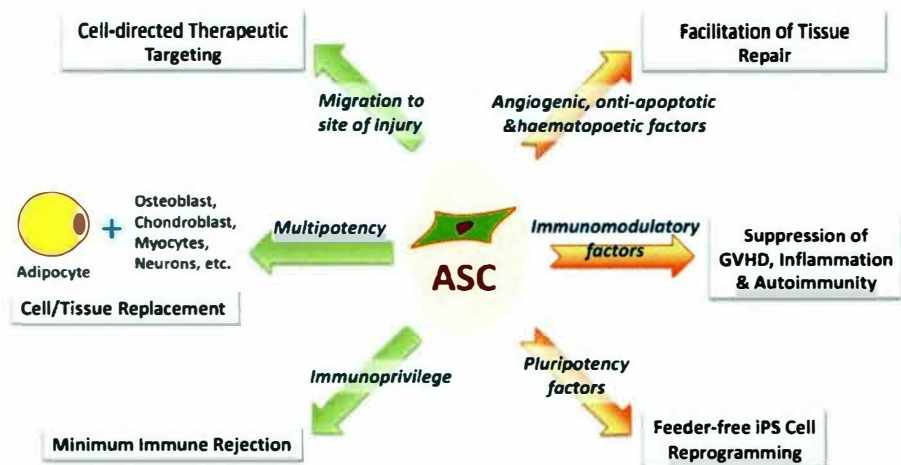
Stem cells and the many supportive cells that are found with them maintain a homeostatic environment which promotes growth and regeneration. But importantly they also possess anti-inflammatory processes which appear to suppress inflammation through the secretion of mediators including IL-10<sup>16</sup>, IL-17<sup>17</sup>, TGF-B superfamily<sup>18</sup>, LIF<sup>19</sup>, soluble HLA-G<sup>20</sup>, and IL-1 receptor antagonist.<sup>21</sup> In addition the expression of immune regulatory enzymes such as cyclooxygenase<sup>22</sup>, and, indolamine 2,3 deoxygenase<sup>23</sup> are seen which help cells “take” to the area and promote regeneration. The cells induce regeneration of “T regulatory cells” which have a profound effect on the local inflammatory environment.

T Regulatory cells (Treg).<sup>24</sup>

Stem cells are capable of directly suppressing the immune systems inflammatory response by depleting certain inflammatory cells (T cells).<sup>25</sup> Because stem cells expressed CD34 receptors they may play a

“Immunosurveillance”

role for circulating CD34+ cells in circulation via activation and differentiation of these cells into dendritic cells (DC) via of toll-like receptors (TLR) agonists.<sup>26</sup>



Although this is a complex subject and part of this article is written for those who have a science background or who are interested in the biochemistry the important concept here is that there is a profound effect on a inflammation in a local environment such as a joint or soft tissue.

## WHAT HAPPENS TO THE FAT CELLS AFTER TRANSPLANTATION INTO MY JOINT OR TENDON?

Again plastic surgeons with all of their experience over the last 20 years provides us the answer as to what happens to fat cells when they are transplanted. Whatever fat cells that we transplant with the rest of the stem cells remain within a joint or soft tissue undergo gradual cell death. The mature adipocytes (fat cells) that undergo this death from anoxia are lost within the first few days following the transfer.<sup>27</sup> Thus, the fat cells are gradually lost but it is important to realize that they serve a function. They serve to fill in tissue defects and gaps and the connective tissue surrounding the fat cells binding to stem cells and act as a scaffolding. In orthopedics this is of critical importance. These cells also provide important cell signaling when they undergo cell death that helps activate other cells around to become more metabolically active and does alter the micro-environment in which they are grafted. Therefore these fat cells and connective tissue provide an important three-dimensional microenvironment for autologous stem cells.<sup>27-29</sup>

