5 Adipose Tissue Complex (ATC): Cellular and Biocellular Uses of Stem/Stromal Cells and Matrix in Cosmetic Plastic, Reconstructive Surgery and Regenerative Medicine

Robert W. Alexander

5.1 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 examination of the homeostatic and replacement mechanisms have afforded us the opportunity to explore how the body successfully maintains and repairs defects from aging, damage or degeneration. Throughout, the value of understanding how various tissues accomplished such task has evolved to one of doing everything possible to encourage vascularization of sites and 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, and 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 in the presence of such elements. At 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 and somewhat inefficient machines were replaced by a number of medical device companies creating smaller, efficient, and more simple centrifugation systems which provided consistent and high concentration abilities. At the same time, this expanded delivery capabilities in more cost-effective and consistent protocols, for use in both the operating room and 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, many

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D. Duscher, M. A. Shiffman (eds.), *Regenerative Medicine and Plastic Surgery*,
https://doi.org/10.1007/978-3-030-19962-3_5

researchers and clinical scientists are developing more sophisticated understanding of optimization of effects, including which product or content element is contributing in critical situations of healing or regeneration. As the applications are tested for safety and efficacy, these values are expected to gradually evolve as are the clinical uses.

Poorly understood is the fact that MANY products have rushed to the market, each claiming capabilities and concentrations that are simply incorrect. During the evolution, many surgeons (especially orthopedic surgery and related areas) were disappointed at the outcome improvement. A significant issue evolved with lesser understanding that simply isolating some platelets did NOT yield high enough, or consistent enough, products which could deliver the most optimal outcomes. It has become very clear that 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 choosing a high hematocrit version or a low hematocrit version have a clear indication tied to the specific locations where they may be utilized. In aesthetic plastic surgery, it is considered that the use of the highest concentrations that can be currently isolated is of significant 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 statistically significant reduction in lipid cysts and microcalcifications, coupled with more stable volume retention in use of autologous fat graft breast augmentation, has enhanced the use of platelet products in such cases. In addition, observational findings of important skin vascularity and texture changes in the areas of the face/neck and the body areas have 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 and 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.

5.2 Evolution of Cell-Based Therapies

Over the past decade, great strides have been made in the understanding and potentials of systemic and 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 to be limited by incomplete repair while requiring a series (often 4–6) to achieve long-term clinical improvement. Current biocellular (orthobiologic) approaches combine the trophic growth factors and important signal proteins which are added with cellular and bioactive matrix elements synergistically to enhance the reparative and regenerative abilities in areas of need. The importance of signaling mechanisms and paracrine responses have been expanding rapidly 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 approach 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." It is very important to understand that many of the biocellular therapies also rely on the presence and reactivity of pre-existing "resident" cell populations which also contribute to local niche (microenvironment) responses. The asymmetric cellular replacement is the reason that one of the cells may be differentiated into a specific cell type or contribute molecular component needs (paracrine effects) locally, while the other one retains its "undesignated" cell capability and remains available for future responses. This is the basis for abilities of tissues & cells to respond to demands and retain the "self-renewal" capabilities ongoing and future homeostasis demands [1].

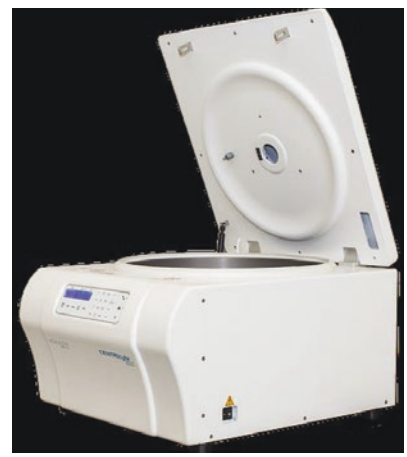
With the advent of FDA-approved tabletop programmable, centrifugal devices for custom high-density platelet concentrations via closed system, use of a simple blood draw yielded more than four to six times patient's own circulating baselines levels. It has been very well shown that the higher the achieved concentrations, the proportionally higher delivery of important factors intrinsically involved in all wound healing and repair (Fig. 5.1).

It has become clear that certain tissue characteristics are most favorable for use in cell-based therapies including easy and safe access coupled with 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 site for repair or regeneration. Researchers are gradually understanding the complexities of contributions of undesignated cellular elements in combination with secretory signaling (paracrine effects) in chemotactic and critical "up or down" regulation of regenerative processes within the environment.

Research has confirmed that the vast majority of such undesignated cells are associated and stored in proximity to the microvascular capillary system and adventitia (Fig. 5.2). Essentially all tissues (with blood supply) have some of these

Adipose Tissue Complex: Mature Adipocytes = 90% Of Volume & Only 10% Of Nuclei In tSVF

[Turnover Rate: 10-20% Per Year]



Centricyte 1000 Closed System For Cell Isolation & Concentration

Fig. 5.1 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 Healeon Medical, Newbury Park, CA, USA)

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.

Enhancement of cellular and biologic therapies comes directly with the ability for providers to be able to identify, target, and guide the cellular vascular fraction (SVF) or cellular-biologic combination to areas of injury or degeneration. Management of protocols has led to improved structural grafting successes in addition to the contribution in orthopedic and sports medicine application using the same cell components. In that regard, ultrasonography has clearly become a MAJOR feature to clinical responses and success. As an example, in medium and deep targets, or those difficult to access, guided MSK ultrasound capabilities offer the optimal integral part of successful responses. Over the past decade, thousands of treatments using Biocellular Regenerative Medicine© techniques have proven safe and remarkably effective. The information provided in this chapter is intended as an introduction to important concepts and describes 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 surgical patients are routinely treated with this combination of cellular or biocellular elements [4–6].

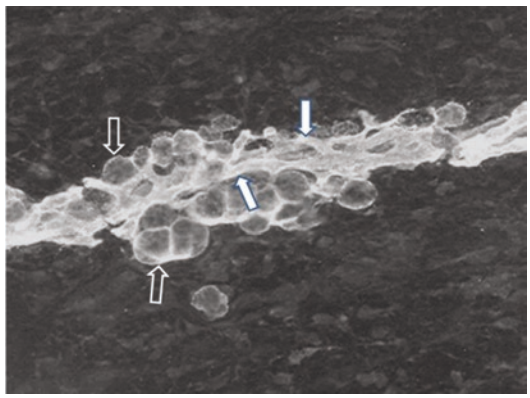


Fig. 5.2 Micrograph demonstrating the relationship of intimate relationship of microcapillaries and attached adipocyte during development (photo: fetal pig during extremity formation)

5.3 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, periadventitial, or mesenchymal cells) found widely spread within our body, and which participate in tissue maintenance, repair, and regeneration. Since 2010, science and medicine have advanced which is termed "translational phase," where proven laboratory science has demonstrated important contributions along with the clinical application of science in human applications in the last decade. Since then there has been controversy concerning the use of the term "stem cells" in the current practice of medicine. Unfortunately, these arguments typically occur with the misuse or overuse of the term "stem cell," as being 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 now widely documented and published (Fig. 5.3).

- 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

Fig. 5.3 General goals in regenerative medical applications

5.3.1 Cellular Components

Biocellular Regenerative Medicine® within the United States currently refers to the use of autologous, ADULT (non-embryonic) multipotent cells capable of participating in maintaining our tissues (homeostasis), healing, and regeneration. Since 2006, the scientific studies demonstrating the values of the highly variable stromal cell populations have 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 bone marrow in MSK and aesthetic-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 contains microvascular supply maintains a reservoir of such cells within the stroma and matrix. That said, we now recognize that adipose tissue complex (ATC) possesses the greatest microvascular organ in the body and serves as an excellent option to access for a variety of uses. Many important peer-reviewed scientific reports suggest that adipose-derived stem/stromal cells of mesodermal origin provide between 1000 and 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 (Figs. 5.4 and 5.5).

Efforts at using mechanical separation (ultrasound, nutational, emulsification efforts) have not proven to be able to separate the very 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) remains the optimal way to isolate and concentrate the desired cellular groups [8] (Figs. 5.6 and 5.7).

