Treatment Coordinator

Handouts in hospital
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HELENE CLINIC

STEM CELL TREATMENT

STEM CELL TREATMENT PRO_HELENE CLINIC SINCE 2013

STEM CELL TREATMENT PRO_HELENE CLINIC SINCE 2013

Regenerative Medicine For You

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MESSAGE *from* Dr. MATSUOKA

One day after Dr. Matsuoka got cancer surgery

After administration of stem cells (back to healthy appearance)

I am Dr. Matsuoka, the representative doctor of Helene Clinic.
I was found with thyroid cancer in 2011. I have been cured after the surgery. To regain my strength and vitality after the surgery, I decided to start Stem Cell therapies. As a patient, I realized and felt that it is a good treatment.The idea has been brought to our team by my own experience, since then we are focusing and developing the therapies at our hospital.
According to the homing theory of stem cells, damaged cells produce an SOS signal, the SDF-1/CXCR4 signal, which is emitted by the damaged cell. It is then detected by the stem cell and sent to the damaged cell to repair it. In other words, it is like a "treatment that focuses on the weak areas of the body".
In adults who are in good health, stem cells are directed to repair tissue in unaffected areas that are not yet symptomatic.
In my case, I was treated with stem cell administration for thyroid hormone imbalance after thyroid cancer surgery. The results were excellent for fatigue and burning sensations. My mind started working quicker and my memory and ideas returned to the way they were about 10 years ago.
I continue to experience stem cell therapy as a patient once a year, and I am pursuing the potential of stem cells to be safer and more effective.
In recent years, stem cell exosome therapy, which is said to be as effective as stem cells, has shown great potential. This is why Helene has also been focusing on stem cell exosome therapy. The body's organs age little by little and eventually end up dying if they are not treated. Stem cell therapy enables their maintenance from an early stage.
Body organs cannot be easily replaced. We need to take good care of them and the best way to maintain our body (them) is to use stem cells for anti-aging effect.

松岡孝明

Legal Advisor

Mr. Ritsuo Hosokawa

Former Minister of Health, Labour and Welfare

13th Minister of Health, Labor and Welfare
Former member of the House of Representatives (7 terms)
Attorney at Law, Director of Koshigaya Sogo Law Office
Trusted Advisor, Democratic Party of Japan, Saitama Prefectural Federation

Technical Advisor

Mr. Ravindra Gupta

Professor at the University of Cambridge

Professor of Clinical Microbiology at the Institute of Immunology and Infectious Disease Therapy, University of Cambridge
Member of the faculty of the African Institute of Health in Durban, South Africa
Time Magazine's "100 Most Influential People of 2020"

STEM CELL TREATMENTS

A treatment that activates weak cells and increases the number of vital cells that are decreasing every day.

01 About Stem Cells

02 How do Mesenchymal Stem Cells work? And their principle

03 Functions of Exosomes

04 Diseases

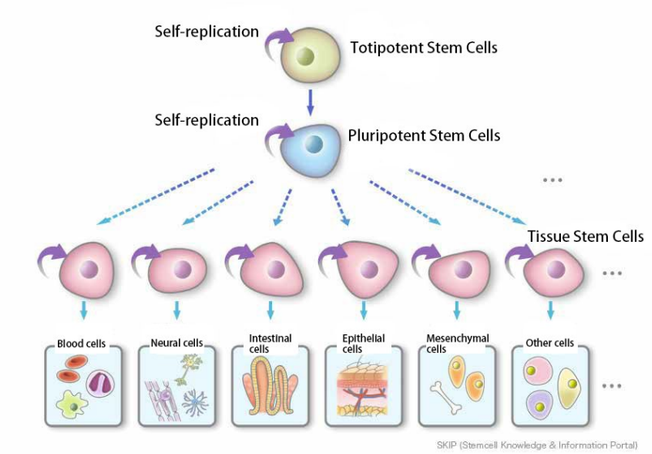
05 Risks of Stem Cell Therapies

[Diseases expected to alleviate symptoms]

