Biocellular Regenerative Medicine

Use of Adipose-Derived Stem/Stromal Cells and It's Native Bioactive Matrix

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KEYWORDS

• Stem cells • Stromal cells • PRP • Regenerative medicine • Nanofat
• Mesenchymal cells • Fat grafts • SVF

KEY POINTS

- Autologous Stem/Stromal Cells and Platelet Concentrates Guided to Targets.
- Combination of Cells & PRP concentrates work better than either alone.
- Biocellular Combination Is Believed To Facilitate Patient's Own Wound Healing/ Regeneration.

EVOLUTION OF CELL-BASED THERAPIES

Over the past decade, great strides have been made in the understanding and potential of targeted cell-based therapies. Starting decades ago, use of an irritant solution to stimulate inflammatory reactions has been replaced in the past few years with transition to injecting various platelet-rich plasma (PRP) concentrates for supporting an effective inflammatory reaction at damaged or degenerative sites. Use of the contained growth factors and signal proteins became recognized as offering a significant improvement in tissue healing responses but seemed limited by incomplete repair while requiring a series (often 4–6) to achieve long-term clinical improvement. Current evolution of combining these trophic growth factors and signal proteins with concentrated undifferentiated cellular/stromal populations seemed like a logical and effective modality, moving into the forefront since 2000. Aesthetic and reconstructive applications led the way, because constant challenges of injury, loss of circulatory capabilities, degenerative, repair, and so forth demanded an optimal approach to regenerative needs. In-depth examination of how the body maintains itself revealed that undesignated cells were integrally important to replacing aging cells (such as

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skin, hair, bowel lining, and so forth). Early on, fat was not thought of as undergoing such homeostatic mechanisms, because typical mitotic activities were not observed. Now it is recognized that rather than a static number of cells varying only in size, mature adipocytes actually undergo total replacement at a rate of 10% to 20% per year but do so in a different form of cell division known as asymmetric cell division. The ability to have resident precursor cells that are capable of responding to local site signals and the ability of providing a replacement cell of the needed type result in potential replacement cell differentiation, while retaining a single precursor cell type. Without that mechanism eventually there would be an uncontrollable stem/stromal cell population.[1]

With the advent of Food and Drug Administration (FDA)–approved tabletop devices for high platelet concentrations via a closed system, use of a simple blood draw yielded more than 4 to 6 times a patient's own circulating baselines levels. It has been well shown that the higher the achieved concentrations, the proportionally higher delivery of important factors intrinsically involved in all wound healing and repair.

It has become clear that certain tissue characteristics are most favorable for use in cell-based therapies, including easy and safe access and plentiful autologous stores of a group of cells possessing multipotent potential. Multipotency 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 damaged or diseased sites for repair or regeneration.

Research has confirmed that a vast majority of such undesignated cells are associated and stored in proximity to the microvascular capillary system (**Fig. 1**). Essentially all tissues (with blood supply) have some of these multipotent cells available to deal with local and isolated demands. The body retains the ability to chemotactically attract and mobilize cells from local and remote 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.[2,3] This is not totally surprising considering that fat also represents the largest microvascular organ in the body.

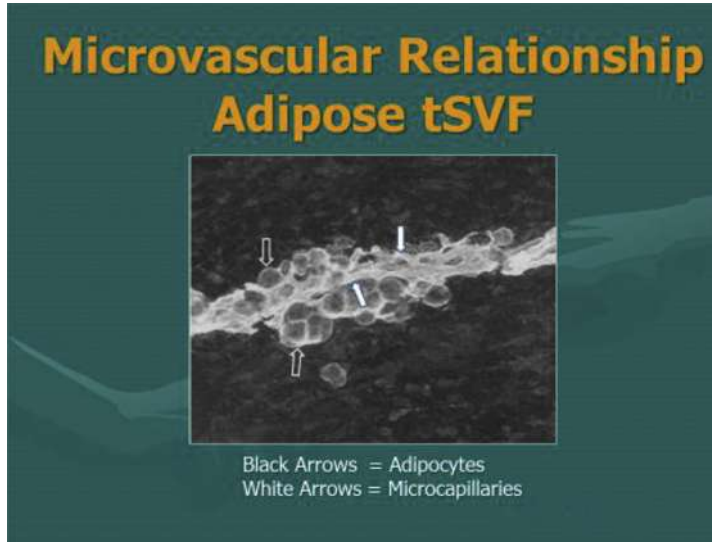

Fig. 1. Microvascular relationships in fetal pig.

Enhancement of cellular and biological therapies comes directly with the ability for providers to be able to identify, target, and guide the cellular-biological combination to areas of injury or degeneration. In that regard, ultrasonography has become a major feature of clinical responses and success. As an example, in medium and deep targets, or those difficult to access, guided musculoskeletal (MSK) ultrasound capabilities offer the optimal integral part of successful responses. Over the past decade, thousands of treatments using biocellular regenerative medicine techniques have proved safe and remarkably effective. The information provided in this article is intended as an introduction to important concepts and describes the current logic believed involved. Major steps have been taken, moving from the laboratory to the bedside. Today, MSK and aesthetic–plastic surgical patients are routinely treated with this combination of biocellular elements.[4–6]

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 (often referred to as adult stem/stromal cells) found widely spread within the body and which participate in tissue maintenance, repair, and regeneration. Science and medicine have recently entered a translational phase, where proved laboratory science has demonstrated important contributions join the clinical application of the science in human applications in the past decade. There has been controversy concerning the use of the term, *stem cells*, in current practice of medicine. Unfortunately, these arguments typically occur with the use of stem cell interpreted as uses of pure embryonic or fetal stem cells, implying destruction of embryo or fetal tissues. In the past decade, the recognition of the safety and efficacy of using a person's own (autologous) adult stem/stromal cells has advanced to the point that it is widely documented and published (**Box 1**).

