Evolution of Cell-Based Therapies

Over the past decade, great strides have been made in the understanding and potentials of guided cell-based therapies. As an evolving expansion of palpation guided injection therapies using mild irritant solutions, many practitioners moved towards use of platelet concentrates. Use of the growth factors and signal proteins became recognized as offering a significant improvement in tissue healing responses. With the advent of FDA approved tabletop devices for high concentration via closed system, use of a simple blood draw yielded more than 4-6 times patient's own circulating baselines levels. It has been very well shown that the higher the achieved concentrations, the proportionally high delivery of important factors intrinsically involved in all wound healing and repair.

Cell-based therapies have long been sought for use at repair and regeneration in humans. Seeking such treatment options produced common goals for the "ideal" cell-based therapy. These included: Autologous source easily obtained via a closed system.
Useful variety of undesignated cells including native extracellular matrix.
Capability of transplantation within the same surgical procedure with NO manipulation.
No requirement of artificial scaffolding.
Capable of injection using high□definition guidance and visible density of product at point of targeted placement.
High viability and concentration of stem/stromal cells in microvascular matrix and, provide an optional ability to isolate, concentrate, culture-expand, and permit intravascular or intrathecal placement.

Examination of the processes of homeostasis, remodeling and self-repair (wound healing) has clearly shown that three major elements are crucial to success:

1. Cellular Elements
2. Biologic Elements
3. Micro-environmental Controls
    a. Extracellular Matrix-Cell Contacts &/or
    b. Bioactive (secretive) Elements

Goals for cellular-based therapy share a number of similarities to those of surgery or medicine in any area. Specifically in musculoskeletal (MSK) cases, some of these important goals include:

1. Return to full function (Shorten Physical Therapy Needs and Time)
2. Eliminate or markedly reduce pain
3. Resist recurrence of injury
4. Reverse, Stabilize, or Resist Degeneration
5. Utilize Autologous tissue for repair
6. Accelerate the healing processes
7. Enhance results of, OR, Reduce open surgical intervention requirements
8. Restore tissues with minimal or no scarring
9. Enhance site vascularization

10. Repair or Replace damaged cell-types within sites with same cell-types or capabilities.

It has become more clear that certain tissue characteristics are most favorable for use in cell-based therapies including ease and safety of access, plentiful stores, cells possessing multi-potency. Multi-potency is important in that such cells have the capability of responding to local signals, and possess the ability to transform or replenish signals needed at a damaged or diseased site.

Research has confirmed that the vast majority of such undesignated cells are associated and stored in proximity to the microvascular capillary system. Essentially all tissues (with blood supply) have some of these multi-potent cells available to deal with local and isolated demands. The body retains the ability to attract and mobilize cells from these storage points in response to chemical and physical signaling in the body. Approximately 15 years ago, an important scientific advance was made by researchers in finding that adipose tissue (fat) contained high numbers of such cells. This is not totally surprising considering that fat represents the largest microvascular organ in the body.

Enhancement of cellular and biologic therapies comes directly with the ability for providers to be able to identify, target and guide the cellular-biologic combination to areas of injury or degeneration. In that regard, it has clearly become a MAJOR feature to clinical responses and success. Particularly in medium and deep targets, or those difficult to access, guided MSK ultrasound capabilities offer the optimal integral part of successful responses. Over the past decade, thousands--of treatments using Biocellular Regenerative Medicine® have proven safe and remarkably effective. The information provided in this chapter is intended as an introduction to the concepts and describe the logic believed to be involved. Major steps have been taken, moving from the laboratory to the bedside. Today MSK patients are routinely treated with this combination of elements.

What is Biocellular Medicine?

The term "Biocellular" refers to the combination of important biological chemicals (such as growth factors, signal proteins and chemicals important to wound healing) with undesignated cells within our body, which participate in tissue repair and regeneration. Science and Medicine have entered into what is termed "translational medicine" where proven laboratory science having demonstrated important contributions joins the clinical application of the science in human applications. There has been much controversy concerning the use of "stem cells" in the current practice of medicine. Unfortunately, this usually is interpreted as being "embryonic" or fetal stem cells, implying destruction of embryo or fetal tissues.