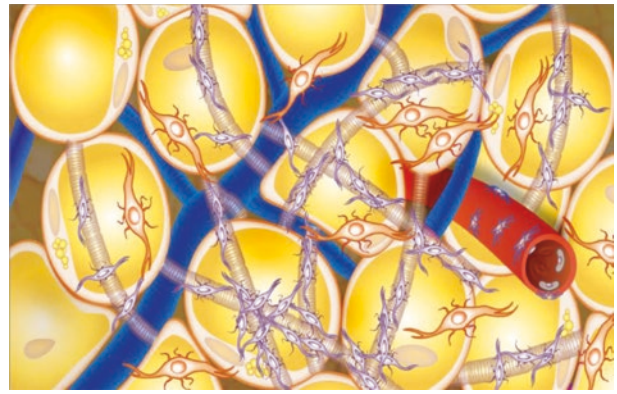
- Easy Harvest Access
- High Quantity Of Viable Stem/Stromal Cells
- Minimum Morbidity Of Donor Site
- Safety After Implantation (Autologous Best)
- Multipotent & Proliferative Cell Groups
- May Be Isolated & Concentrated If Desired
- Prefer Inclusion Stromal Elements (Bioactive)
- Paracrine Functions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

Fig. 5.4 Adipose tissue complex: optimal cell source

The small nucleated cells found closely associated within the vascular tissues are now recognized as serving important roles in maintaining normal tissue content (homeostasis). PLUS have the ability to 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.) in the body. The remarkable design of the human body uses these reservoirs of available, non-differentiated, multipotent cells as the tissue "first responders" in the situations of major or microtrauma 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 first, it was felt that the cellular elements were the critical, and perhaps the only important, ones; experience now shows that the **paracrine** secretory capabilities play critical roles (exosomes, microvesicles). 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.

5.3.2 Biologic Components

The "biological" components in this context refer specifically to the availability of a diverse and



KEY Multipotent Cells Found In AD-tSVF

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

Mature Adipocytes (Not Multipotant Temporary But Contribute In Signaling)

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

** May Be The "Origin" Of All MSCs

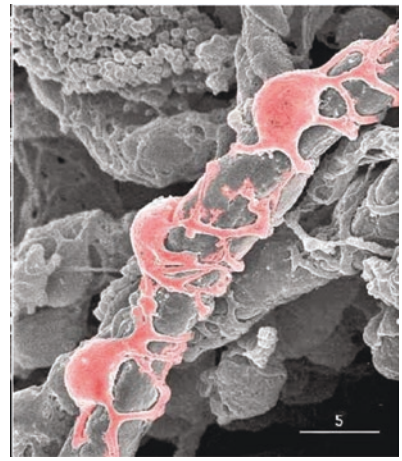


Fig. 5.5 Multipotent cells found in tissue stromal vascular fraction (tSVF)

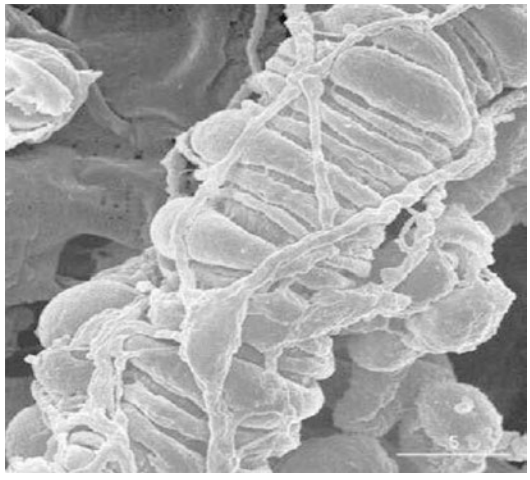
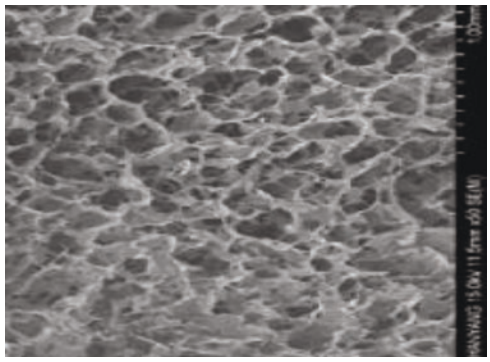


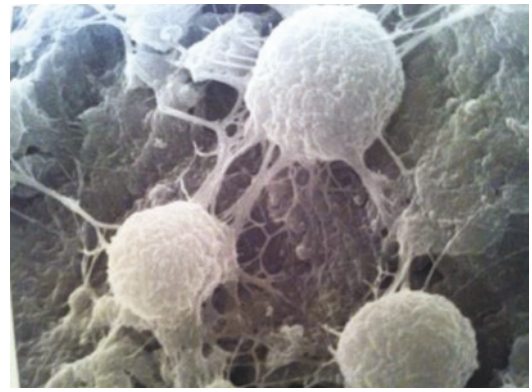
Fig. 5.6 SEM image of pericytes' relationship to micro-capillary. Note: See many complex cell-to-cell and cell-to-matrix attachments

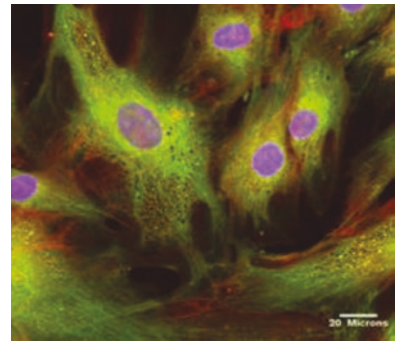
important variety of growth factors and signal proteins which interact with the cells of the degenerative or damaged sites to help recruit needed reparative cells and materials to repair the area. There are two major "biological" components in common use at this time. First is found within recognized 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 that 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 providing a fibrin clot to permit gradual release of platelet contents). Platelets represent a storehouse of small gran-



Native Adipose Extracellular Matrix
(Bioactive – Storage GF - Secretive)

MSCs Cell-To-Matrix
& Cell-To-Cell Contacts





Cell-To-Cell Contacts

Fig. 5.7 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 cell micrograph with NAPI nuclear stain

ules, 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 healing phases. For example, an important chemical available from these granules is essential for blood vessel replacement and repair in order to improve the circulation ability critical to healing of all wounds. Without adequate blood flow, needed oxygen cannot reach the area of damage, nor permit migration of a variety of cells from nearby or distant cell sites (Figs. 5.8 and 5.9).

The second source of biological contributors is found in bone marrow aspirates. Bone marrow has been used for many decades, and it is commonly used in blood-related disorders. However bone marrow does demonstrate microvasculature and, therefore, does have some undesignated reparative cells (stem/stromal cells). The vast majority of stem cells located in marrow belong to the "hematopoietic" stem cell group, and are not considered extremely valuable in the case of regeneration or repair cellular group. However, the desired stem/stromal cells are found in rela-

HD Platelet Concentrates

- Higher Growth Factors & Signal Proteins
- Directly Impact *Proliferation & Migration*
- Platelets "*Quarterback*" Healing Cascade
- Contributes Tissue "Autoamplification" Of Critical *Signaling* Within Repairing Sites

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Fig. 5.8 Why use of high-density platelet concentrates (HD-PRP)?

