



Overview of Cellular Stromal Vascular Fraction (cSVF) & Biocellular Uses of Stem/Stromal Cells & Matrix (tSVF + HD-PRP) in Regenerative Medicine, Aesthetic Medicine and Plastic Surgery

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Abstract

Paper presents an overview of cellular and biocellular uses of both cellular Stromal Vascular Fraction (cSVF) elements and evolving applications of tissue Stromal Vascular Fraction (tSVF) component in repair and regeneration. Use of autologous stem/stromal components in human homeostasis and healing has shaped an evolving paradigm shift in many disciplines of Medicine & Surgery. Mass production or patentability of a patient's own cell and stromal elements is a very unlikely possibility, making the use of these element supported by pharmaceutical companies unavailable.

As a very heterogenous mixture of cells in the cSVF, some actual stem/stromal cell mix offer unique uses which are complimented in the actual multipotent cells and their supportive cells felt to be important in optimizing their functions. Appreciation that the paracrine secretive factors play an integral role in the regenerative milieu, have led to examination of signaling via exosomal & micro vesicle communications.

Discussion of terms, who are providing these services, and the realm of applications evolving is discussed. Appreciation of the contribution of "biologicals" (in this context, meaning platelet-rich plasma concentrates) to enhance the repair and regenerative uses of stem/

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Evolution of Regenerative Medicine in Plastic Surgery

For more than three decades, Aesthetic, Plastic and Reconstructive Surgeons have devoted themselves to understand the intricate management of both acute and chronic wounds, including the fundamentals of healing and repair. Through those years, the importance of examining the homeostatic and replacement mechanisms. Throughout, the value of understanding how various tissues accomplished such task has evolved to one of doing everything possible to encourage vascularization of sites, activations of reparative cells (both remote and locally encouraged via signal proteins and growth factors) which are needed in essentially all groups of patients. In the early 1990s, appreciation of the value of using the most available and rich source of such molecules could be isolated and concentrated, known as Platelet Rich Plasma (PRP), with platelets serving as a very rich source for a multitude of growth factors, cytokines & signal proteins from within their alpha granules stored in their cytoplasm. First reports were examined in the area of maxillofacial and craniofacial bony reconstruction in a variety of situations in the early 1990s. Wound healing was felt to significantly improve the time, users were required to utilize bulky and costly "cell-saving" technology, meaning that major OR uses were a limiting factor, in that most required presence of technicians and cardiovascular ORs to be able to accomplish. In the most complicated surgeries, this was felt by many to be a "value-added" contribution to the intraoperative surgical care and follow up care requirements.

Early in the 2000s, these bulky machines, were replaced by a number of medical device companies creating smaller, efficient, and which provided consistent and high concentration abilities. At the same time, this expanded delivery capabilities in a more cost effective and consistent protocols, for use in both the Operating Room and in the Outpatient Surgical Centers. With the advent of small footprint and affordable systems, the uses and advantages of utilizing these autologous elements expanded exponentially. The area of chronic wound healing, devascularizing injuries and a vast range of soft tissue repair became ubiquitous. As of the writing of this contribution, sophisticated understanding of optimization of exactly which product or content is most important in the various situations, the applications continue to grow and show their values.

Poorly understood is the fact that MANY products have rushed to market, each claiming capabilities and concentrations that are simply incorrect. During the evolution, many surgeons (especially Orthopaedic Surgery and related areas) were disappointed at the outcome improvement. A significant issue is that, simply isolating some platelets did NOT yield high enough, or consistent enough, products which could deliver the most optimal outcomes. By accepted medical definition ABOVE four to six (4-6X) times the individual measured concentration is considered High Density Platelet Rich Plasma. It has become very clear that there are those which provide a low concentration

(single spin centrifugation yielding 1.5 -2.5 times measured baselines) often can be of value in facial and dermal uses, whereas, much higher (>4-6 times baseline) concentrates are now consistently and easily obtained in point of care within outpatient or Operating Room settings.

Since it is now well established that there is a linear increase in available critical growth factors and signal proteins, most surgical and regenerative applications are favored with the higher concentrations. In addition, the level of understanding is being evolved where decisions of whether high hematocrit version, or a low hematocrit version have a clear indication tied to be utilized. In Aesthetic Plastic surgery, it is considered that the use of the highest concentrations to have the highest clinical advantage over the lower concentrations.

Over the past 15+ years, many indications and uses have evolved. One of the most important related to the addition of these platelet concentrates to uses in autologous fat grafting (in small and large volumes) became more clear. The lipid cysts and micro calcification retention in use of autologous fat graft breast augmentation, has enhanced the use of the platelet products in such cases. In addition, observational findings of importance in the areas of the face/neck and the body areas has come to the forefront.

These concepts have made a major contribution that has spread well beyond the wound healing and aesthetic applications. For the past decade and one-half, uses in regenerative medical and surgical fields have become known as highly effective and safe alternatives to use of steroids, non-autologous products, and changed some indications for invasive surgical interventions. This contribution will expand on this background and evolving protocols aimed at skin, tissue supplementation and extensive applications using targeted guidance in chronic pain, musculoskeletal applications, and systemic cellular therapeutic options.

Evolution of Cell-Based & Biocellular Therapies

Over the past decade, great strides have been made in the understanding and potentials of systemic and targeted cell-based therapies. By in depth study of homeostasis and repair mechanisms, it became clear that use of activation of regenerative cells and signals played a core role in our bodies.

Starting decades ago, use of an irritant solution (Dextrose) was injected to stimulate inflammation replaced in the past few years with transition to injecting various Platelet-Rich Plasma (PRP) concentrates for supporting an effective inflammatory reaction at damaged sites. Contained growth factors and signal proteins became recognized as offering a significant improvement. It seemed to be limited by incomplete repair while requiring a series (often 4-6 uses) to achieve long-term clinical improvement. Even then, if a site is severely depleted of cells and available growth factors often does not achieve a long-term improvement. Current evolution of combining these trophic growth factors and signal proteins PLUS concentrated undifferentiated stem/stromal cells seemed like a logical and effective modality, moving into the forefront since 2000. Aesthetic and reconstructive applications led the way, as constant challenges of injury, loss of circulatory capabilities, degenerative changes, repair, etc. demanded an optimal approaches to

structural augmentation and regenerative needs. In depth examination of how our body maintains itself, revealed that undesignated cells were integrally important to replace aging cells (such as skin, hair, bowel lining, etc.). Early on, fat was not thought of as undergoing such homeostatic mechanisms, since typical mitotic activities were not observed.

We now recognize that, rather than a static number of cells varying only in size, mature adipocytes do actually undergo total replacement at a rate of 10-20% per year, but do so in a different form of cell division, known as "asymmetric cell division". The ability to have resident precursor cells which are capable of responding to local site signals, and the ability of providing a replacement cell of the needed type or participate in paracrine actions, resulting in potential replacement cell differentiation, while retaining a single, precursor cell type. At this time, the importance of the paracrine (secretory functions of exosomes and Micro Vesicles) are now thought to be a more important factor in activation, proliferation and functions of the resident undesignated cell groups. Without the asymmetric replenishment mechanisms and cellular signaling activities, we would eventually have an uncontrollable stem/stromal cell population [1].

With the advent of FDA approved platelet programmable, centrifugal devices for custom High Density Platelet Concentrations (HD PRP) 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 higher delivery of important factors intrinsically involved in all wound healing and repair (Figure 1).



Figure 1: Left: Adipose tissue complex (Mature Adipocytes provide approximately 90% by volume; 10% of nuclei in tSVF); Right: Closed system for centrifugation, cell isolation/concentration at point of care (CC1000 Healon Medical, Newbury Park, CA, USA).

