

Activation of Adipose-Derived Stem Cells

Adult Stem Cells (ASCs), by definition, are unspecialized or undifferentiated cells that not only retain their ability to divide mitotically while still maintaining their undifferentiated state but also given the right conditions, have the ability to differentiate into different types of cells including cells of different germ-origin – an ability referred to as transdifferentiation or plasticity.^{1,2} *In vitro*, the conditions under which transdifferentiation occurs can be brought about by modifying the culture medium in which the cells are cultured. *In vivo*, the same changes are seen when the ASCs are transplanted into a tissue environment different to their own tissue-of origin. Though the exact mechanism of this transdifferentiation of ASCs is still under debate, this ability of ASCs along with their ability to self-renew is of great interest in the field of Regenerative Medicine as a therapeutic tool in being able to regenerate and replace dying, damaged or diseased tissue.

Clinically, however, there are a few criteria that ASCs need to fulfill before they can be viewed as a viable option in Regenerative Medicine. These are as follows:³

1. Abundance in numbers (millions to billions of cells)
2. Ease of harvest (through minimally invasive procedures)
3. Ability to differentiate into multiple cell types (which can be regulated and reproduced *in vitro*)
4. Safe to transplant to a different site of the autologous host or even an allogenic host.
5. No conflict with current Good Manufacturing Principles (during procurement, culture or transplantation)

Adipose Tissue Yields an Abundance of ASC's

Compared to any other source, the vast amounts of adipose tissue (depots of fat for storing energy) especially in the abdominal region, by sheer volume of availability, ensure an abundance in numbers of ASCs ranging in the millions per unit volume. The sheer numbers available also has the added advantage of not needing to be cultured in a laboratory over days in order to get the desired number of ASCs to achieve what is called “therapeutic threshold” i.e. therapeutic benefit. In addition, harvesting ASCs from adipose tissue through simple, minimally invasive liposuction under local anesthesia is relatively easier, painless and poses minimal risk to the patient compared to all other possible methods.

Adipose tissue ASCs (AT-ASCs) are extremely similar to stem cells isolated from bone marrow (BMSCs). The similarities in profile between the two types of ASCs range from morphology to growth to transcriptional and cell surface phenotypes.^{4,5} Their similarity extends also to their developmental behavior both *in vitro* and *in vivo*. This has led to suggestions that adipose-derived stem cells are in fact a mesenchymal stem cell fraction present within adipose tissue.⁶

Clinically, however, stromal vascular fraction-derived AT-ASCs have the advantage over their bone marrow-derived counterparts, because of their abundance in numbers – eliminating the need for culturing over days to obtain a therapeutically viable number – and the ease of the harvest procedure itself – being less painful than the harvest of bone marrow. This, in theory, means that an autologous transplant of adipose-derived ASCs will not only work in much the same way as the successes shown using marrow-derived mesenchymal stem cell transplant, but also be of minimal risk to the patient.

AT-ASCs, like BM-ASCs, are called Mesenchymal ASCs because they are both of mesodermal germ-origin. This means that AT-ASCs are able to differentiate into specialized cells of mesodermal origin such as adipocytes, fibroblasts, myocytes, osteocytes and chondrocytes.^{7,8,9} AT-ASCs are also able to, given the right conditions of growth factors, transdifferentiate into cells of germ-origin other than their own. Animal model and human studies have shown AT-ASCs to undergo cardiomyogenic¹⁰, endothelial (vascular)¹¹, pancreatic (endocrine)¹², neurogenic¹³, and hepatic trans-differentiation¹⁴, while also supporting haematopoiesis¹⁵.

Low Risk to the Patient

Autologous transplant of SVF AT-ASCs also poses extremely low risk to the patient when done as a single procedure in a sterile surgical operating room setting. Furthermore, it is postulated that SVF AT-ASCs due to their immunosuppressive properties may be transplanted into not only autologous but also allogenic tissues without initiating a cytotoxic T-cell response.¹⁶ We at AdiStem believe autologous transplant to be the safest and most viable option at this point.