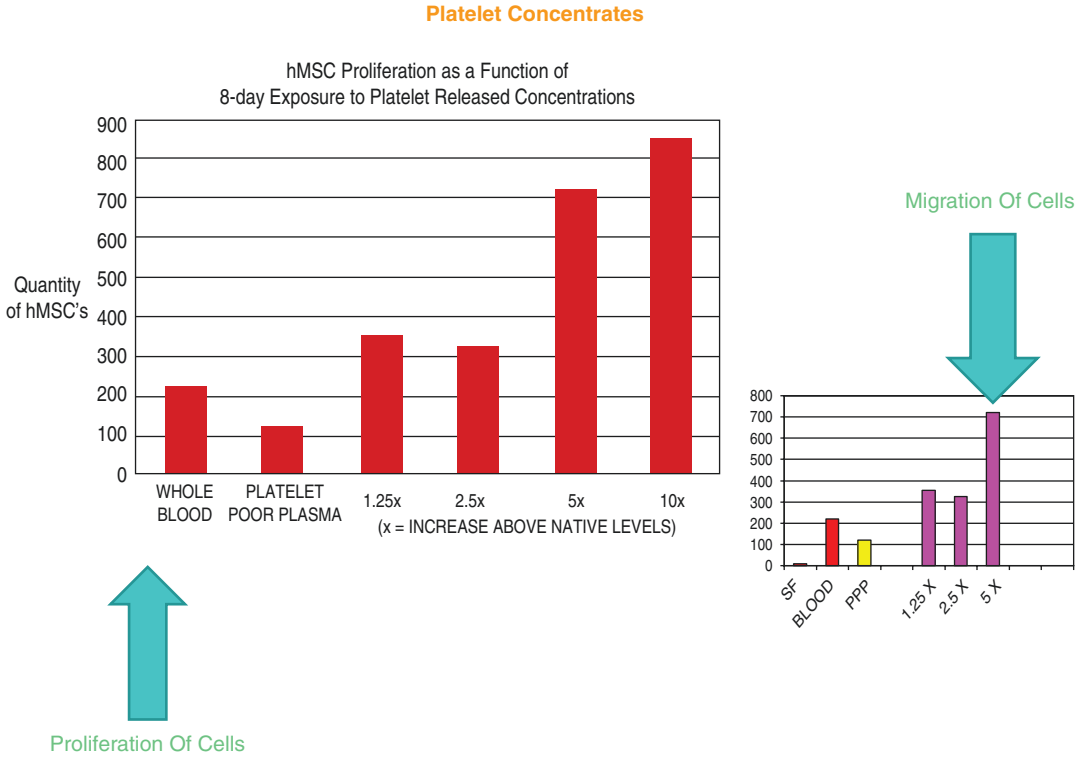


Fig. 5.9 Importance of HD-PRP on proliferation of cells and migration of cells [note: linear response as concentration increases] (Hayneworth, S.E, et al. Mitogenic Stimulation of Human MSCs by Platelet Concentrates. Orth Res Soc. (2002))

tively very low numbers, compared to adipose tissues and its microvasculature. Therefore, many now consider bone marrow as primarily a valuable biologic and platelet source. In order to become a valuable 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, poses higher complication-sequelae rates, and is significantly more expensive to the patients. In addition, the actual number of "reparative" cells (including mesenchymal, pericyte and periadventicial cells) available with the marrow are markedly lower than found in adipose microcapillary tissues. The bone marrow undesignated group is heavily weighted to the hematopoietic stem cell groups. Many now consider that the bone marrow should be considered as a "super" PRP source and included as a valuable biological growth factor (as a platelet concentrate) rather than a primary reparative cell source. At this time there is very little evidence of significant contribution to the regeneration process of MSK tissues derived from the HSC group.

The primary importance and value of concentrates are 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 is their important paracrine secretory influences, rather than contributions of individual cellular components and physical engraftment. Further, it is 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 providing "first responders" to sites 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 in vitro conditions. Host-site interaction with these stem/stromal cells, growth factors, and signal proteins seems to create a complex, heterogeneous precursor population that is considered "site specific" in many of their responses [11].

5.4 How Did Biologic and Cellular Therapeutic Concepts Evolve?

Aesthetic and plastic surgeons have traditionally 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 managed to maintain themselves. For many years, the importance of biologics as a derivative part of platelets was appreciated, not only for its clotting functions, but also for the gradual release of critical chemical components essential to the healing processes in individual sites. These biocellular concentrates are felt 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 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 more clear that site specificity greatly influences cellular changes within the non-designated, heterogeneous, 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 that specific interactions of any single cell or chemical are not able to be determined at this time. At this time, the ability to create an "in vitro" environment to duplicate the "in vivo" niche remains elusive. This makes the ability to develop "optimal" components or interpret activities which can be translated to the in vivo applications impossible. It is well known that the process of cellular isolation and culture expansion likely introduces variables which cannot yet be interpreted accurately.

Key adult multipotent cells are found in essentially every tissue and organ in the body. The 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, 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 capable of reacting to secretions from a senescing adipocyte, allowing the 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 form and abilities. This is logical, in that, if otherwise, we would accumulate a massive number of precursor cells in our tissues (Fig. 5.10).

- Safe & Easy Harvest Via Closed Syringe System
- *Prefer Use Of Own Cells* (Autologous)
- Optimal To Include Native Matrix For Structural Volume Enhancements
- Transplant In *Same Surgical Session, Same Day*
- Predictable & Reproducible Outcomes
- Induce Minimal Recipient Site Inflammation
- Result In Healing With Minimal Scarring
- Optional Ability To Utilize Parenteral Uses For Systemic Disorders
- Not Require Manipulation (But Do Offer Future Culture/Expansion)
- Transplantation Of Intact Micro-Environments

Fig. 5.10 Adipose tissue complex: advantages for use

It was Zuk et al. who identified the multipotent capabilities of 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. (Figs. 5.11 and 5.12). Once this capability was identified, efforts to isolate specific cell types started. This has proven somewhat difficult to interpret, 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 platelet concentrates (HD-PRP) has received a great deal of attention. Identifying stem/stromal cells which can participate in the processes has revealed what an "ideal" cell-based therapy may represent (Fig. 5.13).

In the early 1990s, a method of closed-syringe microcannula lipoaspiration was patented, permitting less traumatic and efficient 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 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 been identified as a

Partial List of stem/stromal cells in tSVF

- Pericytes/Endothelial Cells & Adventitial Cells (Key Group)
- Mesenchymal Stem Cells
- Pre-Adipocytes (Progenitors)
- Fibroblasts
- Macrophages (Type I & II)
- Vascular Smooth Muscle Cells
- T Lymphocytes (TREG)
- Miscellaneous Native Blood Derived Cells
- [NOTE: Reminder - These React With Local Site Cells]

Fig. 5.12 Adipose tissue complex main cellular elements

IMPORTANT

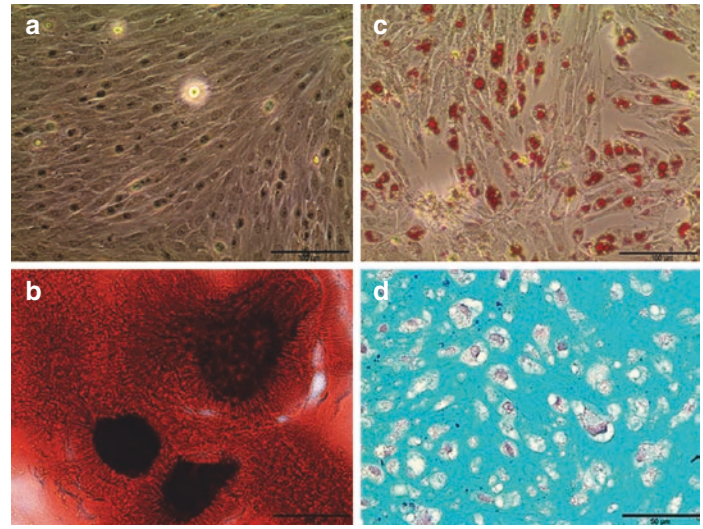
Mesenchymal Stem-Stromal Cells Capabilities Overlap >98+% Regardless Of Tissue Origin

Almost Every Tissue In The Body Contains Pericyte/EPC & MSCs (Vasculary Related)

Adipose & Bone Marrow MSCs Are Virtually *Interchangeable* In Capabilities *In Vitro*

Adipose Provides >1-2000 *TIMES* The Actual MSC Numbers Compared To Bone Marrow (per cc)

Adipose Does *NOT* Require Isolation, Culture-Expansion To Achieve Therapeutic Numbers



a). Control MSC; b) Bone (Alazarin Red); c) Adipose (Oil Red O); Cartilage (Hematoxylin Mayer's & Alcian Blue)

Fig. 5.11 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]

very complex and heterogeneous population, closely related to cellular, adventitial areas, and extracellular matrix contacts. At first, mesenchymal cell group (MSC or ADSC) was thought to be the most important multipotent "stem" cell. Further examination, however, now suggests that these may serve a "sentry" capacity, and that the actual cell group is known as pericyte/endothelial stem/stromal cells [10] (Figs. 5.14 and 5.15).