Biocellular Regenerative Medicine® within the United States, and this chapter, currently refers to use of a person's own (autologous), non-manipulated ADULT (non-embryonic) cells capable of participating in healing and regeneration. The components are derived from and individual's own tissues (usually adipose /fat) tissues as cell source, in combination with concentrated platelets or bone marrow aspirates (as a biologic agent rather than cellular). The small nucleated cells found closely associated with the vascular tissues are now recognized as serving

important roles in obtaining normal tissue content (homeostasis), PLUS have the ability to respond to injury or disease processes in a constant effort to heal or repair damaged cells (as in aging, arthritis, musculoskeletal tissues, neurological disorders, etc.). The remarkable design of the human body uses these reservoirs of available, non-differentiated cells as the first responders in the situations of major or micro-trauma and aging. By secretion of certain chemicals from an injured site, these multipotent (i.e. can become various types of cells) cells can be called upon to participate in the repairs needed to restore tissues and functions. We will explain the cells involved in this process in the content of this information.

The "biological" components refer specifically to the need for a variety of growth factors and signal proteins which interact with the cells of the injury or damaged site to help recruit needed repair cells and materials to repair the area. There are two major "biological" components in common use at this time. First is use of contents of platelets, which store and release a wide variety of needed growth factors and available cells to begin the wound healing processes. For many years we thought the only important roles of platelets were to become "sticky", adhere to each other and participate in clotting mechanisms. We now realize that this may be their LEAST important contribution to wounds and wound healing. Platelets represent a storehouse of small granules, each containing the important growth factors and signal proteins that serve to "quarterback" the entire healing cascade and do this for a prolonged time during the healing phase. For example, an important chemical available from these granules are essential for blood vessel replacement and repair in order to improve the circulation ability critical to healing of all wounds. Without adequate blood flow, needed oxygen cannot reach the area of damage, nor permit migration of a variety of cells from nearby and far cell sites.

The second source of biological contributors are found in bone marrow aspirates. Bone marrow has been used for many decades, and it is commonly used in blood related disorders. Although bone marrow does have some undesignated cells (stem/stromal cells), they are in very small numbers therefore; marrow is considered primarily a valuable biological platelet source (same biologics as in platelet concentrates). They offer relatively few stem cells other than blood forming tissues. In order to become a cell contributor, it is required that they be isolated, concentrated, and cultured to achieve meaningful numbers needed in regenerative and healing applications.

This is technically more invasive to obtain, higher complication rates, and significantly more expensive to the patients. In addition, the undesignated stem/stromal cells, considered of value, are MUCH more rare. The only real difference between concentrated platelets from peripheral blood is a very large store of hematopoietic stem cells (blood forming cells) and platelet rich plasma. At this time there is very little evidence of significant contribution to the regeneration process of MSK tissues. It is primarily of value for the ability to concentrate platelets to be used as. discussed in the previous paragraph as an important additive to cells to work together to provide optimal healing conditions and abilities.

"Workers and Bricks"

A simple analogy is helpful in understanding the importance of both the biological and the cellular elements to achieve more rapid and complete healing and repair.

If you have a brick wall that is beginning to break down, some ' of the mortar holding the bricks together is lost or crumbling. What is needed to repair the wall would be hiring WORKERS to come in, clean up the site, and repair and replace the damaged mortar. Once completed, the wall is repaired, and functions as originally intended. These workers are found in great quantities in platelet concentrates and comprise the "biological" contribution of the Biocellular Regenerative treatments.

In the event, however, that your wall is not only losing mortar holding the bricks in place, imagine if you have lost or broken many of the bricks in the wall. This would require NOT ONLY the “workers” but would also require "BRICKS" to replace the lost and damaged ones. The "bricks" in this analogy come from the cellular source. Combining biologics and cell source have proved more successful than use of EITHER of the agents by themselves.

It is well established that there are many more of these undifferentiated cells located in the largest micro-vascular organ of your body, the adipose (fat) matrix. Therefore, the readily available and safely accessible "cellular" contributor of choice has become adipose tissue retrieved from subdermal fat deposits in the abdomen and/or thigh areas harvest procedure. These are gently removed via closed syringe lipoaspiration, compressed by centrifugation, and mixed with the platelet concentrates (>4-6 times your own circulating platelets) to form the therapeutic mixture known as "Biocellular Regenerative Matrix". This mixture is in current use in aesthetic (plastic), reconstructive, sports & pain medicine, orthopedic medicine and surgery, neurological disorders, musculoskeletal and arthritic applications, and a wide area of overlapping disorders.

What are "adult stem cells"?

These are a diverse group of "non-designated" cells found throughout the tissues of our bodies. They serve as a reservoir of replacement, and repair cells, which react to injury, aging or disease. "ADULT" cells in this category are often referred to as "stem/stromal cells" or "stromal" cells and should be clearly separated from embryonic cells. They are also called "progenitor" cells, which means they have the capability to differentiate into a variety of types

of cells, responding to growth factors and signal proteins within the site where they are located. For example, you have a muscle or ligament tear, these cells might participate in healing or repairing the damage providing replacement muscle or ligament tissues rather than turning into scarified tissue. Scar tissue is not as functional or tolerant of future stresses and is NOT the ideal goal in wound healing. By providing the needed elements to such a site, the body is given the opportunity to fully repair damaged areas, often by cellular and biological events.