Use of the most favorable for use in cell-based therapies including easy and safe access coupled with plentiful autologous stores of a group of cells possessing multi-potent potential. 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 damaged sites for repair or regeneration. Researchers are understanding that the participation in the regenerative properties offered by these cells are not limited to the cell components, but also due to active paracrine secretive activities participating in the chemotactic and signaling processes.

Research has confirmed that stem cells are associated and stored in proximity to the microvascular capillary system and adventitia (Figure 2). 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 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 was made by researchers in finding high numbers of such cells with multiple cellular potentials of differentiation [2, 3]. This finding indicates that multipotent, undesignated cell populations are local on or near perivascular tissues, and is not surprising that the tissue complex represents the largest microvascular organ in the body.

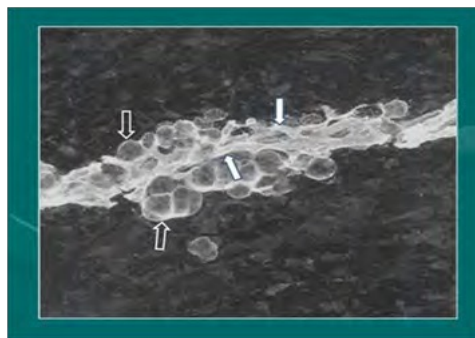


Figure 2: Micrograph demonstrating relationship of intimate relationship of microcapillaries and attached adipocyte during development (Photo: Fetal pig during extremity formation).

Enhancement of cellular and biologic (in this context referring to growth factors, signal molecules such as contained in platelets) therapies comes directly with the ability for providers to be able to identify, target and guide the Cellular Vascular Fraction (cSVF) or cellular-biologic combination to areas of injury or degeneration. Management of protocols have led to improved structural grafting successes in addition to the contribution in orthopedic and sports medicine applications using the same cellular and platelet-derived growth factors & related components. In that regard, ultrasonography has clearly become a MAJOR feature to clinical responses and success. As example, in medium and deep targets, or ultrasound capabilities offers the optimal integral part of successful responses. Over the past decade, thousands of treatments using Biocellular Regenerative Medicine© techniques have proven safe and remarkably effective. The information provided in this chapter is intended as an introduction to important concepts and describe the current logic believed to be involved. Major steps have been taken, moving from the laboratory to the bedside. Today, musculoskeletal (MSK) and aesthetic-plastic with this combination of Cellular or 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, pericyte/endothelial, perivascular, or mesenchymal cells, etc.) found widely spread within our

body and which participate in tissue maintenance, repair and regeneration. Since 2010, Science and Medicine have advanced through what is termed “translational phase”, where proven laboratory science have demonstrated important contributions joins the clinical application of the science in human applications in the last decade. Since, there has been controversy concerning the use of the term “stem cells” in the current practice of medicine. Unfortunately, the misuse of the terms “stem cell” is being interpreted as uses of pure “embryonic” or fetal stem cells, implying the destruction of embryo or fetal tissues. In the past decade, the recognition of adult stem/stromal cells has advanced to the point that it is now widely documented and published (Figure 3) it was confirmed that

- Return To Full Form or Function
- Eliminate Or Markedly Decrease Pain
- Resist Recurrence Of Injury Or Damage
- Reverse, Stabilize, Resist Degeneration
- Avoid Immune Reactions
- Prefer *Autologous* Cells/Tissues For Repair
- Accelerate Healing Processes
- Restore Tissues With Minimal Scarring
- Minimal Tissue Distortion & Morbidity
- Prefer Minimal Manipulation Requirements
- Predictable & Reproducible Outcomes

Figure 3: General goals in regenerative medical applications.

Cellular components

Biocellular regenerative medicine currently refers to use of autologous multipotent cells capable of participating in maintaining our tissues (homeostasis), healing and regeneration. Studies demonstrating the values of the highly variable stromal cell populations has exploded, now to the point that active reports and studies of component cells of adipose origin now exceed the study of non-hematopoietic stromal cells in plastic surgical applications. The importance of such studies and understanding that adipose tissue deposits have gained such recognition due to the much 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 our bodies that contain microvascular supply maintains a reservoir of such cells within the stroma and matrix. That said, we now recognize that Adipose Tissue Complex (ATC) represents the greatest microvascular organ in the body, and serves as an excellent option to access for a variety of uses. Many important studies suggest that adipose-derived stem/stromal cells of mesodermal origin provide between 1000-2000 times the actual numbers found in bone marrow [7]. With the relatively easy collection of adipose tissue, less penetration, widely heterogeneous cellular populations, and important immune-privileged properties, subdermal fat deposits effectively and safely serve as a primary source for gathering stem/stromal cells (Figures 4 and 5).

Efforts at using mechanical separation (ultrasound, nutational, emulsification efforts) have not provided a complex chemical binding of stem-capable group due to the cell-to-cell,

or cell-to-matrix (ECM and periadventitial) attachments. The complex multifaceted connectivity necessary for cells to act on area signaling that exists with the ATC simply will not yield optimal separation and cellular purity needed to create a cSVF pellet. At this point in time, use of incubation, agitation, and digestive processes (blends of collagenase and neutral proteases) remain the optimal way to isolate and concentrate the desired cellular groups (Figures 6 and 7) [8].

- Easy Harvest Access
- High Quantity Of Viable Stem/Stromal Cells
- Minimum Morbidity Of Donor Site
- Safety After Implantation (Autologous Best)
- Multipotent & Proliferative Cell Groups
- May Be Isolated & Concentrated If Desired
- Prefer Inclusion Stromal Elements (Bioactive)
- Paracrine Functions Encouraged
- Secrete Immunomodulatory Factors
- Secrete Pro-Inflammatory Factors (Benign)
- Immunoprivileged Cell Groups Preferred

Figure 4: Adipose tissue complex: Optimal cell source.

KEY Multipotent Cells Found In AD-tSVF

- Mesenchymal Stem/Stromal Cells
- Pericyte-Endothelial Precursor Cells**
- Adipocyte Precursor Cells

Mature Adipocytes (Not Multipotent Temporary But Contribute In Signaling)

Tissue Resident Cell Populations & Bioactive NATIVE Structural Matrix Contribute to the Regenerative Process

** May Be The "Dign" Of All MSCs

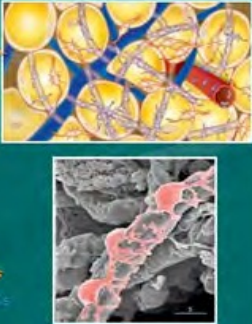


Figure 5: Multipotent cells found in Tissue Stromal Vascular Fraction (tSVF).



Figure 6: SEM Image of pericytes relationship to microcapillary. NOTE: See many complex cell-to-cell and cell-to-matrix attachments.

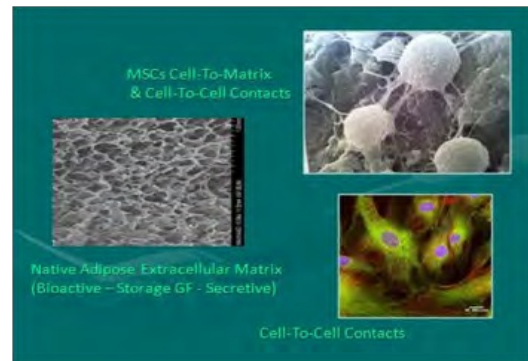


Figure 7: Left: Native scaffolding adipose tissue complex after cellular removal; Right Top: SEM mesenchymal stem cells intimate relationship with extracellular matrix and cell-to-cell elements; Right Bottom: Mesenchymal stem cells micrograph with NAPI nuclear stain.