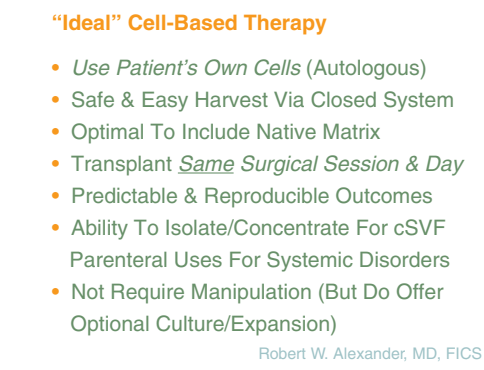


Fig. 5.13 Ideal cell-based therapy advantages

There is much confusion in interpretation of the scientific and clinical published materials caused by a lack of explanation of the difference between *tissue* stromal vascular fraction (tSVF) and *cellular* stromal vascular fraction (cSVF) (Fig. 5.16). For clarification, cSVF is the isolated cellular elements in the ATC created via use of certain collagenase-enzyme blends to separate the complex and multiple attachments comprising the cell-to-cell or cell-to-matrix connections (Fig. 5.7). The utilization of such cSVF is currently the subject of multiple clinical trial applications (see www.clinicaltrial.gov), 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

Fig. 5.14 Closed, sterile disposable, microcannula system (GEMS) (Tulip Medical, San Diego, CA, USA). Left top: Internal lock sample (purple), microcannulas (2.11 mm OD) showing multiport infiltrator, spiral cannula (2.11 mm), and single port injector (1.65 mm), sterile clear luer-to-luer transfer. Top right: External universal non-disposable lock. Bottom right: Microcannula tips

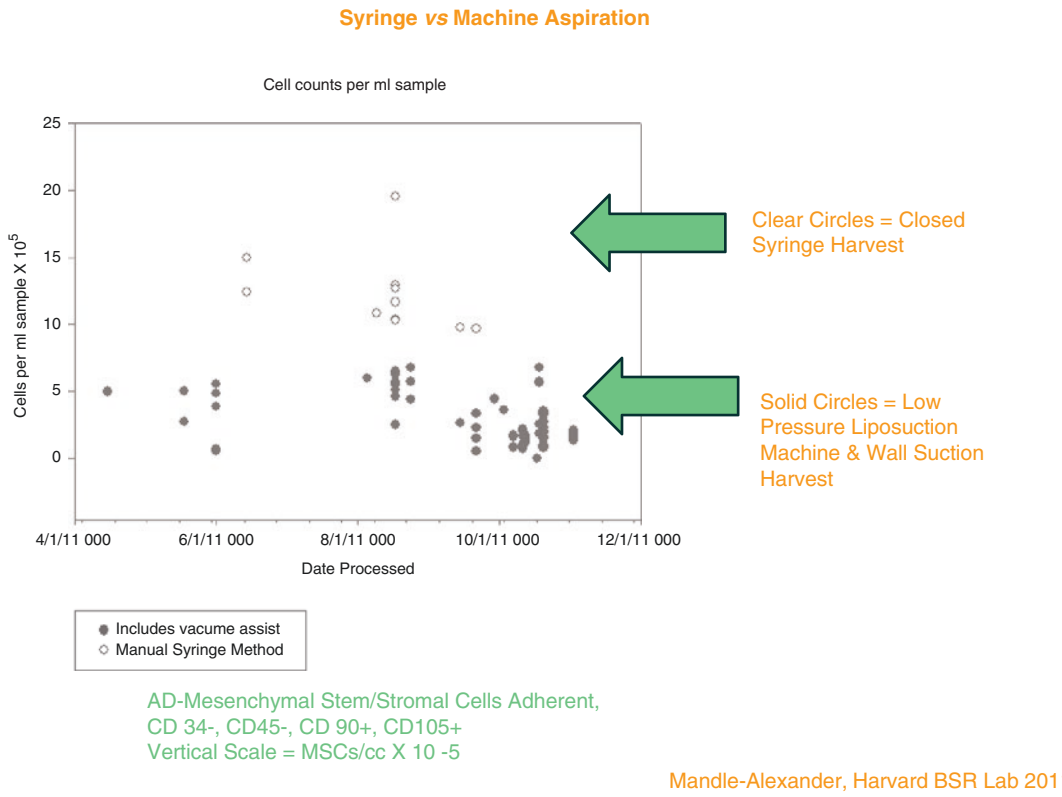


Fig. 5.15 Comparative cellular recovery: closed-syringe microcannula harvest versus machine vacuum on mesenchymal cells in ATC

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*

Robert W. Alexander, MD, FICS

Fig. 5.16 Important understanding of terms: tissue stromal vascular fraction (tSVF) and cellular stromal vascular fraction (cSVF)

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 scientific and engineering publications. It is reported that important information publications are doubling the existing knowledge base every 4-6 months, making the ability to remain current in the scientific and clinical applications most challenging.

In clinical applications, use of AD-tSVF has taken the primary role in aesthetic and 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 due to the actual secretory bioactivity of the tissue. This dual role is considered to be 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 (Fig. 5.17).

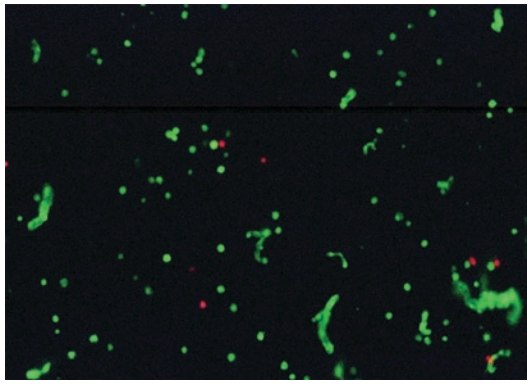


Fig. 5.17 Flow cytometry (live/dead) stain AO-acridine orange and PI-propidium iodide. (NOTE: The green "strings" represent actual viable cells attached to the extracellular matrix, making the grafting a minute, living microenvironment state (tSVF) thought to enhance recipient site responses)

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 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 being called PRP, particularly since most practitioners do not have the capability of confirming actual patient measured baselines to compare with achieved concentrations. To qualify as a true "high-density" PRP, we utilize the minimal concentrations to be four to six times an actual measured baseline, not a calculated extrapolation. This is very important based on the correlation of such concentration to cellular proliferation and migration capabilities. Refinements in HD-PRP options are now recognized as many subdivisions, such as low- and high-hematocrit solutions which have definite clinical implications to tissue tolerance and reactivity [18].

Use of centrifugation has increased in biocellular applications, as it creates a very effective "gravity density separation," which is important to avoid 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 felt to potentially impact site perfusion, as a factor of importance in many plastic surgical, reconstructive poor perfusion wounds, and musculoskeletal (MSK) applications [19].

The final area of importance in MSK applications relates to the ability of optimal targeting of areas of damage, degeneration, or inflammation. Without the 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 [20].

Within the past 2 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 a significant value to an injection site (including when performing 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 remaining after emulsification process confirm 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 not only the AD-tSVF 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 and permits more patient comfort in MSK injections (including small joint targets) [22].

In regenerative medicine, the main goals are relatively well established (Fig. 5.3). Likewise, description of "optimal" features of cellular based therapy in both aesthetic and regenerative applications is 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.

5.5 Understanding the "Workers and Bricks" Analogy

Considering chronic wound and musculoskeletal (MSK) applications, 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 (Fig. 5.18).