It is noteworthy that the protocol devised by AdiStem for the procurement of SVF AT-ASCs does not overlook the therapeutic potential conferred by the cocktail of ingredients present in the SVF. Let us look at this cocktail of cells, proteins and growth factors in a little more detail.

The extracellular matrix of adipose tissue contains different types of Collagen such as Types 1, 3-4, 7, 14-15, 18 and 27 to name a few.⁶ This is important, in Adistem's Fat Transfer protocol where freshly isolated fat is used as a filler in augmentation or post-lumpectomy reconstruction of the breast and in the augmentation of the penis, and where collagen provides the structural support required for cell survival.

Furthermore, the extracellular matrix plays an important role in adipocyte endocrine secretions, and release of growth factors such as transforming growth factor beta (TGF- β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), among others all of which are contained in the SVF.¹⁷ This is consistent with the secretions of cells in the presence of an extracellular matrix. The SVF also contains the various proteins present in the adipose tissue extracellular matrix of which Laminin is of interest due to its ability to help in neural regeneration.⁶

The cellular composition of the SVF ranges from pre-adipocytes to endothelial cells, smooth muscle cells, pericytes, fibroblasts, and AT-ASCs. Typically, the SVF also contains blood cells from the capillaries supplying the fat cells. These include erythrocytes, B and T cells, macrophages, monocytes, mast cells, natural killer (NK) cells, hematopoietic stem cells and endothelial progenitor cells, to name a few. The latter two types of cells, namely the hematopoietic stem cells and endothelial progenitor cells play important roles in supporting the viability of existing blood vessels and helping create new ones respectively.

We believe that these other ingredients that make up the SVF 'cocktail' act as an adjuvant to further augment the effect of the autologous transplant of SVF AT-ASCs.

An important point to note is that there is still debate whether freshly isolated ASCs are functionally similar to ASCs which have undergone expansion.¹⁸ We believe this debate to be of little consequence because of the vast numbers of ASCs we are able to harvest and therefore does not need expansion. Moreover, our own preliminary results in human subjects ($n=32$), where wound-healing was tested by the introduction of freshly isolated ASCs into the wound showed more than promising results. It must be stated however, that isolates from the lipoaspirate on its own proved less effective than when the isolates were introduced into either a proprietary Activation Medium containing known growth factor stimulators of stem cells in addition to the patients' own platelet-derived growth factors (using PRP Kit) for one hour before being re-introduced into the patient.

ASCs Require Activation for Full Functionality

The observations stated above, confirms the theory that Adipose-derived ASCs though large in number lie dormant within the adipose tissue and that they require activation to come into full functionality for more successful implantation into the host tissue and to begin self-renewal by cell division and formation of other cell types by differentiation and transdifferentiation. This is also in line with the theory that ASCs are called into action only when the tissues within which they reside are dying, damaged or diseased. Further preliminary testing to increase the functionality of the Adipose-derived ASCs using specific frequencies of monochromatic light (Laser Technology - **Adistem Laser**) – the specifics of which we prefer not to disclose at this time – has also revealed promising results.

AdiStem Phase I/II Clinical Trials in Humans on the *Safety and Efficacy of Administration of Activated Autologous Adipose-derived Stromal Vascular Fraction Adult Stem Cells* are ongoing and at several stages of completion at various centers around the world for Management of **Type II Diabetes**, Breast Reconstruction Post-Lumpectomy, Management and Healing of Chronic Diabetic Ulcers and Hair regrowth in male and female pattern baldness.

Future research areas which have shown promising results in our initial case studies are Osteoarthritis, Emphysema, Stroke, Heart Failure and early stage Parkinson's Disease.