The small nucleated cells found closely associated within the vascular tissues are now recognized as serving important roles in maintaining normal tissue content (h... respond to injury or disease processes in a constant effort to maintain, heal, or repair damaged cells (as in aging, arthritis, musculoskeletal tissues, neurological disorders, etc.) the body. The remarkable design of the human body uses these reservoirs of available, non-differentiated, multipotent cells as the ti... of major or micro-trauma and aging. By secretion and signaling of certain chemicals from a degenerating or injured site, these multipotent cells (i.e. can become various types of cells) can be called upon to participate in the repairs needed to restore tissues and functions. At frst, it was felt that the ce... perhaps only, importance, experience now show that the paracrine secretory capabilities intrinsically contribute in critical roles (thought via exosomes, microvesicles, etc.). There are many peer-reviewed publications which 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.

Biologic components

The “biological” components, in this paper context, refer specifically to the availability for diverse and important variety of growth factors and signal proteins which interact with the cells of the degenerative or damaged sites to help recruit needed reparative cells and materials to repair the area. There are two major “biological” components in common use at this time. First, 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 [9]. 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 (with exception of provi... release of platelet contents). Platelets represent a storehouse of small granules, each containing very important growth factors and signal proteins that serve to “quarterback” to the healing cascade, and do this for a significant time during the... portant chemical available from these granules are essential for blood vessel replacement and repair in order to improve the circulation

ability critical to healing needed oxygen cannot reach the area of damage, nor permit migration of a variety of cells from nearby or distant cell sites (Figures 8 and 9).

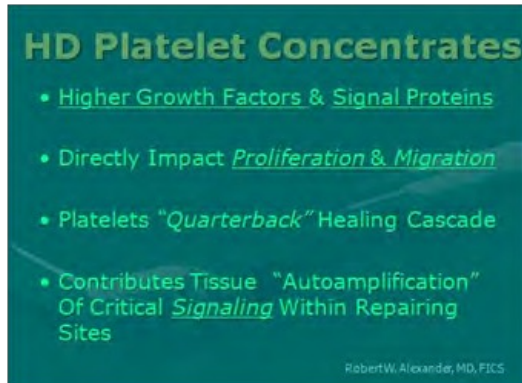


Figure 8: Why use of High Density Platelet Concentrates (HD-PRP)?

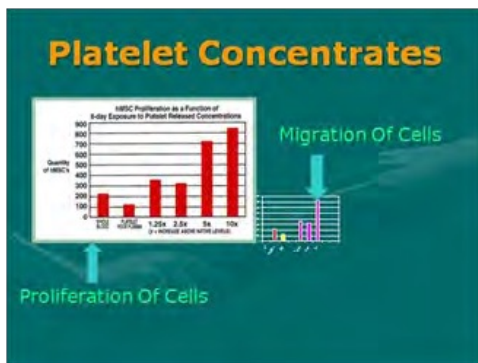


Figure 9: Importance of HD-PRP on proliferation of cells & migration of cells. [NOTE: Linear response as concentration increases.

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. Although bone marrow does demonstrate microvasculature and, therefore, does have some designated reparative cells (stem/stromal cells of the reparative types). That said, the vast majority of “stem” cells located in marrow belong to the “hematopoietic” stem cell group are found there, and not considered extremely valuable in the case of regeneration or reparative cellular group. However, the desired stem/stromal cells are found in relatively very low numbers, compared to adipose tissues and its microvasculature. Therefore, many now consider bone marrow as useful primarily as a valuable biologic and platelet source. In order to become a valuable primary cell contributor for such reparative group, 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 a bit more invasive to obtain, pose higher complication-sequelae rates, and are significantly more expensive - to K t e y e a p d a u l t i t e n n u s l . t i l p n o t a e d n t i t c r e i s s u e t o a r t e r 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), have led to a major trend shift

the primary difference between concentrated platelets and peripheral blood versus marrow concentrates, is a very large store of hematopoietic stem cells (HSCs) while offering almost identical platelet content. At this time there is very little the regeneration process of MSK t

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 appears to be their important paracrine secretory influences, r u a l cellular components and physical engraftment. Further, it well established that the mesenchymal group (MSC) of multipotent cells may actually originate from the pericyte-endothelial cell groups [10]. These offer a great amount of overlapping capabilities, in vitro, suggesting that all tissues having some microvasculature, have resident stem/stromal elements capable of of damage or degenerative effects. It is suggested that the multi-tissue MSC-like groups overlap at greater than 95% in their differential capabilities, at least in vitro conditions. Host site interaction with these stem/stromal cells, growth factors, and signal proteins seem to create a complex, heterogeneous precursor population that is considered “site specific” in many of their r

How did Biologic & Cellular Therapeutic Concepts Evolve?

Aesthetic & Plastic Surgeons have traditionally dealt with wound healing and minimizing 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 (homeostasis). For many years, the importance of biologics as a derivative part of platelets become appreciated, not only for it clotting functions, but for the gradual release of critical chemical components essential to the healing processes with individual sites. These biocellular concentrates are felt to immediately begin to participate in secretions c a p a e r a t i o n , while local cells begin to actively contribute. In addition to these elements, appreciation of the importance of the bioactive, 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 [12 - 14]. It has become influences cellular changes within multipotent populations found in essentially ALL tissues which have microvasculature. In addition, appreciation of the importance of cellular secretions (paracrine and autocrine) within these undifferentiated cell groups has been reported to be of as great, or greater in some instances, as the multipotent cellular differentiation effects [15].

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

to K t e y e a p d a u l t i t e n n u s l . t i l p n o t a e d n t i t c r e i s s u e t o a r t e r 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), have 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, since adipocytes within the ATC were determined not to undergo mitosis, it was assumed that these were relatively static in number, and only changed in size according to lipid storage droplets. It is now clear that adipocytes do have a life cycle, replacing all mature adipocytes every 5-10 years [16]. Examination of how they accomplished this replacement, via a process of “asymmetrical cell division”, was found to be the mechanism, thereby preserving one stem cell during the process. This process results in activation of a precursor cell population is capable of reacting to secretions from a senescing adipocyte, beginning process of the precursor replacement cell to occur, while preserving the precursor cell. This replication by the asymmetric cell division results in a replacement immature adipocyte, while the other portion retaining its precursor forms and abilities. This is logical, in that, if otherwise, we would accumulate a massive number of precursor cells in our tissues (Figure 10).

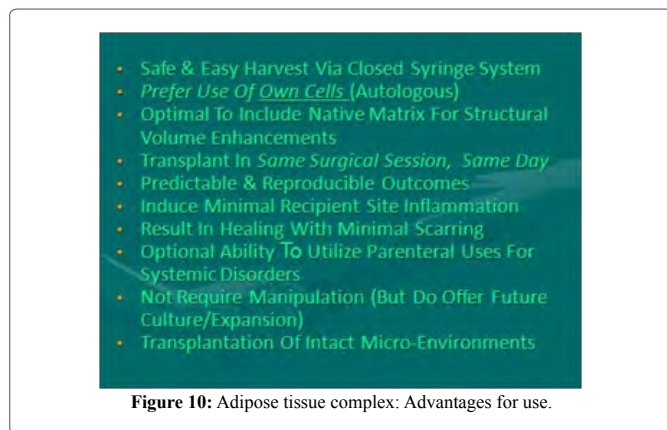


Figure 10: Adipose tissue complex: Advantages for use.