Cellular Components

Biocellular regenerative medicine within the United States currently refers to use of autologous, adult (nonembryonic) multipotent cells capable of participating in maintaining tissues (homeostasis), healing, and regeneration. Since 2006, the number of scientific studies demonstrating the values of the highly variable stromal cell populations has exploded, to the point that active reports and studies of component cells of adipose origin exceed the study of nonhematopoietic stromal cells in bone marrow in MSK and aesthetic–plastic surgical applications. The importance of such studies, and

Box 1
Basic goals in biocellular regenerative medicine

- Return to full form or function
- Eliminate or markedly decrease pain
- Resist recurrence of injury
- Reverse, stabilize, resist degeneration
- Use autologous tissues for repair
- Restore tissues with minimal scar
- Accelerate healing processes

an understanding that adipose tissue deposits have gained such recognition due to the greater numbers of stem/stromal cells (other than blood forming element) in the body, is coupled with the important overlap of potential cellular functions. Essentially every tissue in the body that contains microvascular supply maintains a reservoir of such cells. That said, it is recognized that adipose tissues possess the greatest microvascular organ in the body. Many peer-reviewed scientific reports suggest that adipose-derived (AD) stem/stromal cells of mesodermal origin provide between 1000 and 2500 times the actual numbers found in bone marrow.[7] With the easy collection of adipose tissue, less penetration, widely heterogeneous cellular populations, and important immune-privileged properties, subdermal fat deposits serve as a primary source for gathering stem/stromal cells (**Box 2**, **Fig. 2**).

The small nucleated cells found closely associated within the vascular tissues are recognized as serving important roles in maintaining normal tissue content (homeostasis) plus having the ability to respond to injury or disease processes in a constant effort to heal or repair damaged cells (as in aging, arthritis, MSK tissues, neurologic disorders, and so forth). The remarkable design of the human body uses these reservoirs of available, nondifferentiated multipotent cells as the tissue first responders in the situations of major trauma, microtrauma, and aging. By secretion of certain chemicals from an injured site, these multipotent cells (ie, can become various types of cells) can be called on to participate in the repairs needed to restore tissues and functions. There are many peer-reviewed publications that provide examples of how the cells involved in this process can be enhanced by combined provision of the cellular, native scaffolding, and biologically active components (**Box 3**).

Biological Components

The biological components in this context refer specifically to the availability of a diverse and important variety of growth factors and signal proteins that interact with the cells of degenerative or damaged sites to help recruit needed reparative cells and materials to repair the area. There are 2 major biological components in common use. One is found within recognized contents of platelets, which store and release a wide variety of needed growth factors and proteins to act on available cells to begin the wound healing processes.[8] For many years, the only important role of platelets was believed to become "sticky," that is, adhere to each other and participate in clotting mechanisms. It is now realized that this may be their least important contribution to wounds and wound healing (with exception of providing a fibrin clot to permit gradual release of platelet contents). Platelets represent a storehouse of small granules, each containing important growth factors and signal proteins that serve to

Box 2
Optimal cellular source features

- Ease to get
- High quantity of cells
- Minimum morbidity of donor site
- Safety after implantation (own cells)
- Multipotent and proliferative cell groups
- Secrete immunomodulatory factors
- Immunoprivileged cells preferred

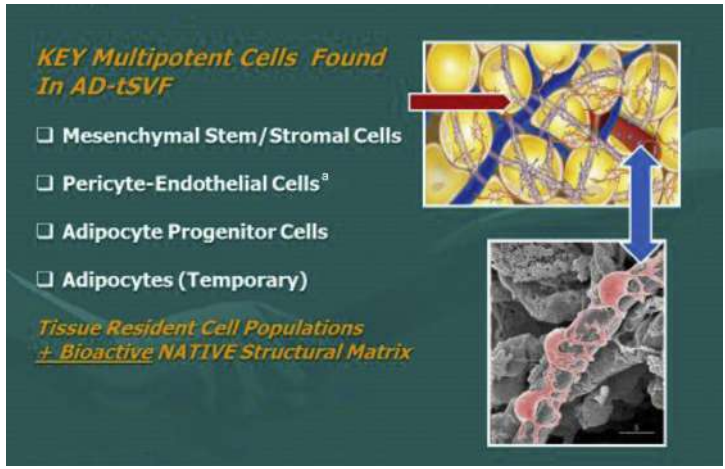

Fig. 2. Locations and components of AD-tSVF. [a] Pericyte & Endothelial Cells may be the origin of all MSCs. Microvasculature (*red arrows*); Highlights pericyte cell attachments (*blue arrows*).

quarterback the healing cascade and do this for a prolonged time during the healing phases. For example, an important chemical available from these granules is essential for blood vessel replacement and repair to improve the circulation ability critical to healing of all wounds. Without adequate blood flow, needed oxygen can neither reach the area of damage nor permit migration of a variety of cells from nearby or distant cell sites (**Figs. 3** and **4**).