Myocardial Infarction
Osteoporosis
Diabetes
Atopic Dermatitis
Anti-Aging (Rejuvenation)
Alzheimer's Disease
Cerebral Stroke
Rheumatism (Arthralgia)
Parkinson's Disease
Renal Failure
Osteoarthritis and other Arthropathies
Immuno-stimulation
Hypertension
Periodontal Disease
Partial Intractable Disease

ABOUT STEM CELLS

Cells and tissues in the body are constantly going on biological renewal. The existing cells have the ability to regenerate and replenish lost cells. Cells with these abilities are called "stem cells".



SKIP (Stemcell Knowledge & Information Portal)

TO BE RECOGNIZED AS STEM CELL, TWO ABILITIES ARE ESSENTIAL

One is the ability to produce various cells that make up our bodies, such as skin, red blood cells, and platelets (differentiation capacity). The other is the ability to divide into cells that have the exact same capabilities as oneself (self-renewal).

STEM CELLS CAN BE DIVIDED IN TWO MAIN TYPES

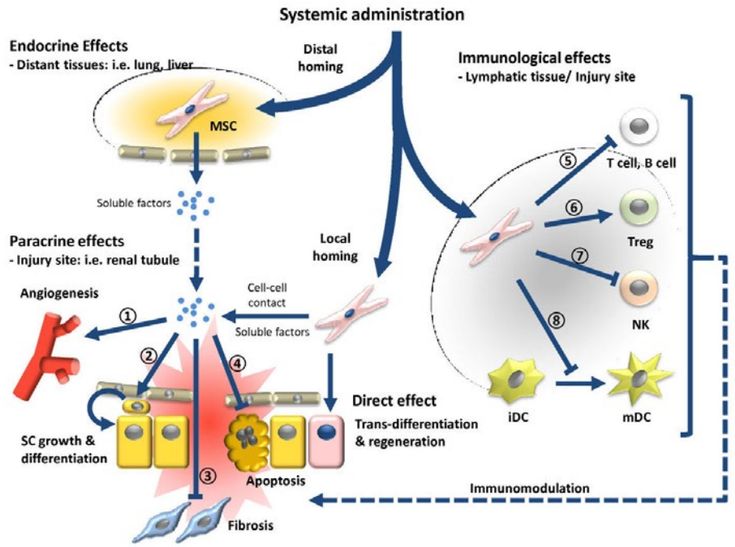
First, there are stem cells that continue to replace missing cells in established tissues and organs, such as skin and blood. This type of stem cells are called "tissue stem cells." Tissue stem cells cannot transform themselves into anything other than they currently are. Hematopoietic stem cells, which produce blood, produce cells of the blood system, neural stem cells, which produce cells of the nervous system, produce cells of the nervous system only, and so on. Secondly, there exist Pluripotent Stem Cells, which can produce any cell in our body, as long as they are embryonic stem cells (ES cells). In other words, pluripotent stem cells can also produce various tissue stem cells in our body. iPS cells (induced Pluripotent Stem Cells) are "pluripotent stem cells" that are artificially created from ordinary cells.

The properties of stem cells are being used in research on new therapies called "regenerative medicine," in which the cells themselves are used as medicine to treat injuries and diseases. Research is also underway to reproduce the state of cells in the body outside the body to investigate the mechanism of diseases.

ABOUT MSC

FULL BODY INJECTION

Systemic administration of Mesenchymal Stem Cells can induce through-blood (endocrine) or local (paracrine) effects, including cell-mediated effects.