It was not until Zuk et al., adipose-derived cellular stromal vascular fraction (AD-cSVF), with capabilities of differentiation to a variety of tissues, including bone, cartilage, tendon-ligament, muscle, fat, nerve, etc. (Figures 11 and 12). Once this capability was identified, efforts to isolate specific types started. This has proven 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 Density Platelet concentrates (HD-PRP) has received a great deal of attention. Identifying stem/stromal cells which can participate in the processes, have revealed what an “ideal” cell-based therapy may represent (Figure13).

In the early 1990s, a method of closed syringe microcannula liposuction was patented, permitting means of acquiring ATC for use as a small particle structural graft [17]. This has since evolved to a disposable, microcannula option which permits safe and effective tSVF, without the potential cleaning and sterilization. In the past 15 years, clinicians and laboratory researchers have identified and interact to provide remarkable contributions in tissue repair and regeneration. These have been heterogeneous population, closely related to cellular, adventitial areas, and extracellular matrix components (Figure 7). The utilization of such cSVF is currently the

(MSC or ADSC) was thought to be the most important multipotent “stem” cell. Further examination, however, now suggest that these may serve a “sentry” capacity, and that the actual cell group known as pericyte/endothelial stem/stromal cells (Figures 14, 15) [10].



Figure 11: Mesenchymal Stem Cell differentiation (MSC) Potentials (Basic):

a) Top Left, Micrograph Control MSC *in vitro*; b) Bottom Left, Bone; c) Top Right, Adipose; d) Bottom Right, Cartilage. [NOTE: Overlapping Differentiation Capabilities of All MSCs Is Extensive].

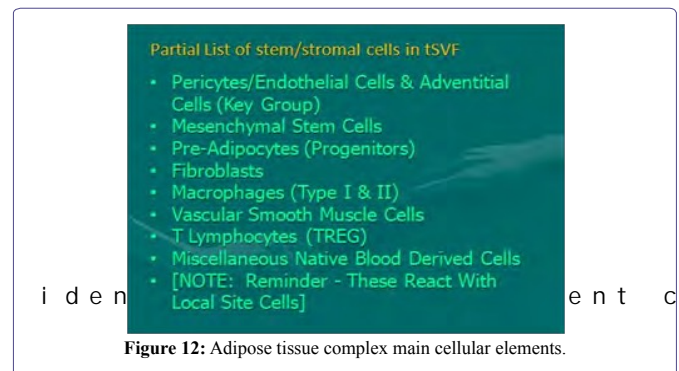


Figure 12: Adipose tissue complex main cellular elements.



Figure 13: Ideal cell-based therapy advantages.

of multiple clinical trial applications, and is heavily utilized 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, they will miss more than 85% of the pertinent information and data evolving on almost a daily basis, as the important advances appear with basic science. It is said that these information channels are currently doubling the existing knowledge base every 4-6 months, making keeping current with scientific and the clinical

In clinical applications, use of AD-tSVF has taken the primary role in aesthetic & regenerative uses, as it is a product that provides the full complement of structural (stroma, ECM) elements plus the resident cellular population of the AD-cSVF. The existing native stroma of ATC is now recognized as of great importance, not only due to the available attachment sites, but ALSO to the actual secretory bioactivity of the tissue. This dual role is considered of great importance, making use of existing native scaffolding of ATC, considering the “mini-microenvironmental attachments” felt to positively interact and contribute to the local recipient sites in need (Figure 17) ultimately to a



Figure 14: Closed, Sterile Disposable, Microcannula System (GEMS) (Tulip Medical, San Diego, CA, USA). Left Top: (2.11 mm OD) showing multiport injector (1.65 mm), sterile clear luer-to-luer transfer; Top Right: External universal non-disposable lock; Bottom Right: Microcannula tips.

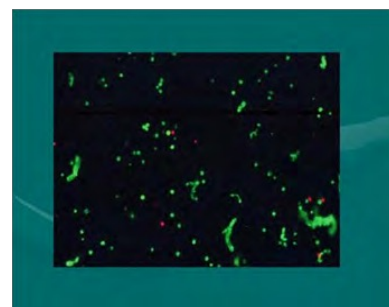


Figure 17: Flow cytometry (Live/Dead) stain (Acridine Orange A/O & Propidium Iodide (PI)) of a Platelet Rich Plasma (PRP) sample. NOTE: The green “strings” are viable cells with extracellular matrix residual attachments. Effectively transplantation of living Microenvironments (tSVF).

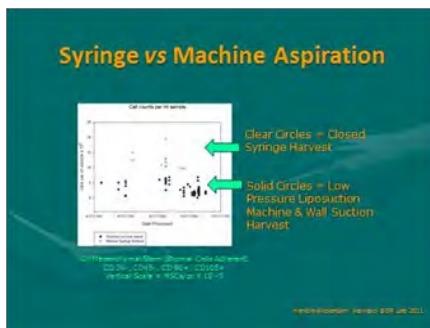


Figure 15: Comparative cellular recovery: Closed syringe microcannula harvest versus machine vacuum on mesenchymal cells in ATC.

TERMS: tSVF & cSVF

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 Being Use As “Cell-Enrichment” Protocols In Tissue Augmentation & In Degenerative Disorders

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Figure 16: Important understanding of terms: Tissue Stromal Vascular Fraction (tSVF) and Cellular Stromal Vascular Fraction (cSVF).

In biological aspects, it is important to clearly recognize that not all platelet-rich plasma preparations and concentrates are the same. It is clearly shown that, the amount of growth factors, signal proteins, and important chemical agents have 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 being called PRP, particularly since most practitioners do not have the capability of confirming actual patient measured baseline concentrations. To qualify as a true “high-density” PRP, we utilize the minimal concentrations to be baseline, not a calculated extrapolation. This is a very important concept to understand, based on the correlation of such concentration to cellular proliferation and the importance of choosing the optimal platelet concentrates for the use, and understanding that NOT ALL PRP CONCENTRATES are equal. Refinements in HD-PRP optical subdivisions, such as low and high hematocrit solutions which have definite clinical implications to

The value of use of centrifugation has increased in Biocellular applications, as it creates a very effective “gravity density separation”, important to avoid cellular 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 or small joint placements. This unneeded load is felt to potentially impact site perfusion (as potential may negatively impact perfusion and healing in sites with small potential spaces. This is a factor of importance in many plastic surgical, reconstructive poor perfusion work applications, such as seen in small joints and limited perfusion sites [19].

The final area of importance is the ability of optimal targeting of areas of damage, degeneration or inflammation. Without use of high resolution imaging, 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

Within the past 4 years, an interesting 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 forming structural fat grafting in aesthetic surgery) as they are gradually lost and removed following their anoxic exposure. It is likewise clear that the stem/stromal cells in the ATC are not as susceptible to those conditions, and in fact, may be stimulated in low oxygen tension environments [21]. Recent publication of viability and numbers of stem/stromal cells remain that the relative numbers of such cells remain statistically the same as those not submitted for emulsification process is that the AD-tSVF not only retains valuable stromal tissue, but 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, hair loss plus permits more patient comfort in MSK injections (incl

In regenerative medicine, the main goals are relatively well established (Figure 3). Likewise, description of "Optimal" features of cellular based therapy in both aesthetic and regenerative applications are becoming standardized. It is important to recognize that the combination of platelet concentrates and AD-tSVF appears to be more effective than either of the entities by themselves.

Understanding the "Workers and Bricks" Analogy

Considering chronic wound and non-healing ulcers, a simple analogy is often helpful in understanding the importance of both the biological and the cellular elements to achieve more rapid and complete healing and repair (Figure 18).