Patents Have Been Filed

AdiStem Ltd. has filed two Australian Innovation Patents and has filed for two International PCT Patents on its methodology of extraction of adipose-derived ASCs from adipose tissue and various methodologies of activation of ASCs.

PRP Kits Available

Using AdiStem PRP Kits, growth factors (GFs) from the patient's own circulating blood platelets are used to activate the adipose-derived ASCs harvested from the same patient.

Wound healing is a complex process, involving a mechanism of complex cascading regulatory events at both the molecular and cellular levels.^{19,20} Growth factors (GFs) are secreted by a wide variety of cells to regulate the wound healing process in an orderly manner.^{21,22} Over the last decade, various GFs, including platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF- β), have been used to accelerate the healing process.²³⁻²⁷

Platelet-rich plasma (PRP), as a storage vehicle of growth factors, is a new application of tissue engineering which was considered for the application of growth factors. PRP is a concentration of platelets in plasma developed by gradient density centrifugation.²⁸ It contains many growth factors, such as PDGF, TGF- β , vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), etc.^{29,30} And it has been successfully used in a variety of clinical applications for improving hard and soft tissue healing.³¹⁻³⁵ Platelet-rich plasma has also been shown to enhance the proliferation of human adipose-derived stem cells.³⁶

The procedure involves the taking of blood during or just prior to the patient's adipose tissue extraction procedure. Platelets are isolated from the blood and then activated to release their growth factors after irradiation with Adistem Laser. The adipose-derived ASCs are then mixed with the growth factor containing plasma at least 30 minutes prior to being administered to the patient.

[See AdiStem Laser Activation Science & Technology »](#)

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AdiStem Laser Activation

The adipose-derived cell population isolated is activated by irradiating the cells with the Adistem Laser, which has certain frequencies of wavelengths in the visible light spectrum (400-1200nm) to stimulate growth and differentiation of stem cells.

Light irradiation or photomodulation can be utilized for significant benefit in the stimulation of proliferation, growth, differentiation, of stem cells from any living organism. Stem cells growth and differentiation into tissues or organs or structures or cell cultures for infusion, implantation, etc (and their subsequent growth after such transfer) can be facilitated or enhanced or controlled or inhibited.

Stem cells can be photoactivated or photoinhibited by photomodulation. There is little or no temperature rise with this process although transient local nondestructive intracellular thermal changes may contribute via such effects as membrane changes or structured conformational changes.

The wavelength or bandwidth of wavelengths is one of the critical factors in selective photomodulation. Pulsed or continuous exposure, duration and frequency of pulses (and dark 'off' period) and energy are also factors as well as the presence, absence or deficiency of any or all cofactors, enzymes, catalysts, or other building blocks of the process being photomodulated.

Photomodulation can control or direct the path or pathways of differentiation of stem cells, their proliferation and growth, their motility and ultimately what they produce or secrete and the specific activation or inhibition of such production.

Photomodulation Can Activate Differentiation or Proliferation of Stem Cells

Our analogy for photomodulation of stem cells is that a specific set of parameters can activate or inhibit differentiation or proliferation or other activities of a stem cell. Much as a burglar alarm keypad has a unique 'code' to arm (activate) or disarm (inhibit or inactivate) sending an alarm signal which then sets in motion a series of events, so it is with photomodulation of stem cells.

Different parameters with the same wavelength may have very diverse and even opposite effects. When different parameters of photomodulation are performed simultaneously different effects may be produced. When different parameters are used serially or sequentially the effects are also different.

The selection of wavelength photomodulation is critical as is the bandwidth selected as there may be a very narrow bandwidth for some applications—in essence these are biologically active spectral intervals. Generally the photomodulation will target flavins, cytochromes, iron-sulfur complexes, quinines, heme, enzymes, and other transition metal ligand bond structures though not limited to these.