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

In the event, however, that your wall is 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 also *"BRICKS"* to replace the lost and damaged ones. The "bricks" in this analogy come from the cellular source (cSVF). Combining biologics + cell sources has proved to be 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 microvascular 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 by the platelet concentrates (>4–6 times patient circulating platelets) to form the therapeutic mixture known as "biocellular regenerative matrix."

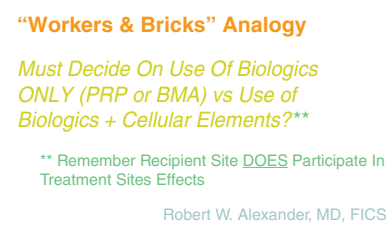


Fig. 5.18 "Workers and bricks" analogy

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

5.6 What Are Adipose-Derived "Adult Stem/Stromal Cells"?

These are a diverse group of "non-designated" cells found throughout essentially all the tissues of our bodies. They serve as a reservoir of replacement and repair cells, which react to injury, aging, or disease. "ADULT" cells in this category are often referred to as "stem/stromal cells" or "stromal" cells, and should be clearly separated from embryonic cells. They are also called by confusing names, such as "progenitor" or "precursor" cells, which means that 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, if you have a muscle or ligament tear, local 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 not as functional or tolerant of future stresses, and is NOT the ideal goal in wound healing. The terms differentiating "benign" versus "toxic" inflammation is becoming important. A highly reactive toxic inflammatory response (such as seen in many animals and young humans) as a protective mechanism, typically result in scarring as the primary result. The term, benign, on the other hand refers to a needed inflammatory reaction, but one modulated by various factors and resulting in little or no scar build up. A core goal of use of concentrated biocellular elements is to encourage the healing while minimizing residual scarring. In example, this is very important in ten-

don repair, where restoration of myotendinous elasticity is critical to avoid re-injury when scar would be exposed to rapid loading of tension (as represented in the Achilles Tendon images above) [23–25] (Figs. 5.19 and 5.20).

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 and 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) [27, 28]. Early reports of improvement in chronic conditions, including pain, arthritis, damaged tendons-ligaments, low back and 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 that this only refers to the use of embryonic tissues or nonautologous 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 than the reparative group of cells.

Considering ready availability of fat and minimally invasive access (using closed-syringe liposuction for example), adipose now has become an

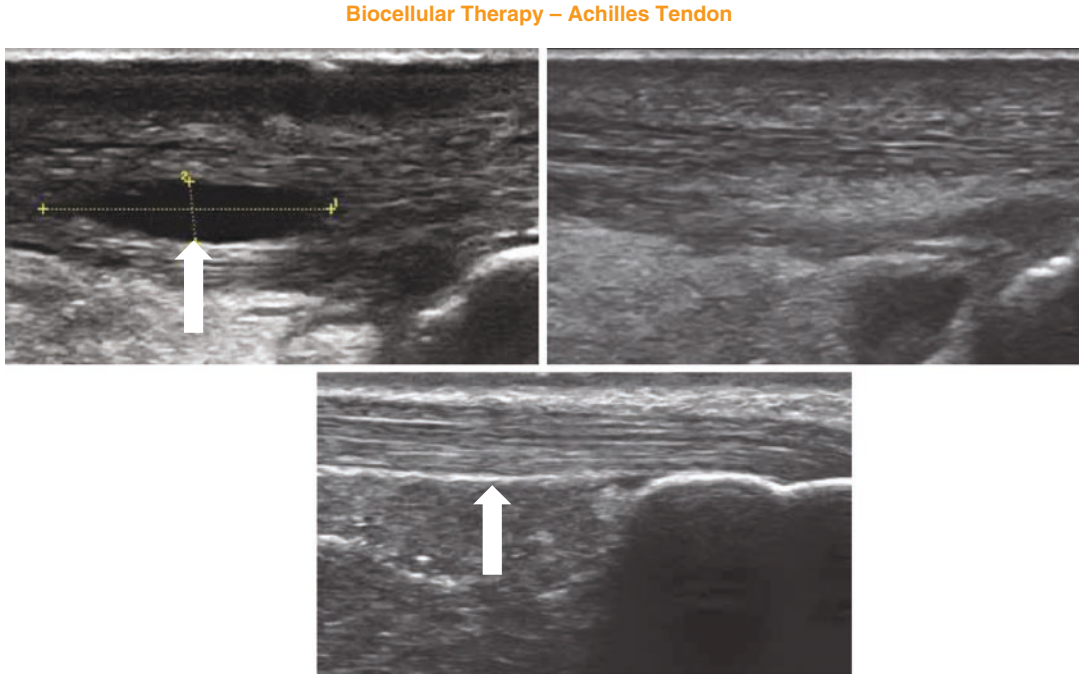


Fig. 5.19 Ultrasound-guided biocellular therapy in torn Achilles tendon. Top left: Tendon tear pretreatment [note: rest of tendon showing tendinosis]. Top right: Posttreatment tendon (at 6–8 weeks); posttreatment 1 year [note: resolution of tear without scarring, rest of tendon returning to more normal fibrillar tendon echotexture]

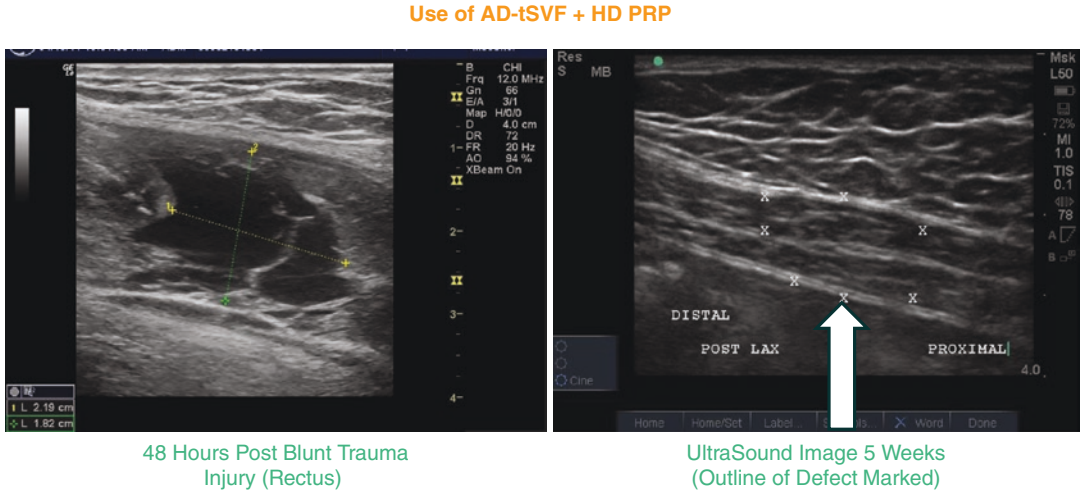


Fig. 5.20 Use of tSVF + high-density platelet concentrate (biocellular therapy) in torn rectus abdominus trauma. Left: Pretreatment image. Right: Area of trauma at 5 weeks post-trauma [note: return of muscle echotexture without scarring]

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 the 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, agitative digestion of cellular contact points) at *point-of-care* (POC) availability, the opportunity of cell enrichment in grafting procedures plus the 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 that have been credited for promotion of healing and repair reside in tissue microenvironments, where they comprise parts of tissues, and organ system identification of pathways, 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 elements specifically targeted (via ultrasound guidance for example) can effectively utilize your own tissues to heal themselves in a more efficient and effective manner.

5.7 What Is Involved in Providing Cellular or Biocellular Regenerative Therapy?

Cellular therapy begins with the exact same harvesting and processing of ATC via microcannu-

las, including centrifugation to remove the remaining suspensory fluid derived from the infusion of very diluted local/epinephrine solution in normal saline. Once removed, the tSVF derivative is exposed to digestion (typically using a blend of collagenase and neutral proteases), incubation, and concentration of the separation of cSVF portions. This cSVF is then neutralized and rinsed to remove the collagenase and protease additives. A final centrifugation results in a concentration "pellet" of cSVF (Fig. 5.21).