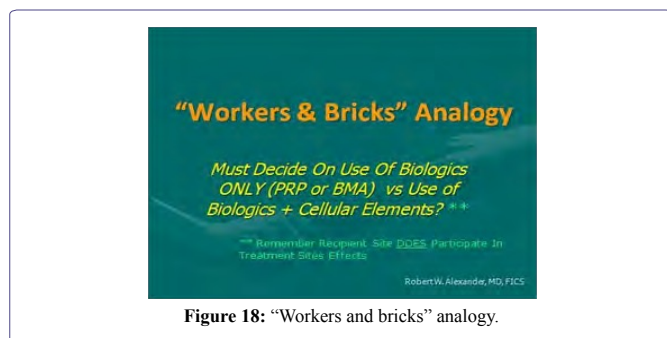


Figure 18: "Workers and bricks" analogy.

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 to 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 MSK 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 the "bricks" aged ones. The "bricks" in this analogy come from the cellular source (cSVF). Combining biologics + cell sources 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, 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 measured circulating platelets) to form the therapeutic mixture known as "Biocellular Regenerative Matrix".

This mixture is in current use in aesthetic (plastic), reconstructive & wound healing, sports & pain medicine, orthopedic medicine and surgery, neurological disorders, musculoskeletal and arthritic applications, and a wide area of overlapping disorders.

What Are "Adult Stem/Stromal Cells"?

These are a diverse group of "non-designated" cells found throughout essentially all the tissues of our bodies primarily associated with perivascular and interstitial areas. They serve as a reservoir of replacement and repair cells, which react to injury, aging or disease. "ADULT" cells in this category are "stromal cells" or "stromal" cells, and should be clearly separated from embryonic cells. They are also called by confusing names, such as "progenitor" or "precursor" cells, which mean they have the capability to differentiate into different types of cells, via responses to growth factors and signal proteins within the actual microenvironment within which they are located. For example, if you have a muscle or ligament tear, ideal tissue components (native to site) plus these non-differentiated cells are felt to participate in healing or repairing the damage providing replacement muscle or ligament tissues, rather than resulting in scarified tissue. Scar tissue is considered a critical factor in how rapidly and effectively the body can repair itself. With that understanding, significant "benign" inflammatory process ("pro-inflammatory"), promoting healing and development with retention of more normal functional abilities. Not only does this improve the quality of the healing, but improve resistance to reinjury. An example is often provided considering that the SVF component include and recruit helpful T-Regulatory (Treg) cellular group (associated with prostaglandin secretive elements). By providing the needed elements to such a site, the body is given the opportunity to fully repair damaged areas, often by combined cellular and biological events (Figures 19 and 20) [23-25].

There are many experiences in subdermal fat in the past 15 years in musculoskeletal area, and for more than 30 years in aesthetic surgical practice. These are often reported on small case series or case reports of treatment & outcomes, and are now being further studied in many clinical trials [26]. Evolving clinical 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 minimally or not respond to conventional therapy (such as Diabetes, Multiple Sclerosis, Alzheimer's disease, Parkinson's disease, Severe Limb Ischemia, Traumatic Brain Injuries, etc.) [27,28]. Early reports of improvement in chronic conditions, including pain, arthritis, damaged tendons-ligaments, low back & facet degeneration, etc., are driving many to select this option to improve surgical outcomes or avoid surgical interventions and shorten the demands for physical therapy.

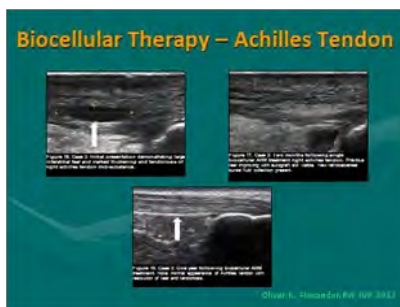


Figure 19: Ultrasound guided; biocellular Achilles Tendon tear pre-treatment [NOTE: Rest of tendon showing tendinosis]; Top Right: Post-treatment tendon (At 6-8 Weeks); Post-treatment one year (1 yr) [NOTE: Resolution of tear without scarring, rest echotexture].



Figure 20: Use of tSVF + high density platelet rich plasma (PRP) for torn rectus abdominus trauma. Left: Pre-Treatment image; Right: Area of trauma at 5 weeks post-trauma. [NOTE: Return of muscle echotexture without scarring].

There are many experiences in such cases over the past 15 years in musculoskeletal area, and for more than 30 years in aesthetic surgical practice. These are often reported on small case series or case reports of treatment & outcomes, and are now being further studied in many clinical trials [26]. Evolving clinical 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 minimally or not respond to conventional therapy (such as Diabetes, Multiple Sclerosis, Alzheimer's disease, Parkinson's disease, Severe Limb Ischemia, Traumatic Brain Injuries, etc.) [27,28]. Early reports of improvement in chronic conditions, including pain, arthritis, damaged tendons-ligaments, low back & facet degeneration, etc., are driving many to select this option to improve surgical outcomes or avoid surgical interventions and shorten the demands for physical therapy.

Many remained confused about the potentials or best source of stem/stromal cells, often believing this only refers to use of embryonic tissues or non-autologous sources such as placental amniotic or umbilical cord-derived cells. In the past 15 plus years, much evidence has led us to understand that our own fat may be a much more plentiful and optimal cell source, avoiding the need to destroy fetal or embryonic tissues, or undergo more invasive marrow access in order to acquire cells and culture-expand them to achieve optimal potential. Further, the stem cells found in bone are heavily weighted to the blood forming side, rather the reparative group of cells.

Considering ready availability of fat, minimally invasive access (using disposable, microcannula (<2.4 mm) closed syringe liposuction for example), adipose now has become an optimal source for these cells with a high safety profile. ATC is the largest micro-vascular organ in the body, and as such, have become well recognized as the largest depositories 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" and "Cell-Based Therapy" for a wide variety of disorders and conditions. With the advent of closed cell isolation/concentration systems (incubation, agitation, digestion of cellular contact points) at Point-of-Care (POC) availability, availability of parenteral intravascular deployments can be achieved. It is most common for these procedures to be performed in outpatient Ambulatory Surgical Centers (ASC) or dedicated clinic procedural facilities.

Specific "Key" cells have been identified and repairs reside in tissue micro-environments, where they comprise parts of tissues and organ systems. However, remains somewhat elusive. The complex components within the AD-tSVF may be considered to offer "smorgasbord" of elements which can become available to any site or tissue. Analyses of growth factors and signal chemicals would suggest that the intact AD-tSVF may offer contributions over and above those as isolated elements [9]. 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 appear to "auto-enhance" 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 underway, addition of needed cell types and biological agents (ultrasound guidance for example), can effectively utilize your own tissues to heal themselves in a natural way.

What is involved in Providing Cellular or Biocellular Regenerative Therapy?

Cellular therapy begins with the exact same harvesting and processing of ATC via microcannulas, including centrifugation to remove the remaining suspensory fluid. The remaining fluid is then diluted with a very diluted local/epinephrine solution in Normal Saline. Once removed, the tSVF derivative is exposed to digestion (typically using a blend of guaranteed sterile enzymes), incubation and concentration of the separation of cSVF portions. This cSVF is then neutralized and rinsed to remove any measurable collagenase and protease activity.

a concentration “pellet” of cSVF. It is important to note, that there are small footprint quality-terminating viability, cell numbers, and cell surface characterization to determine what was actually achieved (Figure 21).