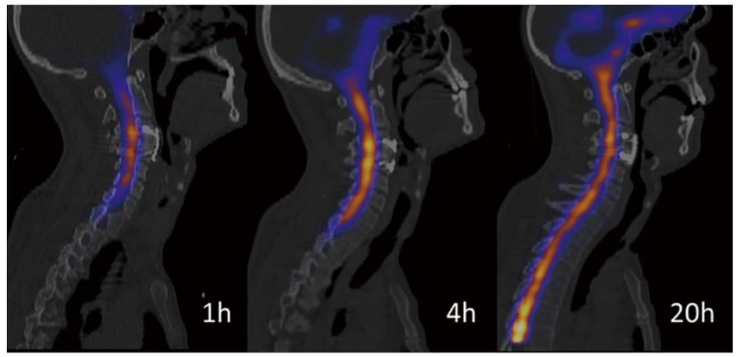


1. Vascular endothelial growth factor (VEGF), insulin -like growth factor 1 (IGF-1), monocyte chemoattractant protein 1 (MCP1), basic fibroblast cell growth factor (bFGF), interleukin 6 (IL6).
2. Stem cell proliferation and differentiation: stem cell factor (SCF), leukemia inhibitory factor (LIF), macrophage colony-stimulating factor (M CSF) Stromal cell-derived factor 1 (SDF1), angiopoietin 1,activin A.
3. Inhibition of fibrosis: hepatocyte growth factor (HGF), the bFGF, adrenomedullin (ADM).
4. Inhibition of apoptosis: VEGF, HGF, and IGF1, transforming growth factor (TGF)-beta, and bFGF, granulocyte macrophage colony-stimulating factor (GM CSF), activin A thrombospondin 1. Immune-mediated effects include the following (5-8).
5. T and B cell suppression: human leukocyte antigen G5 (HLA G5), HGF, inducible nitric oxide synthase (iNOS), indoleamine 2,3 dioxygenase (IDO), prostaglandin E2 (PGE2) , bFGF, TGF-β.
6. TGFβ expression in regulatory T cells (Treg) induces differentiation and proliferation.
7. Inhibition of natural killer (NK) cells by secretion of IDO, PGE 2 and TGFβ.
8. Dendritic cell (DC) maturation by secretion of PGE 2 inhibition.

[Source] Biol Res. 2012;45(3):269-77. Mesenchymal stem cell treatment for autoimmune diseases: a critical review. Figueroa FE, Carrión F, Villanueva S, Khoury M.

DEMONSTRATION OF THE HOMING EFFECT

A 33-year-old male patient with complete tSCI, after a cervical dislocation at the C3-C4 spinal level due to a diving accident, underwent immediate 360 decompression and an instrumented immobilization was performed immediately. Postoperative MRI showed severe spinal cord injury with little spinal cord tissue remaining . Three months after injury, autologous MSCs were harvested from the patient's iliac crest bone marrow and cultured. One third of these were radiolabeled with 600 MBq of 99mTcexametazime HMPAO. At 1 hour, 4 hours, and 20 hours after implantation, whole-body planar images of the head and neck, and hybrid SPECT/hybrid SPECT/CTimaging were performed to follow cell migration.



[Figure] Median sagittal section image of the tSCI patient treated with autologous MSCs. Intrathecal radiolabeled MSCs were administered intrathecally 3 months after acute tSCI. 1 hour, 4 hours, and 20 hours later, hybrid SPECT/CT images of the neck (C3-C4 level) were taken. One hour after administration, a signal (radioisotope) accumulation was observed in the spinal cord at the level of damage (C3-C4) and a slow and gradual increase in the signal was observed after 4 hours of administration. After 20 hours, an extensive signal is observed throughout the spinal canal due to strong background activity. Anteriorly, osteosynthetic material is recognized.