The second source of biological contributors is found in bone marrow aspirates. Bone marrow has been used for many decades, and it is common use in blood-related disorders. Bone marrow does demonstrate microvasculature and therefore does have some undesignated cells (stem/stromal cells). They are, however, in very low numbers compared with adipose tissues. Therefore, many regenerative practitioners consider bone marrow as primarily a valuable biologic and platelet source. To become a valuable cell contributor, it is required that bone marrow aspirates be isolated, concentrated, and culture-expanded to achieve meaningful numbers needed in regenerative and healing applications. This source is technically more invasive to obtain, poses higher complication-sequelae rates, and is significantly more expensive to the patients. In addition to the undesignated stem/stromal cells, content considered of value (such as mesenchymal, periadventitial, and endothelial) is more scarce

Box 3
Ideal cell-based therapy

- Use your own cells (autologous)
- Safe and easy harvest via closed system
- Optimal to include native matrix
- Transplant in same surgical session
- Predictable and reproducible outcomes
- Optional ability to use parenteral uses for systemic disorders
- Require minimal manipulation (offer optional culture/expansion)

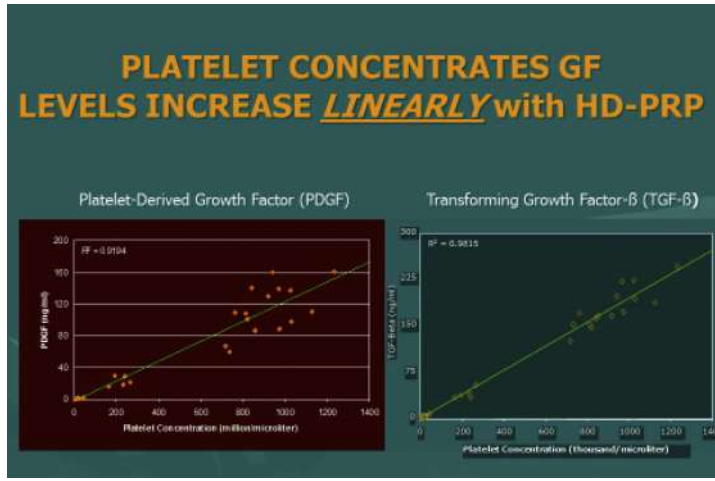

Fig. 3. Platelet concentrations of growth factors linear increase.

compared with millions of resident hematopoietic cells. The primary difference between concentrated platelets from peripheral blood versus marrow concentrates is a large store of hematopoietic stem cells (HSCs) while offering an almost identical platelet content. At this time there is little evidence of significant contribution to the platelet regeneration process of MSK tissues derived from the HSC group.

Concentrates primary importance and value is the ability to provide important growth factors and cytokines/chemokines to optimize earlier healing conditions and abilities. Of even more importance in the cellular therapeutic-based effects seems to be their important paracrine secretory influences rather than contributions of individual cellular components and physical engraftment. Furthermore, it well established

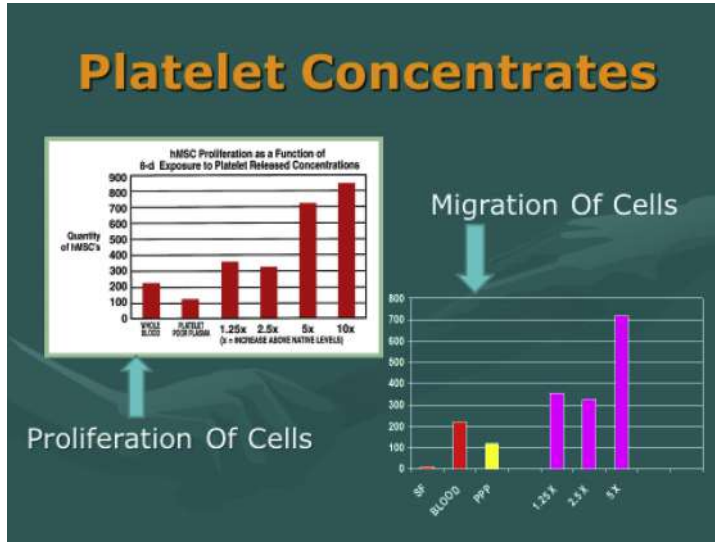

Fig. 4. Platelet concentrate effects on cell proliferation/migration. hMSC, human mesenencymal cells; PPP, platelet poor plasma.

that the mesenchymal group (MSC) of multipotent cells may originate from the pericyte-endothelial cell groups.[9] Stromal cellular elements offer a great amount of overlapping capabilities in vitro, suggesting that all tissues having some microvasculature and have resident stem/stromal elements capable of providing first responders to sites of damage or degenerative effects. It is suggested that the MSC groups overlap at greater than 95% in their capabilities. Host site interaction with these cells, growth factors, and signal proteins seem to create a complex, heterogeneous precursor population that is considered site specific in many of their responses.[10]

HOW DID BIOLOGIC AND CELLULAR THERAPEUTIC CONCEPTS EVOLVE?

Aesthetic and plastic surgeons traditionally have dealt with wound healing and scarring issues for many years. During that time, careful study of the processes of homeostasis, remodeling, and repair led to a better understanding of how the body tissues manage to maintain themselves. For many years, the importance of biologics as a derivative part of platelets become appreciated not only for clotting functions but also for the gradual release of critical chemical components essential to the healing processes with individual sites. These biocellular concentrates are thought to immediately begin to participate in secretions capable of site specific repair and regeneration, while local cells begin to actively contribute. In addition to these elements, appreciation of the importance of the native adipose (3-D) scaffolding (matrix) in provision of essential contact points, which serve to encourage microenvironment changes, including cellular proliferation and chemotactic migration, has come to the forefront.[11–13] Site specificity greatly influences cellular changes within the nondesignated, heterogeneous multipotent populations found in essentially all tissues that have microvasculature. In addition, appreciation of the importance of cellular secretions (paracrine and autocrine) within these undifferentiated cell groups has been reported to be as great, or greater in some instances, as the multipotent cellular differentiation effects.[14]

Once the complex processes of repair and regeneration were examined closely, it became apparent that determination of specific interactions of any single cell or chemical is not able to be determined. At this time, the ability to create an in vitro situation that can clinically duplicate the in vivo microenvironment, making selection of optimal components impossible.