There are many experiences in such cases over the past several years in the musculoskeletal area, and for nearly 20 years in aesthetic surgical practice. These are often reported on small case series or case reports of treatment and outcome and are being further studied in many clinical trials. These trials include both guided placement of stem/stromal elements and biological agents in orthopedic medicine and surgery, but also intravenous and central nervous system placement in a variety of complex disorders which do not respond to conventional therapy (such as Diabetes, Multiple Sclerosis, Alzheimer's disease, Parkinson's disease, Severe Limb Ischemia (loss of blood flow), Traumatic Brain Injuries, etc.). Early reports of improvement in chronic conditions, including pain, arthritis, damaged tendons-ligaments, etc., are driving many to select this option to improve surgical outcomes or avoid surgical interventions and shorten the demands for physical therapy.

Many are confused about the potentials or best source of stem/stromal cells, often believing this only refers to use of embryonic tissues. In the past 10-15 years, much evidence has led us to understand that your own fat may be a more plentiful and ideal cell source, avoiding the need to destroy fetal or embryonic tissues to ac9uire cells of great potential.

With the ready availability of fat, minimally invasive access (using closed syringe liposuction), fat now has become an optimal source for these cells with a high safety profile for patients. Fat is the largest micro-vascular organ in the body, and as such, has become well recognized as the largest deposit of undifferentiated stem/stromal cells in the entire body. The ease of gathering fat tissues on an outpatient basis and local anesthetic procedures has led to the evolution of Biocellular Therapy (sometimes called Cell-Based Therapy) for a wide variety of disorders and conditions. It is most common for these procedures to be performed in outpatient ambulatory surgical centers or designated clinic facilities.

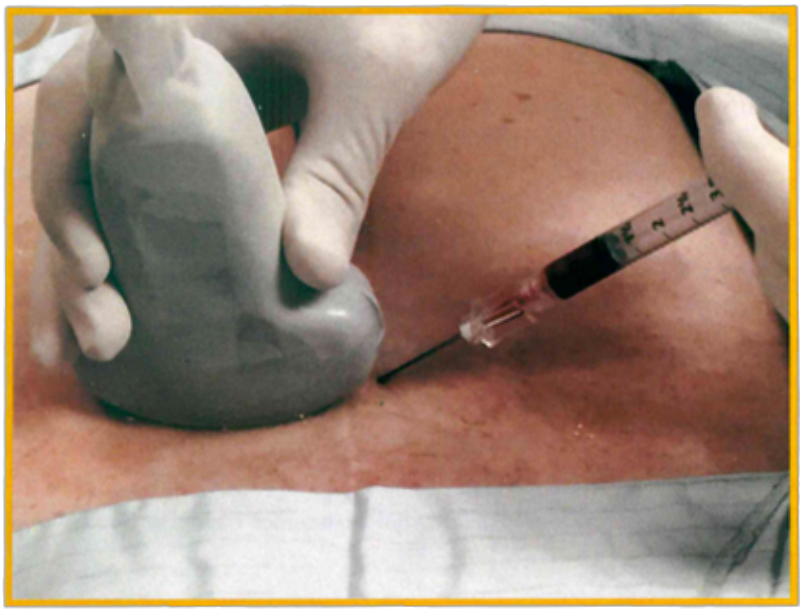
Key cells needed to promote healing and repair reside in tissue micro-environments, where they comprise parts of tissues and organ systems. These are also called "niches" and are the locations where injury or disease must be addressed to permit the body to repair or regenerate itself. It is believed that when that process is underway, addition of needed cell types and biological elements specifically targeted (via ultrasound guidance for example), can effectively utilize your own tissues to heal themselves.

What is involved in Biocellular Regenerative Therapy?

First, the platelet concentrates are created in an FDA approved, sterile, closed device from a standard peripheral blood draw (like you have when your physician tests blood). Thousands of patients have undergone treatments using these concentrates with quality results in many

inflammatory or aging conditions. Next, the cellular sample is harvested from subdermal fat deposits under sterile protocols using the patented closed syringe system for minimal tissue disruption. This is often referred to as microcannula liposuction or harvesting. This fat is cleaned, compressed (centrifuged) and unwanted liquid layers separated by centrifugation. This process not only helps with removal of unwanted liquids, but also compresses the adipose cellular components to provide a more effective cell and bioactive matrix.