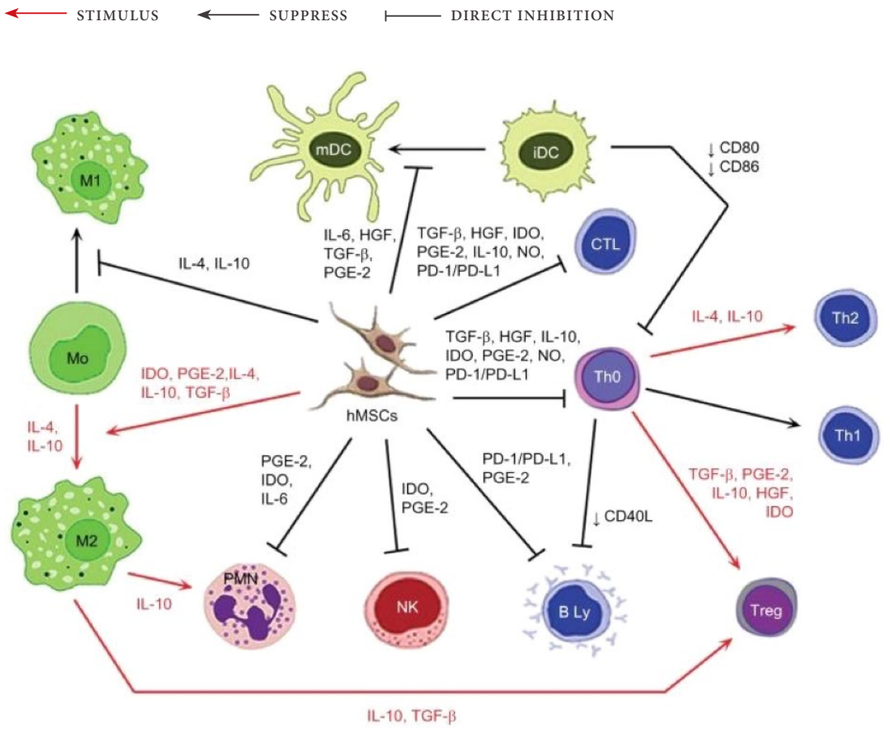
These results suggest the presence of a homing signal that promotes MSC migration and engraftment at the site of spinal cord injury 3 months after acute tSCI.

[Source] Homing of mesenchymal stem cells after acute traumatic cervical spinal cord injury a case report. May 2020Cytotherapy 22(5):S89 S90. DOI:10.1016/j.jcyt.2020.03.151.Project: Mesenchymal stem cell therapy for spinal cord injury site.

ACTIVATED IMMUNOMODULATORY ACTION OF MSC

[Figure] "Activated Immunomodulatory Action of MSCs"

MSCs can affect the functions of many activated T cells, B cells, NK cells. They can also affect the functions of dendritic cells, monocytes/macrophages, neutrophils, and mast cells.



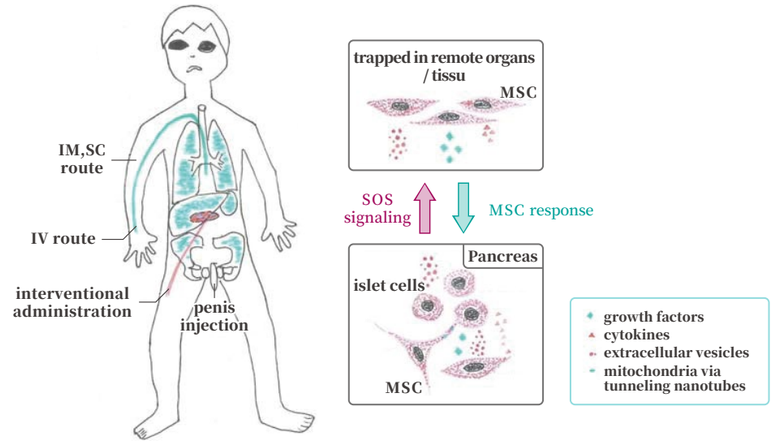
[Abbreviations]