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 resuspend 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 (150 μm) ensure removal of any fibrin or other components in a manner common during blood product transfusions (which utilize a standard 170 μm 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 developed and reported (***see www.ClinicalTrials.gov***).

The biocellular therapies refer to the use of tSVF (harvested via microcannula syringe systems) plus the mixed additive of HD PRP (or lower concentrations when used on radiation damage and hair and for dermal uses). The tSVF may be used after a centrifugation (typically 800–1200 g-Force for 3–4 min), mixed with an appropriate concentration (by volume percentage) of PRP, and then guided or intradermally placed to targets. Plastic surgery literature has

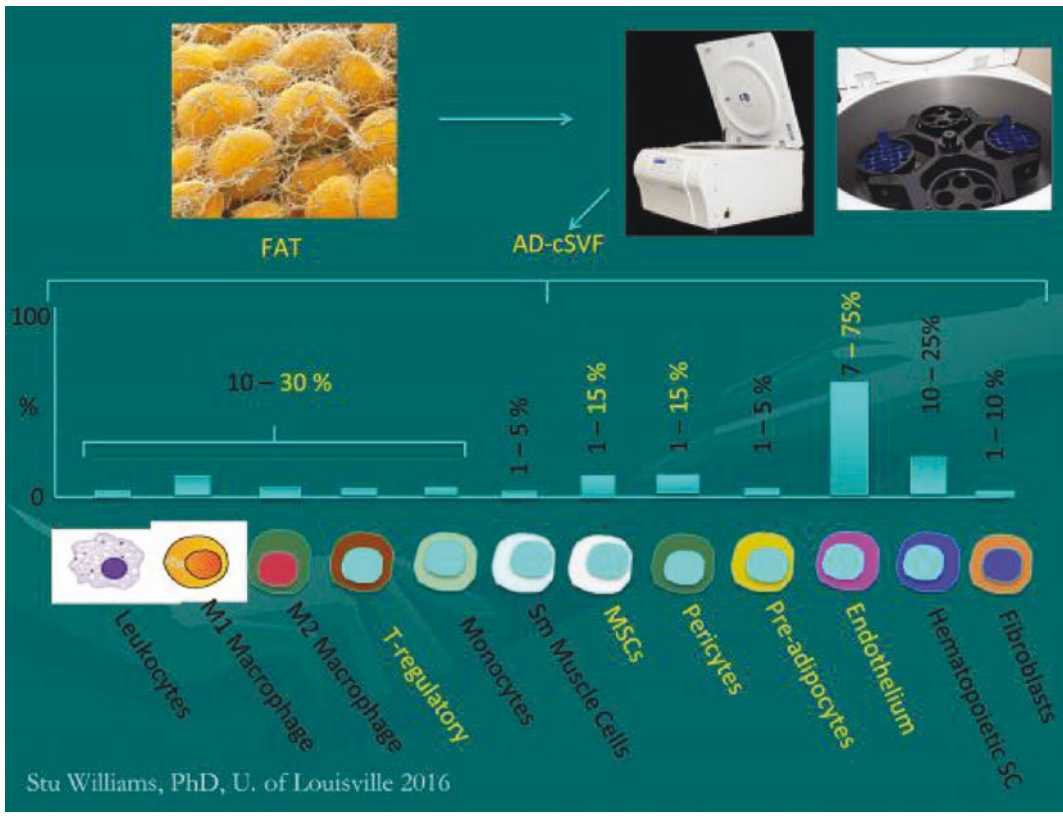


Fig. 5.21 Main component cells in cellular stromal vascular fraction [note: approximately one-third have multipotent capabilities, balance supportive cells of importance] (courtesy of Dr. Stu Williams, University of Louisville)

many examples of improved structural grafting accomplished using cell-enriched tSVF as previously discussed (Fig. 5.22).

As the platelet concentrates are understood to be a key contributor for provision of critical healing growth factors and signal proteins, we recommend striving for greater concentrations achieved 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 "auto-amplification" effect, wherein the site-specific needs are boosted in response during 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 microvessel formation and improved perfusion. Thousands of patients have undergone treatments using these concentrates with quality results in many inflammatory or aging conditions.

An autologous tSVF sample is easily harvested from subdermal fat deposits under sterile protocols, using the patented closed-syringe system. This is often referred to as microcannula lipoaspiration or lipoharvesting [17]. This adipose tissue complex (ATC) may be cleaned and 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 fluid load (Fig. 5.23). By effectively 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 delivery to targets [29].

Cellular and biocellular therapies are commonly performed in many areas of within and outside of the United States; FDA-suggested *guidelines* being discussed currently confuse these issues employing digestive chemicals. Many sites are actively providing these services in the United States, often acting within controlled Institutional Research Based (IRB) trials or study groups within specialties. The author currently stresses the importance of information documentation and reporting on safety and efficacy. Until these existing trials are concluded and reported, it is common to perform these newer options within an approved IRB channel. Following multiple

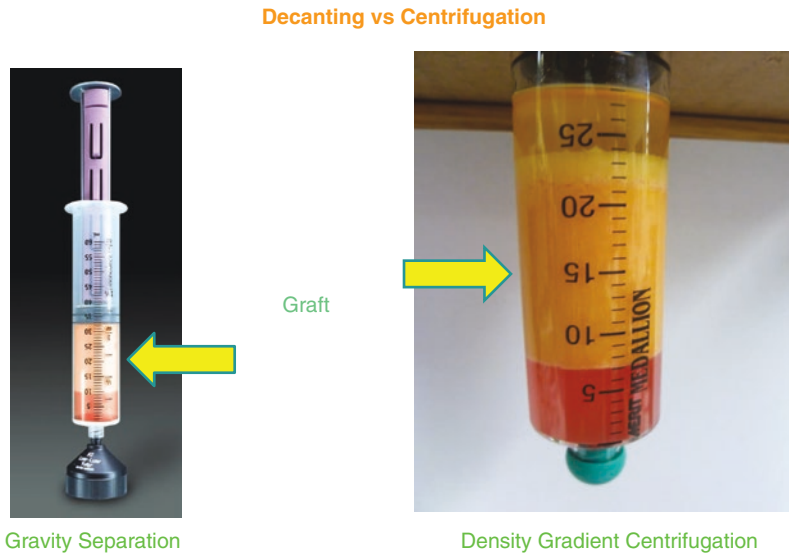


Fig. 5.22 Value of centrifugation for layer separation compared to gravity decantation. Note: Density gradient separation at 800–1000 g-Force/3 min provides effective excess liquid/debris (infranatant), compressed graft (tSVF), and well-separated unwanted free lipid (supranatant)

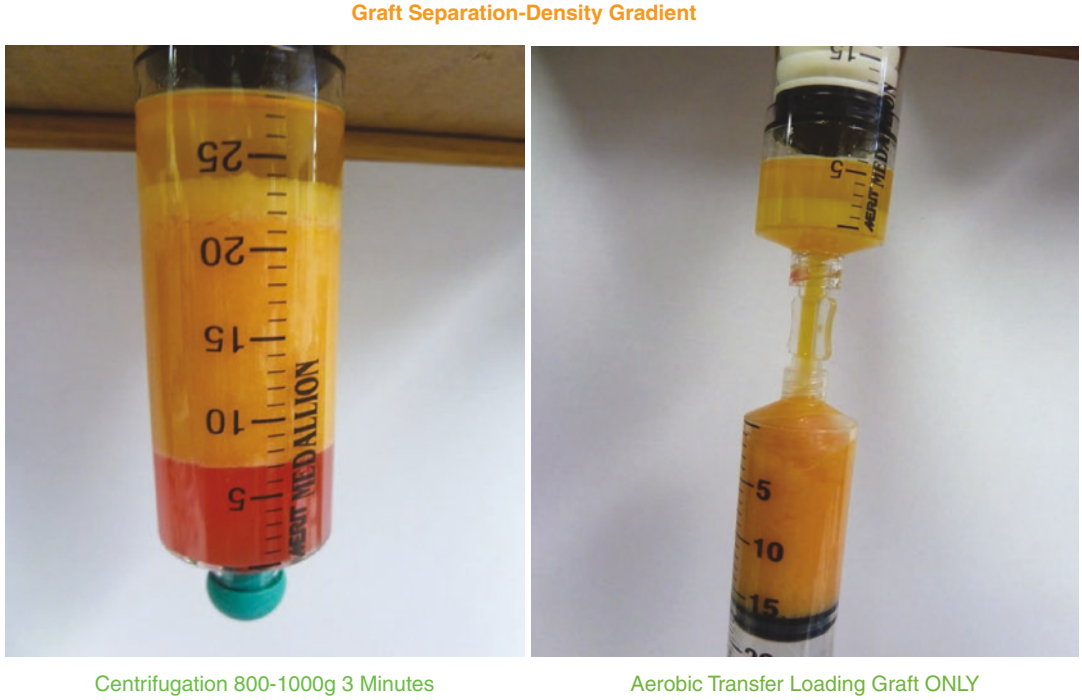


Fig. 5.23 Left: Showing centrifuged specimen. Right: Loading of compressed graft via clear luer-to-luer transfer, leaving supranatant layer (not desired for injection)