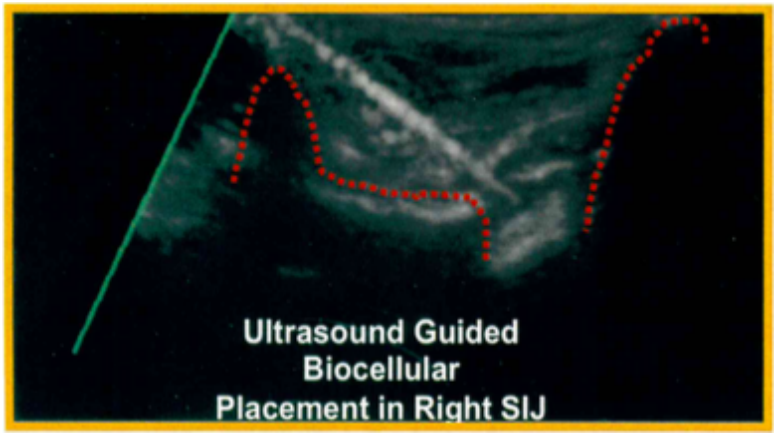
By effectively reducing the volume of injection materials, earlier recovery of comfort and ambulation is common. Biologicals such as high-density platelet concentrates are then added via closed syringe transfer to create a mixture of cells, and the important growth factors and signal proteins provided by the HD PRP. In the future, a portion of the fat harvested can be separated into another syringe, exposed to digestive agents, warmed and shaken to permit further isolation and centrifuged to form a "cellular pellet" forming a concentrated package of the mononuclear undifferentiated cellular collection. Once created, these cells are available for intra-vascular uses OR to be added back to the adipose graft (which still has its bioactive matrix). This is termed "cell-enriched", and which are simply added back to your own adipose tissue, mixed with HD PRP, and carefully guided into identified target sites to assist your own tissue healing using only your own tissues. This is commonly performed in many areas outside of the United States, but at this moment, the FDA regulations prohibit the addition of the digestive chemicals. Some sites are working to be able to provide these. services in the United States but are acting within controlled Institutional Research Based (IRB) studies. We are currently seeking the ability to provide both options within the United States, following International Cellular Medicine Society (ICMS) IRB guidelines plus providing the full protocols in approved sites outside the continental United States.



Who should consider having Biocellular Treatment?

Patients and providing doctors (e.g., primary care health care provider, orthopaedic surgeon, sports medicine specialist, pain management specialists, wound care centers, etc.) decide whether candidates have a condition which has good potential to be improved through use of biologic or stem/stromal cellular treatment. Pre-operative evaluations are very important in diagnostic capability and should include appropriate MSK ultrasound imaging to determine the specific locations of problems. Use of high 9uality MSK ultrasonography is considered a KEY part of such evaluation, particularly considering that this modality plays a central role for

providers to effectively "hit" the desired targets. Optimal placement correlates with earlier and improved outcomes, making it an essential part of Biocellular Therapy.



Most times these procedures are completed under local anesthesia, nitrous oxide, or light sedation depending on patient needs and desires. These cases are designed and planned to be completed within the same day. Providers handle tissues using standard aseptic protocols. Latest advances in use of cellular therapies now include clinical trials using a person's own isolated stem-stromal cells from fat or bone marrow for uses in a very wide variety of medical conditions and diseases, many of which are resistant to traditional medical therapies. These trials are an extension of current biocellular protocols in which adipose tissues are harvested under sterile techni9ue, followed by separation into two portions. The first half is centrifuged to compress the tissue SVF (tSVF), and the second used in a closed system to isolate and concentrate the attached stem-stromal cells into pellet form. This pellet may be returned to the original graft (called "cell enrichment"), mixed with high-density platelet concentrates, and utilized in the same manner as the biocellular treatments using ultrasound guidance for accurate placement. Safety and efficacy are the major parameters for evaluation of treatment protocols being developed and published. In many cases, unusual and resistant disorders are now treated with these isolated cells to be given in parenteral pathways (IV, IA, IP, IT, etc.). Many IRBs are now reporting in areas of heart disease and attacks, critical ischemia's, neurodegenerative (MS, Alzheimer’s, Parkinson's, Trauma and Stroke, ALS, etc.), chronic wound care, Diabetes, bowel inflammatory disease, autoimt11une disorders and many others. It is believed that some conditions involving systemic problems, degenerative issues, and regeneration will become high prominent features in the future. Combination treatments are currently available within the United States, and its Regulatory agencies, but only under recognized IRS trials.

"USING YOUR OWN TISSUES TO HEAL" represents major health care paradigm change, and is one of the most exciting minimally invasive options currently available.

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