- iDC, Immature dendritic cells
- HGF, Hepatocyte growth factor
- PGE-2, Prostaglandin E2
- NO, nitric oxide
- IL, Interleukin
- TGF-β,Transforming growth factor-β
- IDO, indoleamine 2,3-dioxygenase
- PD-L1, programmed death ligand 1
- hMSC, human mesenchymal stem cell
- Treg, Tregulatory
- Th, T helper
- CTL, Cytotoxic T cell
- mDC, Mature cotyledon, Mature dendritic cell
- PD-1, Programmed cell death protein 1
- PMN, Polymorphonuclear leukocyte
- NK, NK cell

[Source] J Inflamm Res. 2016; 9: 231–240. doi: 10.2147/JIR.S121994
Activation, homing, and role of the mesenchymal stem cells in the inflammatory environment.
Lukáš Zachar, Darina Bačenková, and Ján Rosocha

ELUCIDATION OF THE MECHANISMS OF MSC ADMINISTRATION

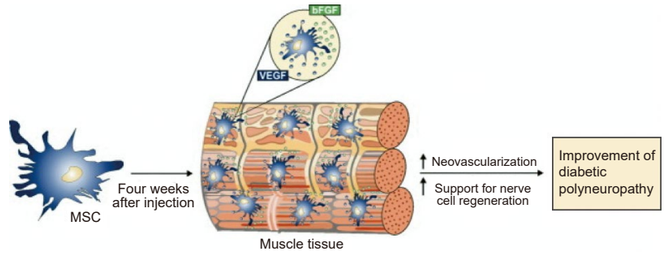
In interventional administrations, mesenchymal stem cells are directed to the pancreas, but by other routes (IV/ IV injection, IM intramuscular, SC/ subcutaneous, penile), most mesenchymal stem cells are trapped in the lungs, liver, spleen, and bone marrow. Damaged islet cells send SOS signals, and in response to these signals, mesenchymal stem cells secrete beneficial growth factors, cytokines, and extracellular vesicles to repair the damage. Mesenchymal stem cells transplanted into the pancreas can send out tunnel-shaped nanotubes containing mitochondria to repair or promote repair of pancreatic damage.



[Source] Pawitan JA1, Yang Z2, Wu YN2, Leed EH2. Towards Standardized Stem Cell Therapy in Type 2 Diabetes Mellitus: A Systematic Review Curr Stem Cell Res Ther. 2018 May 2. doi: 10.2174/1574888X13666180502143657.

EFFECTS OF MSC TREATMENTS ON DIABETIC POLYNEUROPATHY

Four weeks after intramuscular injection, MSCs are deposited in the gaps between muscle fibers via the generation of bFGF and VEGF, inducing angiogenesis and neuronal regeneration resulting in amelioration of diabetic polyneuropathy. IV injection of MSCs is also a potential therapy for diabetes.



[**Abbreviations**] bFGF : basic fibroblast growth factor
VEGF : Vascular endothelial growth factor
MSC : Mesenchymal Stem Cells

[Source] Stem Cells. 2011 Jan;29(1):5 0. Concise review: Mesenchymal stem cell treatment of the complications of diabetes mellitus. Volarevic V, Arsenijevic N, Lukic ML, Stojkovic M.

EXOSOMES

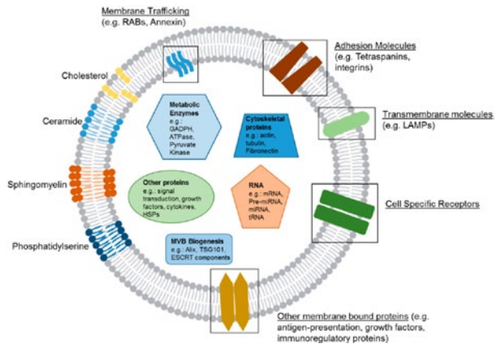
ABOUT EXOSOMES

	Exosomes	Vesicles	Apoptotic Cells
Molecular diameter (nm)	40 - 150	100 - 1,000	500 - 4,000
Generation paths	Living Cells (Endocytosis)	Living Cells (Part of the membrane budding)	Apoptotic Body