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

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, and are gradually being reported in translational clinical journals in the medical literature. As the cellular therapy group is well recognized as favoring immune privilege and pro-"benign" inflammation, those local, systemic, and autoimmune issues are included in many clinical studies (Figs. 5.24 and 5.25).

How the cSVF actually works is under intense study at this time. At first, the thought that the cellular components were the most important (like the incorrect belief that autologous fat grafting relied on the presence of mature adipocytes) has proven not to be correct. In fact, it is now believed that the actual graft successes are a result of the

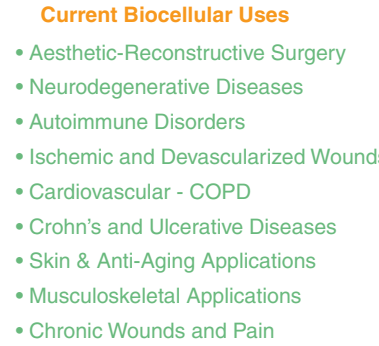


Fig. 5.24 Sample of current cellular and biocellular uses

stem/stromal cells and bioactive matrix (extracellular matrix and 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, 31].

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 exchange of these elements is the means of communication and stimulation of nuclear change leading to the proliferative and trophic effects to guide the differentiation for cell activities or secretions in specific sites. There are 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 [32] (Figs. 5.26 and 5.27).

In the discussions on "nonhomologous" 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 cell groups in applied regenerative efforts in wound healing and orthopedic applications. The concept of these terminally differentiated cells (mature adipocytes) playing a key role in repair and regeneration of musculo-

Fig. 5.25 Disposable system for emulsification (micronization, "Nanofat"). Top: screen (offset 600/400 μm screen). Bottom: shows "partial-emulsification" luer-to-luer series of progressively smaller internal sizes (2.4, 1.4, and 1.2, respectively) (photo provided by Tulip Medical, San Diego, CA, USA)

Exosomes & Microvesicles

- Directly Relate To *Paracrine Functions*
- Located In All Cells, Including Resident Stem Cells & MSC/PC/EPC Groups
- Essential To *Inter*cellular Communication
- Means By Which Stem Cells Send & Receive Messages
- Uses mRNA & miRNA Exchanges To Initiate Their Effects On Nuclear Differentiation & Proliferation

Fig. 5.26 Paracrine secretions and intercellular communications: exosomes and microvesicles as means of signaling for stem cell activation and proliferation

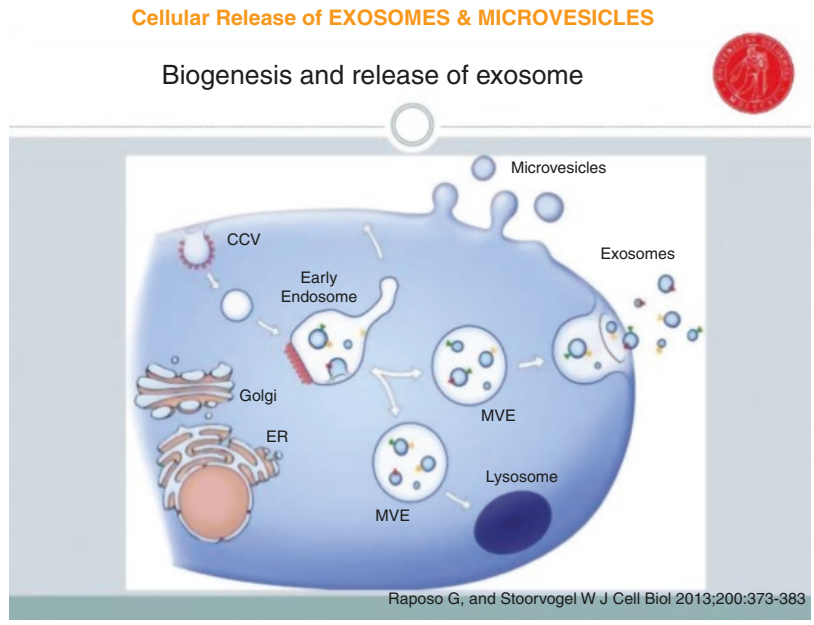


Fig. 5.27 Diagrammatic representation of exosomes and microvesicles

skeletal 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 nonhomologous use based on the fact that fat cells "cannot secrete milk" as the only function or tissue comprising the breast is ludicrous.

5.8 A Recent Advance in Use of Biocellular Uses: Micronized and "Nanofat™" (Emulsified AD-tSVF)

Over the past few years, several alternative advances in processing the lipoaspirated tSVF via mechanical emulsification have evolved [32]. However many applications still favor 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 detrimental impact on stem/stromal cellular numbers or viabilities while markedly reducing the volume of ATC provided by mature adipocytes (Fig. 5.25).

Recent advances now offer the opportunity to have a disposable system to achieve maximal lysis of adipocytes while preserving the much smaller cSVF component (Figs. 5.28 and 5.29). It is important to note that this emulsified (micronized) tSVF product is NOT ABLE to produce true cSVF and, therefore, CANNOT be safely used in *any* intravascular uses. Although not believed to be as effective as using larger fragment tSVF for structural grafting, it can still be combined with PRP concentrates to provide improved dermal vascularity and configuration.

With the ability to inject through small-bore needles, patient comfort in dermal and hair injections is enhanced, and it offers a range of radia-

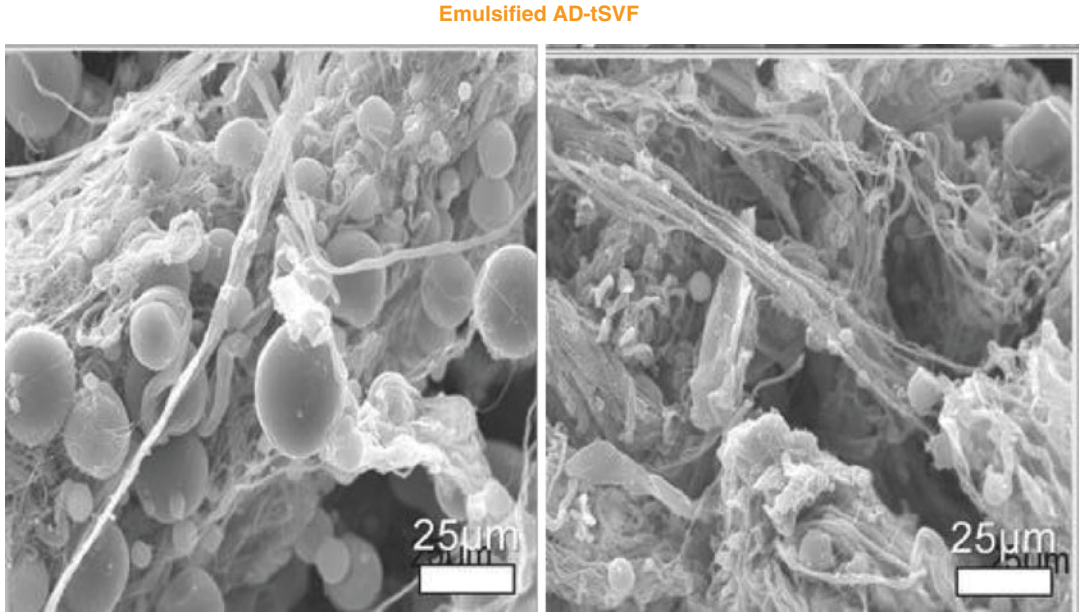


Fig. 5.28 Emulsified adipose tissue complex (tSVF). Left: Shows SEM of AD-tSVF prior to emulsification. Right: SEM of AD-tSVF after emulsification [note: essential removal of all intact mature adipocytes, leaving perivascular and extracellular matrix (ECM) intact] (Feng, J, Doi, K. et al. Micronized Cellular Adipose Matrix as a Therapeutic Injectable in Diabetic Ulcer. Regen Med. 2015; 10(6))