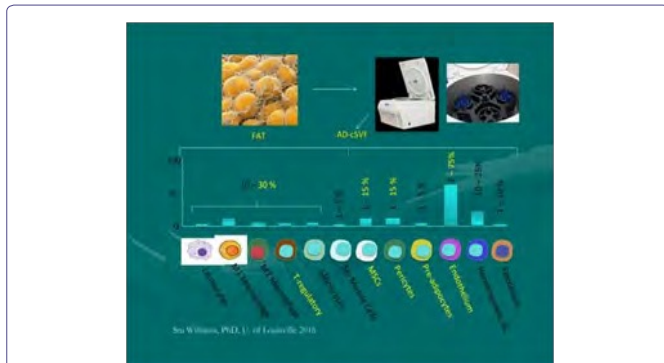


Figure 21: Main component cells in cellular stromal vascular fraction: [NOTE: Approximately one-third have multipotent capabilities, balance supportive cells of importance] Courtesy of Dr. Stu Williams

At this point, cellular isolates/concentrates may be used in multiple applications, such as cell enrichment (where these isolates are added back to a tSVF portion) to provide higher stem/stromal cell numbers for potential graft and injection applications. The other option, is to re-suspend this cSVF group in 500 cc Normal Saline for delivery parenterally (IV, IA, Intrathecal, Intraperitoneal, or mucosal spray) depending on desired locations and uses. If IV or IA, typical very fine in line tubing filters (1.50 micron) ensure removal of fibrin or other components in transfusions (which utilize a standard 170 micron filter).

In the case of parenteral deployment, most clinical researchers are using this within an FDA/NIH approved Clinical Trial situation, where safety and efficacy data is derived.

The Biocellular Therapies refer to the use of tSVF (harvested via microcannula syringe systems) (or lower concentrations when used on radiation damaged, hair and dermal uses). The tSVF may be used after a centrifugation (typically 800-1200 g-Force for 3-4 minutes), mixed with an appropriate concentration (by volume percentage) of PRP, then guided or intradermally placed to targets. Plastic Surgery literature has many examples of improved structural grafting accomplished using cell-enriched tSVF as previously discussed (Figure 22).

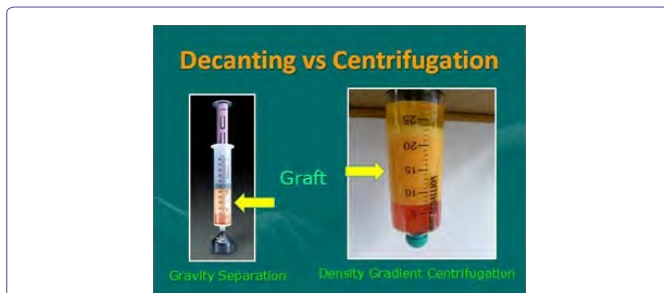


Figure 22: Value of centrifugation for layer separation compared to gravity decantation. NOTE: Density gradient separation at 800-1000 g-Force/3 min provides effective excess liquid/debris (Infranant), compressed graft (tSVF), and well separated unwanted free lipid (Supernatant).

As the platelets concentrates are understood to be a key contributor for provision of cellular healing growth factors and signal proteins, we recommend striving for greater concentrations achieved translates directly with linear increases of those elements. Acting as a central component in the inflammatory and and maintain the healing processes in conjunction with the local site stroma and cells. This effect is effect, wherein the site specific the most important regenerative or healing processes. Such factors as Vascular-Endothelial Growth Factor (VEGF) and many others contribute to this process with encouragement of micro-vessel formation and improved perfusion. Thousands of patients have undergone treatments using these concentrates with inflammatory or aging conditions.

An autologous tSVF sample is easily harvested from subdermal fat deposits under sterile protocols, using the patented, disposable closed syringe system. This is often referred to as microcannula lipospirospiration or lipoharvesting [17]. This Adipose Tissue Complex (ATC) may be 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 with less intercellular reducing the volume of injection materials, earlier recovery of comfort and ambulation is common. Biologicals such as High-Density Platelet Concentrates (HD-PRP) can then be 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 [29].

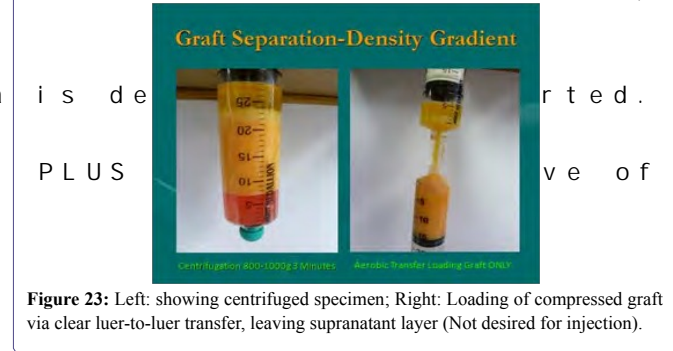


Figure 23: Left: showing centrifuged specimen; Right: Loading of compressed graft via clear luer-to-luer transfer, leaving supernatant layer (Not desired for injection).

Cellular and Biocellular Therapies are commonly performed in many areas of within and outside guidelines being discussed currently confuse these issues implying that they all are employing digestive chemicals. Many sites are actively providing these services in t controlled Institutional Research Based (IRB) trials or study groups within specialties. The author currently stresses the importance of information documentation and reporting these existing trials are concluded and reported, it is common to perform these newer options within an approved IRB channel. Following multiple institutional and organizational IRB guidelines using specific trial studies by providing the approved trial protocols, both within the United States and international.

The cellular isolates are currently being utilized for a wide variety of human clinical applications on a global basis. Following a myriad

of basic research studies, animal models were tested and reported in the bioscientific literature, traditional clinical Journals in the medical literature. As the Cellular Therapy group is well recognized as favoring immune privilege and pro "benign" inflammation (resulting in healing and reported in the literature), these local, systemic and autoimmune issues are included in many clinical studies (Figures 24 and 25).



Figure 24: Sample of current cellular & biocellular uses.



Figure 25: Disposable system for emulsification (Offset 600/400 micron screen er-to-luer series of progressively smaller internal sizes (2.4, 1.4, and 1.2 Respectively)). Photo Provided By Tulip Medical

How the cSVF factually works is under intense study at this time. At first, the thought that the important (like the incorrect belief that autologous fat grafting relied on the presence of mature adipocytes) have proven to not be correct. In fact, it now believed that the actual graft successes are a result of the stem/stromal cells and bioactive matrix (Extracellular Matrix & Peri-adventitial Cells) and their secretory abilities to impact healing and graft sites. This secretory action is considered of greater importance than the undesignated cell populations within the tSVF [30].

The importance of this understanding has led to appreciation of the paracrine capabilities via exosomes and microvesicles, and their roles in transferring signals via mitochondrial RNA (miRNA) and messenger RNA (mRNA). It is now believed that the exchanges of these elements are the means of communication and stimulation of nuclear change leading to the proliferative and trophic effects to guide the differentiation for cell activation. This secretory action is currently important studies utilizing both autologous and non-autologous exosomes and microvesicles as an important contribution to the activation and guidance of cells to respond (Figures 26 and 27) [31,32].



Figure 26: Paracrine secretions and inter-cellular communications: Exosomes and microvesicles as means of signaling for stem cell activation and proliferation.

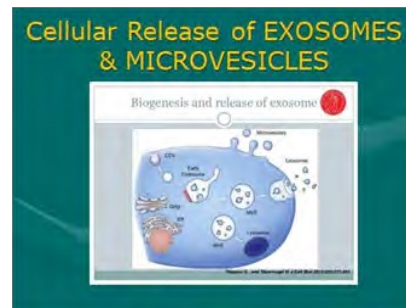


Figure 27: Diagrammatic representation of exosomes and microvesicles.