[Source] Mesenchymal Stem Cell Derived Extracellular Vesicles: A Novel Cell Free Therapy, Immunological Investigations, DOI: 10.1080/0882 0139.2020.1712416

INTERCELLULAR COMMUNICATION

Proteins on the plasma membrane receive signals from the extracellular environment. The process of endocytosis and exocytosis fusion releases exosomes. Exosomes contain substances that help the transmission of information between cells. Exosomes usually have the same properties as the parent cell where the cells of the exosomes differentiated, and contents also vary depending on the original cell. MSC exosomes contain many cytokines and growth factors.



[Source] https://www.nobelprize.org/prizes/medicine/2013/press release/

NOTABLE RESEARCH AREAS

The 2013 Nobel Prize in Physiology or Medicine has been awarded to three scientists for their discovery of a mechanism that regulates the transport of extracellular vesicles.

> *"Large, busy ports need a system to ship the right cargo to the right destination at the right time." What Rothman, Schekman, and Schudhoff discovered was a system that ingeniously controlled the transport and delivery of cellular cargo.*
>
> -The Nobel Prize in Physiology or Medicine 2013

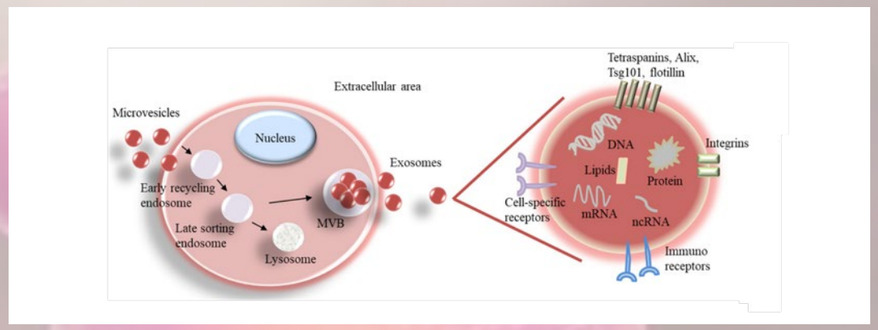
[Source] https://www.nobelprize.org/prizes/medicine/2013/press release/

MSC EXOSOMES CHARACTERISTICS

MSC exosomes are nearly identical to their parental cells in their ability to release cytokines, regulate immune responses to maintain homeostasis capabilities, and play roles in erythroid maturation, antigen presentation in immune responses, coagulation, inflammation, and angiogenesis.

01 TARGETED MOBILITY

Even though the majority of cells can emit exosomes, exosomes do not happen to randomly interact with neighboring cells but conduct messages selectively.



[Source] Schwarzenbach H, Gahan PB. Predictive value of exosomes and their cargo in drug response/resistance of breast cancer patients. Cancer Drug Resist 2020;3:63 82. http://dx.doi.org/10.20517/cdr.2019.90

02 NANOPARTICLES

A typical cell is about 10,000 to 30,000 nanometers (nm) in size, whereas an exosome is 40 to 150 nanometers (nm), only about 1/200th the size of a cell. Exosomes also have a bilayer lipid membrane structure and can pass through the gaps between endothelial cells to reach the target cells, thus having a great effect on the human body. In addition, some studies have shown that exosomes can cross the blood-brain barrier (BBB) to reach target cells.