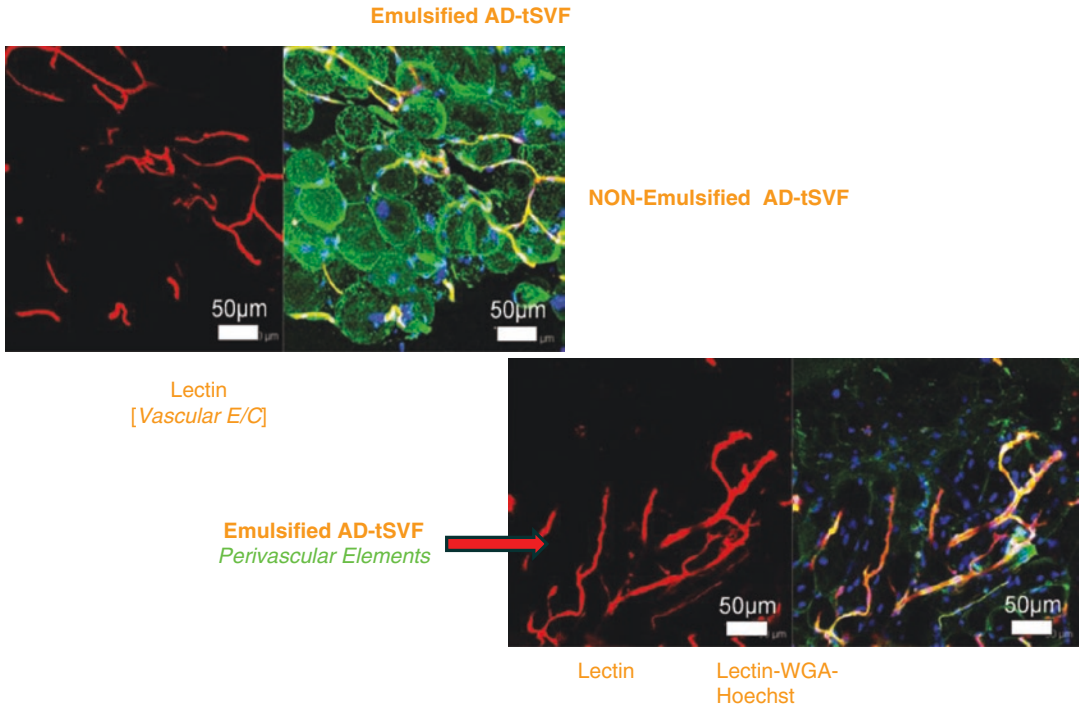


Fig. 5.29 Emulsification of adipose tissue complex (tSVF). Top left: Shows AD-tSVF prior to emulsification (stain showing vascularity on left, and intact adipocytes (green) on right of micrograph). Bottom right: Shows AD-tSVF after emulsification of mature adipocytes [note: multiple blue-stained nuclei remaining intact (small fragment tSVF, not a cSVF)]

Fig. 5.30 Sample of post-radiation skin aging/damage (sun). Top: Pretreatment biocellular mix. Bottom: Posttreatment biocellular grafting (1 year) of cheeks, lips, and nasolabial folds [note: to show true texture change as a result of biocellular mix, placed subdermally]

tion/solar-damaged skin and small joint targeted applications in orthopedic medicine. The abilities of biocellular modalities to promote wound healing and regenerative capabilities via intradermal placement have opened opportunities to permit improved skin circulation and texture; improve skin aging and radiation damage and hair regeneration; and participate in chronic wound applications, as well as many small joint and superficial targets in musculoskeletal applications (Fig. 5.30).

5.9 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 semiautomated 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 extensive equipment and technician costs. Today such isolation is being done in the United States under Institutional Review Board settings to 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.

With the changes in legislation and within the FDA, new and exciting possibilities of IND/RMAT designations are being considered and suggested guidelines are evolving. Timing of these changes is optimal, as much of the safety and early efficacy opportunities will already be in place by the time such designations are better understood. The twenty-first-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 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 of a pivotal value to insure very accurate diagnostics and guided placement to defined targets. Ultrasonography, with its dynamic abilities 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-thecal, intra-peritoneal, etc.) pathways, and become effective for a very expansive treatment for such disorders as neurodegenerative diseases (MS, Alzheimer's, ALS, Parkinson's, brain injuries/stroke, etc.), diabetes, chronic lung disease, heart disease and damage, chronic wound healing, fibromyalgia/causalgia, ulcerative bowel disease, Crohn's, colitis, and so forth.

5.10 Who Provides Biocellular Treatment?

Initially the realm of cosmetic plastic surgeons advanced in uses for sun damage, and deep structural and superficial dermal injections, and a variety of indications have been adopted by a very wide variety of providers. Patients are now cared for by a diverse group of providing doctors (e.g., primary care healthcare providers, internists/neurologists, aestheticians, hair regenerative specialists, aesthetic-plastic surgeons, general surgeons, orthopedic surgeons, emergency/sports medical specialists, pain management specialists, wound care centers, etc.). Those nonsurgical trained practitioners are now deciding whether candidates have a condition which has reasonable potential for improvement through the use of combinations of biologic and stem/stromal cellular treatment. Thorough physical and pretreatment 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 orthopedic applications, use of metrics such as range of motion, indicated MRI studies, and high-quality ultrasonographic imaging combined can determine the specific locations of problems and guide proper placement. In the vast majority of orthopedic cases, use of high-quality MSK ultrasonography is considered a KEY part of such treatment. Proper diagnostic imaging and ultrasound evaluation are 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; tar-

geted and tracked therapy consistently correlates with earlier and improved clinical outcomes. Use of metrics 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, and 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 a variety of mechanical emulsification systems have evolved, it has become easier to permit guided (ultrasound) targeting of damaged or degenerative tissues via very small bore needles (e.g. 23-30 gauge). In addition, this mechanical reduction in particle size of the tSVF does not statistically reduce the numbers, viability or clinical outcomes from it use. The ability to provide improved patient comfort and access to skin, hair, and small joints is rapidly becoming an appealing option.

5.11 Conclusion

"USING YOUR OWN TISSUES TO HEAL" represents a major healthcare paradigm change, and is one of the most exciting minimally invasive options currently available. Both cellular and biocellular regenerative therapies are rapidly improving in documentation and cellular analyses, and gaining very good safety and efficacy profiles. Once considered 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 to be of great value going forward in providing evidence of the specific area of optimal use.

There are many evolving clinical trials (NIH/FDA oversight) 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 it is not clear that it possesses very few of the non-hematologic (reparative) stem cells, and it is 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 three-dimensional scaffolding at this time. This inability limits the development of understanding how to mimic a true in vivo culture/expansion capability, and continues to delay the 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 leave 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.

Acknowledgements The author wishes to thank our clinical and laboratory staff, Ms. Nancy L. Smith and Ms. Susan Riley, for their endless hours and efforts in order to facilitate patients, sample gathering, and testing. Without such devotees, clinical papers cannot be reported.

There are no conflicts of interest reported in this chapter or its content.

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