## Stem cells around blood vessels:

It also turns out that the small blood vessels that are aspirated during the time we are extracting fat are of critical importance. They also have concentrations of perivascular elements representing pools of adult stem cells and stromal cells ready to be recruited and committed when exposed to certain growth factors after transplantation. These areas of “multifocal cells” are often under the regulation of transforming growth factor (TGF-B) family. Regulation of these pools of cells effect cellular activation, differentiation and recruitment of other cells as well as the organization within the host in which these tissues are transplanted. There is a complex cell to cell communication under the influence of various growth factors and cytokines that alter the microenvironment in which they are transplanted. They also provide signals for “homing” of other cells that are recruited for the needs of the cellular elements at the target site.<sup>30</sup>



The reason the stem cells are present all over blood vessels is that blood vessels are constantly changing and adapting in tissue throughout the body on a regular basis. And, it would seem logical that there would be a concentrated stem cell system around these vessels to respond to injury and to maintain homeostatic mechanisms throughout the body.<sup>30</sup> We just take advantage of their existence.

#### IN SUMMARY:

In summary, common methods of stem cell therapies being used today involve “autologous” cells obtained from the patient’s body. Common methods being utilized involve aspiration of bone marrow blood and or adipose tissue that are then processed and concentrated for the regenerative cell population contained within these tissues. Unfortunately the number of actual multipotent stem cells available in these tissues are quite low and therefore depending on the volume obtained, skill of the physician and laboratory processing knowledge of the physician, the the actual stem cell numbers can significantly vary. For example, even after cell processing, centrifugation and concentration of stem cell population the actual number of cells that can be considered “multipotent stem cells” maybe only one percent of the population that is isolated. For this reason some individuals seek care overseas in an attempt to try to find quality facilities that can do culture and expansion of stem cells which has led to a great deal of economic abuse to the American consumer. Allogeneic stem cells (stem cells in a bottle) has not been found to be a viable alternative in our practice since we have been unable to culture any stem cells from these products to date. Adipose tissue has historically been excellent source for stem cells and we have enjoyed success with this technique in the past with an a good safety profile. We have unfortunately had to prematurely abandon our studies and use of adipose tissue as the source of stem cells until regulatory questions have been answered by the FDA. We hope in the future to return back to exploring some clinical uses of adipose tissue derived stem cells in the future. Stem cells elicit a bio cellular strategy to affect inflammation, pain, soft tissue and cartilage healing. Stem cells often work by altering the micro-and biochemical environment modulating immune function, inflammation to improve healing potential.<sup>19, 31</sup>

Patients seeking stem cell therapies often have strong belief systems based on media hype and a misunderstanding of stem cell technology. This can be further perpetuated by a misunderstanding and lack of knowledge of the technology and use of the technology by physicians themselves. Many physicians began the use of stem cell technology’s after taking a weekend course and have little to no knowledge of the advanced principles and science behind regenerative medicine. Patient’s should be carefully evaluating the background and experience of the physicians who they are seeking stem cell therapies from. Patient’s need to get “beyond stem cell therapy” and realize that the stem cells utilized is just one component to the regenerative medicine technology being utilized. Stem cells injected into a joint does not necessarily mean that a patient is going to expect significant regeneration of the cartilage nor significant structural changes in the joint. There are many adjuncts to the stem cell therapy procedure that often need to be Incorporated to enhance the effectiveness of this intervention.

#### RESEACH SUPPORT IS NEEDED:

Although significant money has been available for research scientists to do basic bench research in the United States there has been little funds made available for head-to-head clinical trials and important randomized clinical control trials using this important new technology. Although companies are becoming active in the US that are for profit they sell specific patented and proprietary laboratory techniques to doctors with no comparative research as to whether their product is any more superior than another. It is critical that an institution become established in the US that is unbiased and will perform this important research and progress this science. We have established 5 research and development centers around the US and a nonprofit foundation to help answer many of the questions



of clinical application of stem cell therapies. These institutions will be working together to develop consensus guidelines, techniques and methods that will substantially effect the clinical application of stem cell various other regenerative medicine techniques we will be utilizing in the future. We will be conducting unbiased randomized clinical control trials over the next few years to answer important questions that is needed to advance this field. We also hope to build a foundation in the future that will be able to also offer care to individuals who typically have no access to this new technology.

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