03 LOW IMMUNOGENICITY

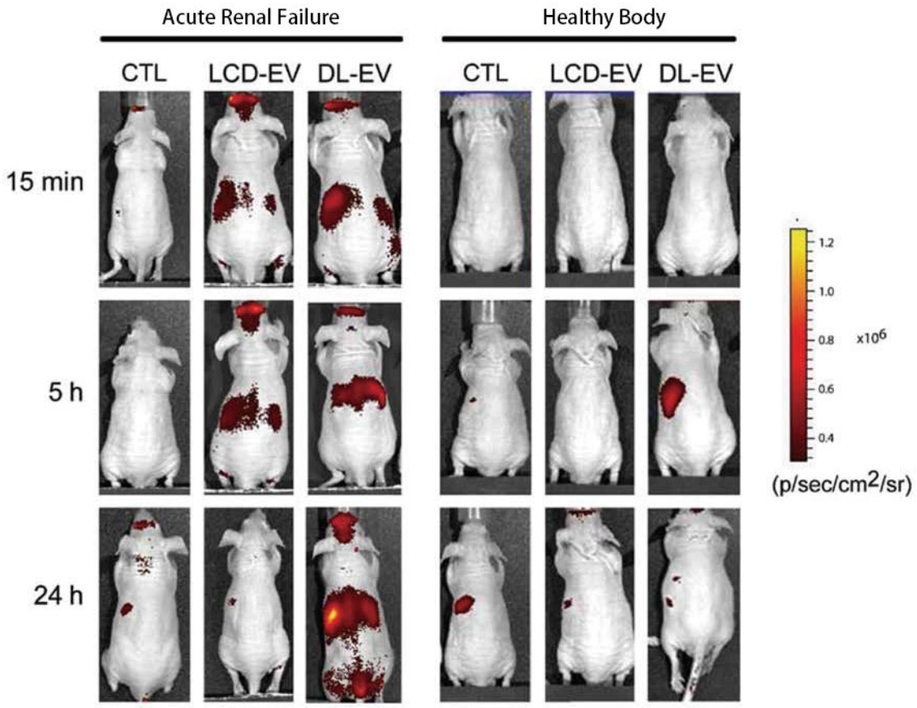
Exosomes are not cells. They serve only as message carriers and have few antigens on the outer membrane surface, making them universal and difficult for the immune system to recognize, and therefore, the risk of injecting them into the human body is low.

04 REGULATION OF IMMUNE FUNCTIONS

- Remove unwanted proteins during the cell maturation process.
- Heat shock proteins in exosomes activate natural killer (NK) cells and macrophages.
- Dendritic cell (DC)-derived exosomes activate T cells.
- Exosomes derived from infected cells elicit a specific antimicrobial response.

RELATED RESEARCH

MSC EXOSOMES CONCENTRATE IN DAMAGED TISSUES



[Source]

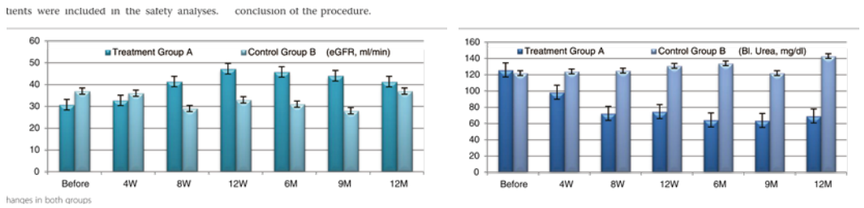
Biodistribution of mesenchymal stem cell derived extracellular vesicles in a model of acute kidney injury monitored by optical imaging. DOI: 10.3892/ijmm.2014.1663 · Source: PubMed

MSC-derived exosomes show a similar homing effect. Once MSCs migrate to the site of injury through the CXCR4-SDF-1 axis pathway, they release exosomes to attempt to control the immune system and send a message to the affected cells. MSC-derived exosomes release adhesion molecules such as CD29, CD44, CD73. Homing to damaged or inflamed tissue then becomes possible. In the photo above, in a mouse model of acute renal failure, MSCEV is mainly accumulated in the inflamed kidneys. The red area indicates that MSC-derived exosomes are accumulating in the injured kidney.

CHRONIC KIDNEY DISEASE

Twenty patients with chronic kidney disease for more than 6 months were treated with MSC exosomes twice (1 week apart). Patients improved their eGFR ratios and BUN levels within one year of treatment with MSC exosomes.