Key adult multipotent cells are found in essentially every tissue and organ in the body. Determination that some of the highest concentrations of these adult stem/stromal cell populations were found within adipose tissue complex (ATC) has led to a major trend shift to more closely evaluate the activities of such tissues and how they can be easily and safely acquired and concentrated for uses in wound healing and repair. Early on, because adipocytes within the ATC were not thought to cell divide, it was assumed that these were static in number and only changed in size according to lipid storage droplets. At this point, it is clear that adipocytes do have a life cycle, replacing all mature adipocytes every 5 to 10 years.[15] Examination of how they accomplish this replacement, via a process of asymmetric cell division, was found that the precursor cell population reacting to secretions from a senescent adipocyte. This replication by cell division results in a replacement immature adipocyte, whereas the other portion retains its precursor form and abilities. This is logical, in that if otherwise, a massive number of precursor cells would accumulate in the tissues (**Box 4**, **Fig. 5**).

Zuk and colleagues[16] identified the multipotent capabilities of AD–tissue stromal vascular fraction (tSVF), with capabilities of differentiation to a variety of tissues, including bone, cartilage, tendon-ligament, muscle, fat, nerve, and so forth (**Fig. 6**).

Box 4
Tissue stromal vascular fraction: adult stem/stromal cell elements

*Stromal vascular fraction **AD-tSVF** — very heterogeneous*

- Mesenchymal stem cells (a key cell group)
- Pericytes/endothelial cells and adventitial cells
- Preadipocytes (adipose progenitors)
- Fibroblasts
- Macrophages
- Vascular smooth muscle cells
- Miscellaneous native blood derived cells
- Extensive bioactive-secreting ECM

Once this capability was identified, efforts to isolate specific cell types started. This has proved difficult in that it is currently impossible to imitate the in vivo microenvironment in the laboratory. Over the past decade, exploration of concentrating the identified ATC undifferentiated cell population coupled with high-density (HD) platelet concentrates (HD-PRP) has received a great deal of attention. Identifying stem/stromal cells that can participate in the processes has revealed what an ideal cell-based therapy may represent (see **Box 4**).

In the early 1990s, a method of closed syringe lipoaspiration was patented, permitting a less traumatic and efficient means of acquiring ATC for use as a structural graft.[17] This has evolved to a disposable, microcannula option that permits safe and efficacious low-pressure acquisition of AD-tSVF. In the past 15 years, clinicians and laboratory researchers have identified several important cell types, which interact to provide remarkable contributions in tissue repair and regeneration. These have

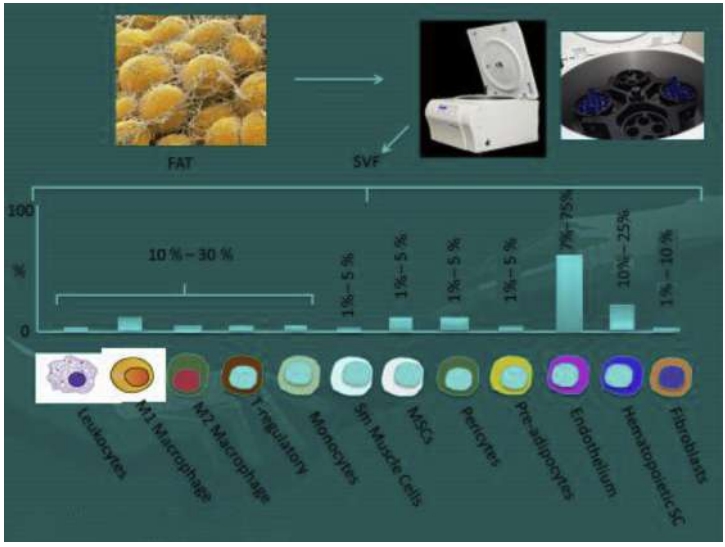

Fig. 5. Range of cellular components in AD-tSVF.

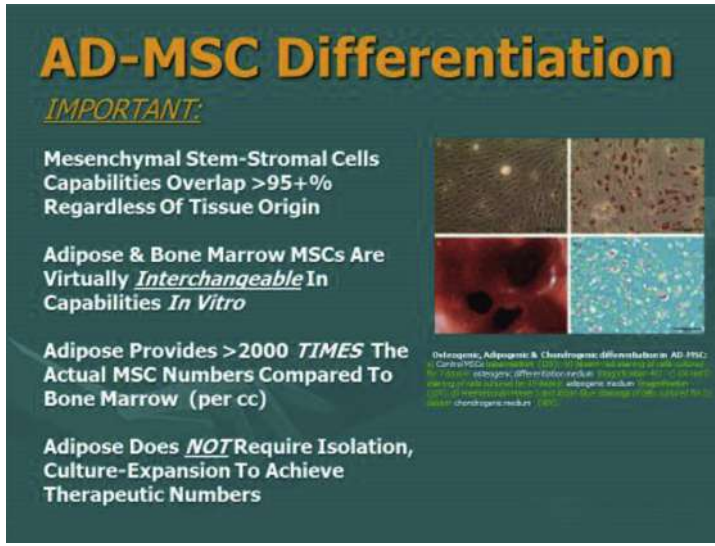

Fig. 6. Examples of tissue differentiation from AD-cSVF.

been identified as a complex and heterogeneous population, closely related to cellular, adventitial areas and extracellular matrix contacts. At first, mesenchymal cell group (MSC or AD stem cell) was thought the most important multipotent stem cell. Further examination, however, suggests that it may serve a sentry capacity and that the actual cell group is known as pericyte/endothelial stem/stromal cells[18] (**Box 5**, **Figs. 7** and **8**).