In the discussions on "non-homologous" uses when using tSVF or cSVF, it is most misunderstood, that the actual cells that are of importance are NOT the adipocytes, but rather the supportive and undesignated cell group found in association with the ECM and peri-adventitial elements that actual stimulate precursor replacement cells of adipose or other cells groups in applied regenerative efforts in wound healing and orthopaedic applications. The concept of these terminally differentiated cells (mature adipocytes) playing a key role in repair and regeneration of musculoskeletal components has been mostly discarded. The FDA concept that adipose structural grafting should not be allowed in large volume breast augmentation is based on the statement that it is a non-homologous use based on the fact that fat cells "cannot secrete milk" as the only function or tissue comprising the breast, is ludicrous.

A Recent Advance in Use of Biocellular Uses: Micronized & "Nanofat™" (Fully or Partially Emulsified)

Over the past few years, several alternative advances in processing the lipoaspirated tSVF via mechanical [31]. While many application still favoring the same Biocellular product creation (including use of additive advantages offered by addition of HD-PRP concentrates) while retaining small fragment tSVF capable of injection via small bore needles (down to 30 gauge). Recent published evidence has shown that creation of the mechanically emulsified "nanofat" does not have a cellular numbers or viabilities while markedly reducing the volume of ATC provided by mature adipocytes (Figure 25).

Recent advances now offer the opportunity to have a disposable system to achieve maximal lysis of adipocytes, while preserving the much smaller cSVF component (Figures 28 and 29). It is important to note that this emulsified (tSVF) component is NOT A done in the United States under insure reporting of patient safety and determining effectiveness. Clinical trials are gradually being released at this time, many requiring several years to acquire data, compile and report. The vast majority of such reports are providing clear clinical evidence of patient safety and effective clinical treatment outcomes.

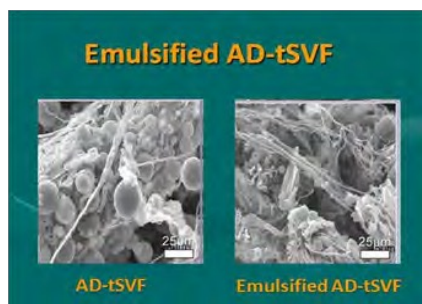


Figure 28: Emulsified adipose tissue component (tSVF) prior to emulsification; Right: Essential Removal of All Intact Mature Adipocytes, Leaving Perivascular and Extracellular Matrix (ECM) Intact].



Figure 29: Emulsification of adipose tissue component (tSVF) prior to Emulsification (Stain (Green) on right of micrograph); Bottom: Emulsification of mature adipocytes. [NOTE: Multiple blue stained nuclei remaining intact (Small fragment tSVF, NOT a cSVF).

With the ability to injection through small bore needles, patient comfort in dermal and hair injections is enhanced, plus offers a range of radiation/solar damaged skin and small joint targeted applications in orthopaedic medicine. The abilities of Biocellular modalities to promote wound healing and regenerative capabilities via intradermal placement has opened opportunities to permit improved skin circulation and texture, improve skin aging and radiation damage, hair regeneration, participate in chronic wound applications, as well as many small joint and superficial applications (Figure 30).

What Is the Future in Stem/Stromal Cellular and Biocellular Treatments?

There are now safe, reproducible capabilities of sterile, closed isolation of the large numbers of stem and stromal cells from the adipose tissues. Within such semi-automated and automated closed systems,

this ability is becoming practical even in outpatient procedural rooms and carefully prepared within sterile protocols. Once this was exclusively possible only in very costly laboratory settings, requiring expensive equipment and technician costs. Today such isolation is NOT A done in the United States under insure reporting of patient safety and determining effectiveness. Clinical trials are gradually being released at this time, many requiring several years to acquire data, compile and report. The vast majority of such reports are providing clear clinical evidence of patient safety and effective clinical treatment outcomes.



Figure 30: Sample of post-radiation skin aging/damage (Sun). Top: Pre-treatment biocellular mix; Bottom: Post-treatment biocellular grafting (1 Year) of cheeks, lips and nasolabial folds. [NOTE: To show true texture change as result of biocellular mix, placed subdermally].

With the changes in legislation and within the FDA, new and exciting possibilities of IND/RMAT designations are being considered and suggested guidelines evolving. Timing of these changes is optimal, as much of the safety and efficacy data already be in place by the time such designations are better understood. The 21st Century Cures Act and the “Right to Try” 2018 Legislation are examples of some of these changes.

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 musculoskeletal application exceeding 150,000 human clinical uses, with a remarkable efficacy and safety profile. These results, from case series or reports, and should not be discarded out of hand, simply because they are not participating in a pivotal value to insure that very accurate diagnostics and guided placement to defined targets. - Ultrasonography during examination, will remain as a needed core competency for those taking care of musculoskeletal and chronic wound cases.

In the future, it is very likely that such isolated cells will provide parenteral (intravenous, intra-arterial, intra-theal, intra-peritoneal, etc.) pathways, and become effective for a very expansive treatment in such disorders as neurodegenerative diseases (MS, Alzheimer’s, ALS, Parkinson’s, brain injuries/stroke, etc.), diabetes, chronic lung disease, heart disease and damage/gia/causalgia, ulcerative bowel disease, Crohn’s, colitis, and so forth.

Providers of Biocellular Treatment?

Initially, within the realm of Cosmetic-Plastic Surgeons, advances in uses for sun damage, deep tions, and a variety of indications were presented in didactic settings and peer-reviewed publications, have been adopted by a very wide variety of surgical and non-surgical providers. Patients are now cared for by a diverse group of providing doctors (e.g., primary care health care providers, internists/Neurologists, Aestheticians, Hair Regenerative Specialists, Aesthetic-Plastic Surgeons, General Surgeons, Orthopaedic Surgeon, Emergency/Sports Medical Specialist, Pain Management specialists, Wound Care centers, etc.). Those non-surgical trained practitioners are now deciding whether candidates have a condition which has reasonable potential for improvement through use of combinations of biologic and stem/stromal cellular treatment. Thorough physical and pre-treatment evaluations, proper training, and informed consent delivery to patients are essential in diagnostic, treatment planning, and care delivery. Circulatory, neurological, and indicated systemic conditions should be documented and thoroughly evaluated regardless of the background of the medical provider. In the case of orthopaedic applications, use of metrics such as range of motion, indicated MRI studies and high quality ultrasonographic imaging, combine to determine guide proper placement. In the vast majority of orthopaedic cases, use of high quality MSK ultrasound such treatment. Proper diagnostic imaging and ultrasound evaluation is considered a key to the most success, particularly considering that this modality plays a central role for providers to effectively "hit" the desired targets. Palpation may provide fairly accurate placement in experienced providers, targeted and tracked therapy consistently correlates with earlier and- rics that are more objective, successful monitoring of many patients including range of motion, remodeling of tissues in repeated interval ultrasound studies, and return of strength, range of motion, comfort during function provide very valuable informative standards. For many years, simple dextrose prolotherapy major benchmarks were limited to patient reported pain levels, activity levels and perceived improvement as their primary metrics.

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 variety of mechanical guided injections through very small bore needles (25 gauge-30 gauge) without statistically reduced viabilities or clinical outcomes. The ability to provide improved patient comfort and access to skin, hair and small joints is rapidly becoming an appealing option.