These act much like chlorophyll and other pigments in photosynthesis as 'antennae' for photo acceptor molecules. These photo acceptor sites receive photons from electromagnetic sources such as those described in this application, but also including radio frequency, microwaves, electrical stimulation, magnetic fields, and also may be affected by the state of polarization of light. Combinations of electromagnetic radiation sources may also be used.

The photon energy being received by the photo acceptor molecules from even low intensity light therapy (LILT) is sufficient to affect the chemical bonds thus 'energizing' the photo acceptor molecules which in turn transfers and may also amplify this energy signal. An 'electron shuttle' transports this to ultimately produce ATP (or inhibit) the mitochondria thus energizing the cell (for proliferation or secretory activities for example). This can be broad or very specific in the cellular response produced.

The as yet unknown mechanism, which establishes 'priorities' within living cells, can be photomodulated. This can include even the differentiation of stem cell population.

Photomodulation parameters can be much like a "morse code" to communicate specific instructions to stem cells. This has enormous potential in practical terms such as guiding or directing the type of cells, tissues or organs that stem cells develop or differentiate into as well as stimulating, enhancing or accelerating their growth (or keeping them undifferentiated).

Trials are Ongoing

AdiStem Ltd. has a large on-going international research project looking at the effects of different frequencies of monochromatic lights on stem cells. It has now found five frequencies (three are present in the Adistem Laser) that can activate stem cells in vitro and two frequencies that inhibit them. AdiStem Ltd. is also exploring the direct effect of different low level frequencies of light on endogenous stem cells (in vivo).

[See Adipose-Derived Stem Cell Activation Science & Technology »](#)

TB4-7™ Peptide from AdiStem

TB4-7™ Peptide applied topically or given systemically promotes angiogenesis and wound healing, cardioprotective and immuno-potentiating effects, hair growth and thickness, hair coloration change (from grey back to its original darker colour) and has very potent anti-aging, anti-wrinkle and anti-scarring properties. Trials amongst body builders have also shown increased muscle growth and strength together with improved energy levels and other benefits.

TB4-7 (patent pending) is a synthetic peptide analogue of the active region of Thymosin B4.

Thymosin β 4, a ubiquitous 4.9-kDa polypeptide of 43 amino acids length originally isolated in 1981 from the thymus gland, is a potent mediator of cell migration and differentiation¹⁻⁶.

It is a member of a ubiquitous family of 16 related molecules with a high conservation of sequence and localization in most tissues and circulating cells¹³ and circulating levels in the serum reduce with age. TB4 binds to actin, blocks actin polymerization, and is the actin-sequestering molecule in eukaryotic cells¹⁴⁻²⁰).

It was identified as a gene that was up-regulated four- to six fold during early endothelial cell tube (blood vessel) formation and found to promote angiogenesis. It is present in wound fluid⁷, and when applied topically or given systemically, it promotes angiogenesis and wound healing³).

Thymus extracts (which contain small amounts of Thymosin beta 4), have been used successfully both orally and by injection in patients since the 1960's in Europe (see [Biofactor](#)). Derived from our

autologous adipose-derived stem cell **clinical trials and research**, we have uncovered that one of the main factors that the stem cells secrete to induce repair and an anti-aging effect were various peptides belonging to the TB4 family. TB4-7 (patent pending) was the most potent fragment from this family of secreted peptides that we discovered.

Wound-Healing

TB4 elicits cell migration through a specific interaction with actin in the cell cytoskeleton^{8,9}. Recently, it was demonstrated that a central small amino acid long actin binding domain has both angiogenic and wound healing activity while other domains of the protein are inactive¹⁰.

In angiogenesis and in wound healing, TB4 acts by accelerating the migration of endothelial cells and keratinocytes and increasing the production of extracellular matrix-degrading enzymes^{4,9}. TB4 has also shown to increase corneal epithelial cell migration in vitro and in vivo, with activity in the nanogram range¹. Thymosin beta 4 is found in high concentrations in platelets²². TB4 also has anti-inflammatory activity^{1,11} and more recently has also been shown to stimulate epidermal stem cell differentiation¹².