There is confusion in interpretation of the scientific and clinical published materials caused by a lack of explanation of the difference between tSVF and cellular stromal vascular fraction (cSVF) (**Box 6**). For clarification, cSVF is the isolated cellular elements in the ATC created via use of certain collagenase-enzyme blends to separate the attachment comprising the cell-to-cell or cell-to-matrix connections. The use of such cSVF is the subject of multiple clinical trial applications (see clinicaltrial.gov) and is heavily used in cell isolation, culture expansion, and cell characterization studies. This creates an information gap between clinical applications and those strictly of research value. If clinicians read only the peer-reviewed clinical journals,

Box 5
Advantages of closed syringe microcannula use

- Disposable system (prevents contamination)
- Reasonably priced, many optional openings
- Maintains closed harvest and uses
- Ability to reduce vacuum pressures
- Cell-friendly surfaces protect tissues
- SuperLuerLok (Tulip Medical, San Diego CA) provides optimal vacuum
- Easily permits additives (HD-PRP or bone marrow aspirate)
- Has decades of clinical experiences that prove safety and efficacy

Fig. 7. GEMs (Tulip), microcannula closed aspiration syringe system.

they miss up to 85% of the pertinent information and data evolving on almost a daily basis, because the important advances appear in basic scientific and engineering publications.

In clinical applications, use of AD-tSVF has taken the primary role in aesthetic and regenerative uses, because it is a product that provides the full complement of structural (stroma and extracellular matrix [ECM]) elements plus the resident cellular population of the AD-cSVF. The existing native stroma of ATC is considered of great importance due not only to the available attachment sites but also to the actual secretory bioactivity of the tissue. This dual role is considered of importance, making use of existing native scaffolding of ATC to positively contribute to the local recipient sites in need.

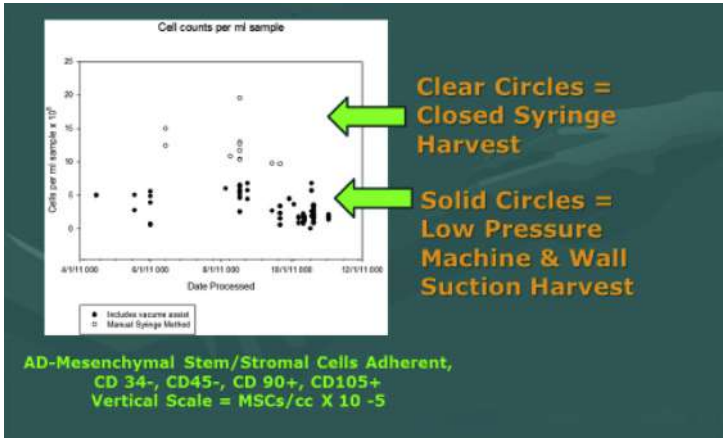

Fig. 8. Syringe versus machine pump aspiration. (*Courtesy of* R. Mandle, PhD and R. Alexander, MD, Harvard BSR Lab 2011, Cambridge, MA.)

Box 6
Understanding terms: tissue stromal vascular fraction and cellular stromal vascular fraction

Tissue stromal vascular fraction (tSVF)

- Includes all cellular components of tissue
- Includes all biologic components
- Includes native bioactive matrix (secretive)
- Requires no manipulation

Cellular stromal vascular fraction (cSVF)

- Requires digestion, incubation, isolation
- Common uses reported in research settings
- Does not have native matrix component
- Often used as cell-enrichment protocols

In biological aspects, it is important to recognize that not all PRP preparations and concentrates are the same. The amount of growth factors, signal proteins, and important chemical agents has a direct linear relationship to the concentration of platelets actually achieved. It is confusing to follow the variety of processes used in creating what is called PRP, particularly because most practitioners do not have the capability of confirming actual patient measured baselines to compare with achieved concentrations. To qualify as a true HD- PRP, the minimal concentrations used are 4 to 6 times an actual measured baseline, not a calculated extrapolation. This is important based on the correlation of such concentration to cellular proliferation and migration capabilities (see **Fig. 4**).

Use of centrifugation has increased in biocellular applications, because it creates an effective gravity density separation, which avoids cellular debris, unwanted fluids and local anesthetics, and isolation of the unwanted free lipid layer from the upper portions of the lipoaspirate. In addition, it permits decrease of the interstitial fluid load, a factor requiring overcorrection of grafts or small joint placements. This unneeded load is thought to potentially have an impact on site perfusion, a factor of importance in many plastic surgical reconstructive wounds and MSK applications.[19,20]

The final area of importance in MSK applications relates to the ability of optimal targeting of areas of damage, degeneration, or inflammation. Without use of high-definition ultrasonography, it is virtually impossible to assure accurate placement of the biocellular therapeutic modality. With the use of ultrasonography, coupled with compressed and thoroughly mixed biocellular components, patients respond more rapidly, show metrics of responses, and achieve earlier final outcome than when placed via palpation only.[21]

Within the past 2 years, an option of removing the unwanted mature adipocytes from the AD-tSVF has become available. It is well documented that the large, mature adipocytes do not contribute significant value to an injection site (including when performing structural fat grafting in aesthetic surgery) because they are gradually lost and removed after their anoxic exposure. It is likewise clear that the stem/stromal cells in the ATC are not as susceptible to those conditions and may be stimulated in low oxygen tension environments. Recent publication of viability and numbers of stem/stromal cells remaining after emulsification process confirms that the relative numbers of such cells remain statistically the same as those not submitted for emulsification.

One of the advantages of this process is that the AD-tSVF not only retains valuable stromal tissue but also the entire specimen (mixed with HD-PRP) can be easily injected through small-bore needles (25–30 gauge). This facilitates uses in scars, radiated damage skin, and hair loss plus permits more patient comfort in MSK injections (including small joint targets).[22]

In regenerative medicine, the main goals are well established (see **Box 1**). Likewise, description of optimal features of cellular-based therapy in both aesthetic and regenerative applications is becoming standardized. The combination of platelet concentrates and AD-tSVF seems more effective than either of the entities by themselves.