Conclusion

"Using your own tissues to paradigm change, and is one of the most exciting minimally invasive options currently available. Both cellular & biocellular regenerative therapies are rapidly improving in documentation, cellular analyses, and gaining very good safety purely experimental, it has 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/Surgical interventions. Clinical Trials will

prove of great value going forward in providing evidence of the specific area of optimal use.

There are many evolving clinical trials (NCT01714069) recognizing the very remarkable abilities of cellular components of adipose tissue complex (ATC). The number of active and evolving trials using ATC is surpassing the uses of bone marrow due mostly to the much higher numbers of reparative cells within the marrow. Marrow remains the gold standard for various blood-related diseases, but is not clear that it possess very few of the non-hematologic (reparative) stem cells, and it thought by many to be only useful as a biologic contributor to the wound healing and regenerative processes discussed in this chapter.

To date, researchers and bioengineers are not able to effectively reproduce or provide true 3-dimensional scaffolding at this time. This inability limits development of understanding how to mimic a true in vivo culture/expansion capability, and continues to delay understanding of how these cells act and are controlled at the local tissue level. Translation from tissue culture and the inherent changes potentially introduced in the process leaves us with the need to cautiously advance therapeutic modalities into clinical practices. Classic tissue culture/expansion remains problematic, in that it is known to introduce variables which are not able to be translated into how the body uses these undesignated cell populations.

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ENM, TEN & MEME O BONE

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References

- Alexander RW (2012) Understanding a fraction (AD-SVF) cell biology and use on the basis of cellular, chemical, structural and paracrine components: a concise review. *Journal of Prolotherapy* 4: 855-869.
- Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, et al. (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 7: 411-421.
- Zuk P (2013) Adipose-Derived Stem Cells in Tissue Regeneration: A Review. *Stem Cells* Pg no: 35.
- Alexander RW (2013) Understanding A Fraction (SVF) Cell Biology In Reconstructive and Regenerative Applications On the Basis of Mononucleated Cell Components. *J of Prolo* 10: 15-29.
- Alderman DB, Alexander RW, Harris GR, Astourian M (2011) Stem Cell Prolotherapy in Regenerative Medicine: Background, Theory and Protocols. *J. of Prolotherapy* 3: 689-708.
- Sadati KS, Corrado AC, Alexander RW (2011) Utilized to promote greater graft volume retention in autologous fat grafting. *American Journal of Cosmetic Surgery* 23: 203-211.
- Alexander, Robert W (2011) Autologous Fat Grafting, in *Autologous Fat Grafting*, Textbook, Shiffman, M. ed., Springer, Berlin 14: 87-112.

8. Alexander RW (2016) Biocellular Adipose-Derived Stem/Stromal Cells and Its Native Bioactive Matrix. *Phys Med Rehabil Clin N Am* 27: 871-891.
9. Blaber SP, Webster RA et al. (2012) Analysis of In Vitro Secretion Profiles for TransAMed 10: 172.
10. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, et al. (2008) A Perivascular Origin For Mesenchymal Stem Cells In Multiple Human Organs. *Cell Stem Cell* 3: 301-313.
11. Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, et al. (2009) Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell* 5: 17-26.
12. Brizzi MF, Tarone G, Deflippi P and growth factors as tailors of the stem cell niche. *Curr Opin Cell Biol* 24: 645-651.
13. Kim SH, Turnbull J, Guimond S (2009) Signaling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J Endocrinol* 209: 139-151.
14. Alexander RW (2009) Fat Transfer with Platelet-Rich Plasma for Breast Augmentation. *Breast Augmentation Pg* no: 451-469.
15. Kato H (2011) Short- and long-term cellular events in adipose tissue modeling after non-vascularized grafting. Paper presented at the International Federation for Adipose Therapeutics and Science Miami, 9th Annual Symposium on Adipose Stem Cells and Clinical Applications of Adipose Tissue; Nov 4-6; Miami, Florida, USA.
16. Alexander RW (1994) Liposculpture System for Improvements in Fat Removal and Free Fat Transfer. *Am J Cosm Surg* 11: 127-134.
17. Alexander RW, Harrell DB (2013) Autologous fat grafting: use of closed syringe microcannula system for enhanced autologous structural grafting. *Clin Cosmet Investig Dermatol* 6: 91-102.
18. Alexander, Robert. 2019: *Unpublished Data*
19. Kurita M, Matsumoto D, Shigeura T et al. (2008) Influences of centrifugation on cell viability and tissues in liposuction aspirate. *Plast Reconstr Surg* 121: 1033-1041.
20. Alexander RW (2015) Contributing Author, Textbook "Introduction To Biocellular Medicine", in Sonography of the Extremities: Techniques & Protocols 4th Ed., Moore, R., Editor, General Musculoskeletal Imaging Inc., Cincinnati Pg no: 97-104.
21. Eto H, Suga H, Inoue K, Aoi N, Kato H, et al. (2011) Adipose injury associated factors mitigate hypoxia in ischemic tissues through activation of adipose-derived stem/progenitor/stromal cells and induction of angiogenesis. *Am J Pathol* 178: 2322-2332.
22. Alexander RW (2016) Biocellular Adipose-Derived Stem/Stromal Cells and Its Native Bioactive Matrix. *Phys Med Rehabil Clin N Am* 27: 871-891.
23. Breen EJ, Kuah D, Teat-Der O, Ivede E, KIL, PAO, P, X, and Dens. RW (2013) Adipose-Derived Tissue Stromal Fraction Plus High Density Platelet-Rich Plasma or Bone Marrow Concentrates in Achilles Tendon Tears. *J of Prolotherapy* 5: 895-912.
24. Albano JJ, Alexander RW (2011) Autologous Fat Grafting As a Mesenchymal Stem Cell Source and Living Bioscaffold In a Patellar Tendon Tear. *Clin J Sport Med* 21: 359-361.
25. Alderman D, Alexander RW (2011) Advances in Regenerative Medicine: High-density Platelet-rich Plasma and Stem Cell Prolotherapy for Musculoskeletal Pain. *Journal of Pain Management (JPM)* 10: 49-90.
26. Alexander RW (2011) Autologous Fat Grafts as Mesenchymal Stromal Stem Cell Source For Use in Prolotherapy. *Liposculpture for Pain Management* 1: 680-688.
27. Jackson MV, Morrison TJ, Doherty DF, McAuley DF, Matthay MA, et al. (2016) Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the *In Vitro* and *In Vivo* Models of ARDS. *Stem Cells* 34: 2210-2223.
28. Alexander RW (2017) Use of Software (PIXYL) Without Contrast As Objective Metric in Neurological Disorders and Degenerative Diseases. *Int Phys Med Rehab J* 2: 203-206.
29. Tobita M, Tajima S, Mizuno H (2015) Adipose Tissue-Derived Mesenchymal Stem Cells and Platelet-Rich Plasma: Stem Cell Transplantation Methods That Enhance Stemness. *Stem Cell Res Ther* 6: 215.
30. Alexander RW (2017) Use of PIXYL software (with and without contrast) as valuable metric in clinical trial tracking in study of multiple sclerosis (MS) and related neurodegenerative processes. *Clin Trials Degener Dis* 2: 1-6.
31. Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, et al. (2013) Nanofat Grafting: Basic Research and Clinical Applications. *Plast Reconstr Surg* 132: 1017-1026.
32. Lamichhane TN, Sokic S, Schardt JS, Raiker RS, Lin JW, et al. (2015) Emerging roles for extracellular vesicles in tissue engineering and regenerative medicine. *Tissue Eng Part B Rev* 21: 45-54.



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