Together, these data show that TB4 is a potent, naturally occurring wound repair factor. It is different from other repair factors, such as growth factors, in that it promotes endothelial and keratinocyte migration, does not bind to the extracellular matrix, and has a very low molecular weight, and thus can diffuse relatively long distances through tissues^{4,21}.

Hair Growth

It has also been demonstrated that TB4 promotes hair growth in various rat and mice models including a transgenic TB4 overexpressing mouse²⁴. The mechanism by which TB4 acts to promote hair growth is by its stimulatory effects on follicle stem cell growth, migration, differentiation, and protease production.

Anti-Aging Properties

Applied topically on the skin (as a spray or cream), TB4-7b has very potent anti-aging, anti-wrinkle, anti-scarring and wound healing properties. No adverse or unwanted effects have been observed in case studies and in a phase I/II clinical trial involving 30 patients. Applied topically on the scalp, promotion of hair thickness, change in coloration (from grey back to its original darker colour), and hair growth have also been clearly observed.

Large Clinical Trial About to Be Initiated

When injected into patients it promotes activation of circulating and various organ stem cells in a safe manner, leading to a very potent anti-aging effect. Benefits have so far been observed in patients with a variety of degenerative diseases, such as heart disease, neuromuscular disease, diabetes and emphysema, to name a few. A large clinical trial under FDA regulations is about to be initiated on the efficacy of TB4-7 in patients with Type II diabetes, Duchenes Muscular dystrophy and emphysema.

As has been shown in cardiac tissue, TB4-7 promotes the activation of cardiac muscle and endothelial progenitor cells, and their survival, and decreases muscle apoptosis²⁵. Similarly in the cornea, TB4 promotes cell migration and wound healing, has anti-inflammatory properties, and suppresses apoptosis²⁶. Another group's results indicate that TB4 also promotes the survival and neurite outgrowth of cultured spinal cord neurons²⁷. We have observed similar promotive effects of TB4 on the survival and stimulation of skeletal muscle progenitors and myocytes.

Already Used By Body Builders

Human trial candidates have noticed the following in an ongoing trial in body builders where they inject 10mg of TB4-7 twice weekly for four weeks:

- Increased muscle girth.
- Faster recovery time (wear and tear) from training.
- Increased muscular endurance.

Increased muscular strength.
Decreased musculoskeletal pain.
Improved hand-eye coordination.
Increased energy levels.
Improved mood (decreased anxiety).
Improved sleeping patterns.
Improved concentration span and short-term memory.
Improved hair growth.

AdiStem TB4-7™ Peptide is an injectable formulation of TB4. A similar formulation has just completed Phase I clinical trials and has been shown to be safe and well-tolerated. The product candidate has been developed to address medical indications where subcutaneous administration is warranted. AdiStem Ltd. is currently planning a Phase I/II clinical trial to evaluate AdiStem TB4-7™ in type II diabetic patients although other indications such as motorneurons disease, emphysema, and Duchene's muscular dystrophy have been identified as potential targets.

Available in a Cream for Hair Growth through ActiStem Ltd.

AdiStem TB4-7™ Peptide is now available in a cream for hair growth. You can purchase or view more information on our distributor's website, ActiStem.com.

About AdiStem Ltd.

Aside from autologous adipose-derived stem cell therapy, AdiStem Ltd. is focused on the discovery and development of novel stem cell derived peptides to accelerate tissue and organ repair. Currently, AdiStem Ltd. is developing a product candidate, TB4-7, for Type II diabetes. AdiStem Ltd. is also developing other product candidates for use on other diseases. These product candidates are based on TB4, a synthetic derivative copy of the 43-amino acid, naturally occurring peptide. AdiStem Ltd. holds and has applied for over 6 world-wide patents and patent applications related to novel peptides to date.

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