"WORKERS AND BRICKS" ANALOGY

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 a brick wall is beginning to break down, some of the mortar holding the bricks together is lost or crumbling. What is needed to repair the wall is 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.

Imagine, however, that the wall not only is losing mortar holding the bricks in place but also many of the bricks in the wall are lost or broken. This would require not only the workers but also bricks to replace the lost and damaged ones. The bricks in this analogy come from the cellular source. Combining biologics and cell source has proved more successful than use of either of the agents by themselves (**Box 7**).

It is well established that there are many more of these undifferentiated cells located in the largest microvascular organ of the body, within 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 thigh areas. These are gently removed via closed syringe lipoaspiration, compressed by centrifugation, and mixed the platelet concentrates (>4–6 times patient circulating platelets) to form the therapeutic mixture known as biocellular regenerative matrix.

This mixture is in current use in aesthetic (plastic), reconstructive, sports, and pain medicine, orthopedic medicine and surgery, neurologic disorders, MSK and arthritic applications, and a wide area of overlapping disorders.

WHAT ARE ADIPOSE-DERIVED ADULT STEM/STROMAL CELLS?

AD adult stem/stromal cells are a diverse group of nondesignated cells found throughout the tissues of the body. 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 or precursor cells, which means

Box 7
"Workers and bricks" analogy

It is important to note that use of combined cellular and biologic elements work better than either cellular or biologic alone.

they have the capability to differentiate into different types of cells via responses to growth factors and signal proteins within the microenvironment where they are located. For example, with a muscle or ligament tear, local and the nondifferentiated cells are thought to participate in healing or repairing the damage providing replacement muscle or ligament tissues rather than resulting in 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 (**Figs. 9** and **10**).[16,23,24]

There are many experiences of such cases over the past 10 years in MSK area and for more than 25 years in aesthetic surgical practice. These are often reported in small case series or case reports of treatment and outcome and are being further studied in many clinical trials.[25,26] Evolving clinical trials include not only 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 that do not respond to conventional therapy (such as diabetes, multiple sclerosis, Alzheimer disease, Parkinson disease, severe limb ischemia, traumatic brain injuries, and so forth). Early reports of improvement in chronic conditions, including pain, arthritis, damaged tendons/ligaments, and so forth, are driving many to select this option to improve surgical outcomes or avoid surgical interventions and shorten the demands for physical therapy.

Many clinicians and researchers remained confused about the potentials or best source of stem cells, often believing only refers to use of embryonic tissues. In the past 10 to 15 years, evidence has led to understanding that the body's own fat may be a more plentiful and optimal cell source, avoiding the need to destroy fetal or embryonic tissues or undergo more invasive marrow access to acquire cells and culture-expand them to achieve optimal potential.

Fig. 9. Use of biocellular combination in Achilles tendon injuries. (*Data from* Oliver K, Alexander RW. Combination of autologous adipose-derived tissue stromal vascular fraction plus high-density platelet-rich plasma or bone marrow concentrates in achilles tendon tears. Journal of Prolotherapy 2013;5;e895–912)

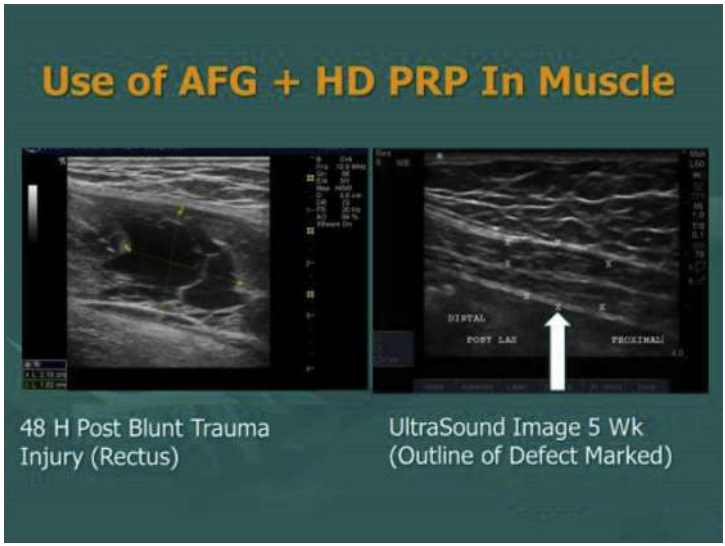

Fig. 10. Use of biocellular use in muscle tears, abdominal wall. Note: Minimal Scar Evidence Residual.

Considering the ready availability of fat, minimally invasive access (using closed syringe liposuction, for example) adipose has become an optimal source for these cells with a high safety profile for patients. As previously described, ATC is the largest microvascular organ in the body and, as such, has become well recognized as the largest depository of undifferentiated stem/stromal cells in the entire body. The ease of gathering fat tissues on an outpatient basis and local anesthetic has led to 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 dedicated clinic procedural facilities.

Specific key cells needed to promote healing and repair reside in tissue microenvironments, where they comprise parts of tissues and organ systems remain elusive. The complex components within the AD-tSVF may be considered to offer a smorgasbord of elements that can become available to any site or tissue. Analyses of growth factors and signal chemicals suggests that the intact AD-tSVF may offer contributions over and above those as isolated elements.[8] The cell groups participating in the healing or repair are subject to important contributions of native cell components in vascularized tissues and, by introduction of concentrates of cells and biologics, seem to autoenhance the site controls and effects. These native site cell groups 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 under way, addition of needed cell types and biological elements specifically targeted (via ultrasound guidance for example) can effectively use the body's own tissues to heal themselves in a more efficient and effective manner.

WHAT IS INVOLVED IN PROVIDING BIOCELLULAR REGENERATIVE THERAPY?

Because the platelets are appreciated as key contributors in provision of critical healing growth factors and signal proteins, the author recommends striving for greater concentrations achieved translates directly with linear increases of those elements.

Acting as a central component in the inflammatory and healing cascade, they help begin and maintain the healing processes in conjunction with the local site stroma and cells. This effect is recognized as an autoamplification effect, wherein the site specific needs are boosted in response during the most important regenerative or healing processes. Factors such as vascular-endothelial growth factor contribute to this process with encouragement of microvessel formation and improved perfusion. Thousands of patients have undergone treatments using these concentrates with quality results in many inflammatory or aging conditions.

Next, the autologous 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 lipoaspiration or lipoharvesting.[27] ATC may be cleaned and compressed (centrifuged) and unwanted liquid layers separated by centrifugation (**Box 8**). 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 with less intercellular fluid load (**Fig. 11**). By effectively reducing the volume of injection materials, earlier recovery of comfort and ambulation is common. Biologics, such as HD-PRP, are then added via closed, sterile luer-to-luer transfer to create a mixture of cells and the important growth factors/signal proteins provided from within the platelet alpha-granules. There is a direct correlation between concentration achieved and the delivery to targets (RW Alexander, unpublished data, 2012).[28]

There are now the capabilities to submit some volumes of the fat harvested (AD-tSVF) that can be separated into another syringe, expose the ATC to certain digestive agents, incubated, shaken to permit cellular isolation, and then neutralized, rinsed, and recentrifuged to create a cellular pellet (AD-cSVF). Enzymatic release of cell-to-cell and cell-to-matrix contacts forms a concentrate of the heterogeneous, mononuclear undifferentiated cellular collection. Once created, these cells are available for intravascular uses or be added back to adipose graft (AD-tSVF) (which still has its bioactive matrix). This cellular additive has been studied and reported in preclinical and clinical trials. This is termed *cell-enrichment* when simply added back to the AD-tSVF, mixed with HD PRP, and carefully guided into identified target sites to assist tissue healing using autologous tissues. This is commonly performed in many areas outside the United States, while FDA suggested guidelines discussed currently are confusing these issues regarding “manipulation” when employing digestive enzymes. Many sites are actively providing these services in the United States, often within controlled Institutional Review Board (IRB) trials or study groups within specialties. The author currently provides both options, following multiple institutional

Box 8
Advantages of centrifugation of adipose derived–tissue stromal vascular fraction

- Creates a density gradient of SVF
- Markedly improved layer separation of
  - Infranatant fluids
  - Red blood cells and cellular debris
  - Lipids, proteases, and lipases (separator disk)
- Optimal centrifugation 800 g to 1000 g for 3 to 4 minutes
- Favors transplantation of maximum stroma
- Decreases fluid load to site and reduces exposure to local anesthetics

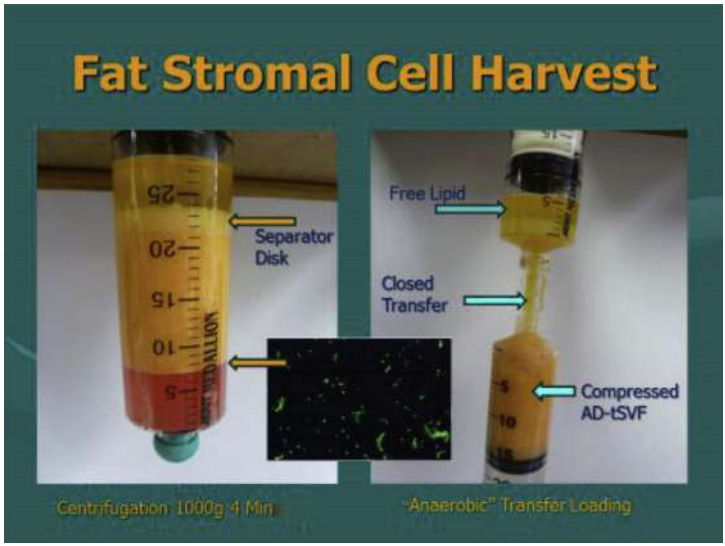

Fig. 11. Centrifuged (compressed) adipose graft layered separation.

and organizational IRB guidelines using specific trial studies by providing the approved trial protocols, both within the United States and internationally.

Termed AD-cSVF, the cellular isolates are currently used for a wide variety of human clinical applications on a global basis. After myriad basic research studies, animal models were tested and reported in the bioscientific literature and gradually reported in translational clinical journals in the medical literature. The parenteral uses (including intravenous, intra-arterial, intrathecal, and intraperitoneal) are reported on a regular basis. Because the cellular group is well recognized as favoring immune privilege, those systemic and autoimmune issues are included in many clinical studies (**Box 9**).

WHAT IS THE FUTURE IN STEM/STROMAL CELLULAR AND BIOCELLULAR TREATMENTS?

There are now capabilities of closed isolation of the large numbers of stem and stromal cells from the adipose tissues. Within such semiautomated and automated closed

Box 9
Current biocellular regenerative therapeutic applications

- Antiaging, unstable scarring and aesthetic surgery
- Skin, hair, radiation applications
- MSK - musculoskeletal uses, joints and structures
- Wound and chronic pain care
- Neurodegenerative diseases
- Disorders - Chonic obstructive pulmonary disease (COPD)
- Crohn's disease and ulcerative bowel disorders

systems, this ability is becoming practical even in outpatient procedural rooms and carefully prepared within sterile protocols. Once this was exclusively possible only in costly laboratory settings, requiring extensive equipment and technician costs. Today such isolation is done in the United States under IRB settings to ensure reporting of patient safety and effectiveness. Clinical trials are gradually being released, many requiring several years to acquire data, compile, and report. A vast majority of such reports are providing clear clinical evidence of patient safety and effective clinical treatment outcomes.

Isolation of these cells permits creation of what is termed, *cell-enriched biocellular grafts*. These grafts, higher in numbers of the heterogeneous undifferentiated cells, are believed to provide an even more potent guided injectable therapy. For example, there are many peer-reviewed clinical articles providing strong evidence of enhanced outcomes within the aesthetic–plastic surgical literature. Over the past decade, there are estimated numbers of use of biocellular therapies in MSK application exceeding 150,000 human clinical uses, with a remarkable efficacy and safety profile. These are reported in case series or reports and should not be discarded out of hand, simply because they are not participating in specific trial settings. It remains a pivotal value to insure that accurate diagnostics and guided placement to defined targets. Ultrasonography, with its dynamic abilities during examination, will remain a needed core competency for those taking care of MSK and chronic wound cases.

In the future, it is likely that such isolated cells will provide parenteral (intravenous, intra-arterial, intrathecal, intraperitoneal, and so forth) pathways and become effective for an expansive treatment in disorders, such as neurodegenerative diseases (multiple sclerosis, Alzheimer disease, amyotrophic lateral sclerosis, Parkinson disease, brain injuries/stroke, and so forth), diabetes, chronic lung disease, heart disease and damage, chronic wound healing, fibromyalgia/causalgia, ulcerative bowel disease, Crohn disease, colitis, and so forth (**Box 10**).

A RECENT ADVANCE IN USE OF BIOCELLULAR USES: NANOFAT (EMULSIFIED ADIPOSE DERIVED–TISSUE STROMAL VASCULAR FRACTION)

Over the past 2 years, major advances in processing the lipoaspirated AD-tSVF via mechanical emulsification has evolved. Although still favoring the same biocellular product creation, including use of additive advantages offered by addition of HD-PRP concentrates while retaining small-fragment AD-tSVF capable of injection via small-bore needles. Recent published evidence has shown that creation of the mechanically emulsified nanofat does not have a detrimental impact on stem/stromal cellular numbers or viabilities while markedly reducing the volume of ATC provided by mature adipocytes (**Fig. 12**).

Box 10
Trends in stem/stromal biocellular uses

- Targeted applications cells + HD-PRP
  - Skin, hair restoration, and scarring
  - MSK injuries and regenerative needs
- Cell-enrichment potentials increasing
- Emulsified AD-tSVF and systemic uses
- Culture/expansion and cryopreservation of AD-cSVF
- Increased uses in devascularized wounds and tissues

Fig. 12. ACM emulsification system (Healeon Medical, Newport Beach, CA) AD-tSVF with HD-PRP additive.

With the ability to inject through small-bore needles, patient comfort is enhanced along with offering a range of intradermal and small joint–targeted applications. The abilities of biocellular modalities to promote wound healing and regenerative capabilities via intradermal placement have created opportunities to permit improved skin circulation and texture, skin aging and radiation damage, and hair regeneration and participate in chronic wound applications as well as many small joint and superficial targets in MSK applications (**Fig. 13**).

Fig. 13. Emulsified AD-tSVF intradermal injection use. EC, endothelial cells; MSC, mesenchymal stromal cell; TNC, total nuclear count; RBC, red blood cell. (*Courtesy of* Tonnard P, 2012.)

Box 11
Biocellular success is a three-legged stool

- Highest-quality graft harvest
- Achieve a high density of PRP
- Exact placement in target sites

WHO PROVIDES BIOCELLULAR TREATMENT?

Patients and providing doctors (eg, primary care health care providers, internists/neurologists, aesthetic–plastic surgeons, general surgeons, orthopedic surgeon, emergency/sports medical specialist, pain management specialists, wound care centers, and so forth) decide whether candidates have a condition that has reasonable potential for improvement through use of combinations of biologic and stem/stromal cellular treatment (see **Fig. 10**). Thorough physical and pretreatment evaluations are essential in diagnostic and treatment planning. Circulatory, neurologic, and indicated systemic conditions should be documented. In cases orthopedic applications, use of metrics, such as range of motion, indicated MRI studies, and high-quality ultrasonographic imaging, combine to determine the specific locations of problems and guide proper placement (see **Fig. 9**). Use of high-quality 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. Palpation may provide fairly accurate placement with experienced providers; targeted and tracked therapy consistently correlates with earlier and improved clinical outcomes. Use of metrics that are more objective and successful monitoring of many patients, including range of motion, remodeling of tissues in repeated interval ultrasound studies, and return of strength, provide valuable informative standards. For many years, prolotherapy major benchmarks were limited to patient-reported pain levels, activity levels, and perceived improvement as their primary metrics (**Box 11**).

Most times these procedures are completed on outpatient, ambulatory basis using local anesthesia, nitrous oxide, or occasionally 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. Since the advent of using a mechanical emulsification system, which permits guided injections through very small-bore needles (25 gauge–30 gauge) without statistically reduced viabilities, the ability to provide improved patient comfort and access to small joints is rapidly becoming an appealing option (**Box 12**).

Box 12
Biocellular therapies change treatment paradigm

Things have changed

- Biocellular therapy
  - Combination more potent than either alone
  - Shown to be the most potent regimen
  - Offers reduction toxic inflammation
  - Effectively heal with minimal scar formation
  - Provides needed cells for specific site
- Parenteral applications for systemic issues rapidly evolving

SUMMARY

"Using your own tissues to heal" represents a major health care paradigm change and is one of the most exciting minimally invasive options currently available. Biocellular regenerative therapies are rapidly improving in documentation and cellular analyses and are gaining good safety and efficacy profiles. Once considered purely experimental, they have entered into an accepted, translational period to clinical providers, backed by improving science supporting the basic hypotheses. It is a well-recognized and reported alternative to many traditional medical/ interventions.

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