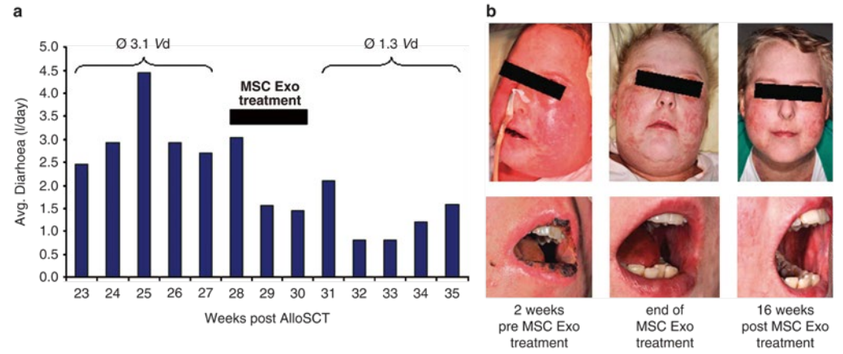
- eGFR is an indicator for diagnosing chronic kidney disease. Normal value is 100-120, and a value lower than this value indicates a decline in kidney function.
- BUN is a diagnostic indicator of renal function; a higher value indicates lower renal function.



[Source] Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. doi :10.1186/s40824 016-0068-0

GRAFT VERSUS HOST DISEASE (GvHD)

MSC exosomes are administered to patients with severe GvHD reactions to allogeneic transplants.



[Source] MSC derived exosomes: a novel tool to treat therapy refractory graft versus host disease Leukemia (2014) 28, 970 973; doi:10.1038/leu.2014.41

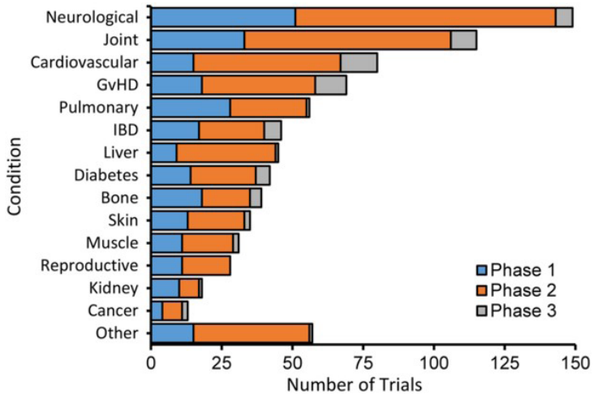
APPLICATION IN DRUG DELIVERY SYSTEMS (DDS)

Stem cells can reach the site of injury by receiving signals (SDF-1). Upon receiving a signal, the stem cell decides which active substances (cytokines, growth factors, etc.) to load into the exosomes, to the "address" of the location. After exosomes leave the stem cell, they follow their address to the damaged area, release cytokines and growth factors, and repair the damage. But that only happens when stem cells are injected into the body. Would this happen if we inject only exosomes?

At Helene Clinic, we use passive exosome culture technology to culture exosomes together with active substances needed by the target cells. We make sure (that only) to collect purified exosomes which then (can be returned to) accurately reach the damaged site and repair the lesion.

MSC CLINICAL TRIALS

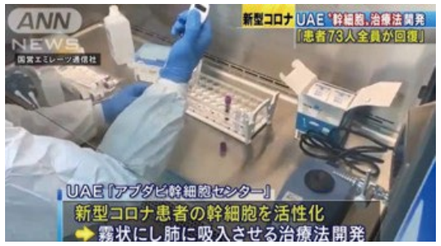
Indications of mesenchymal stem cells in clinical trials. Data of clinical registry 916.



[Source] Trends in mesenchymal stem cell clinical trials 2004 2018: Is efficacy optimal in a narrow dose range? https://doi.org/10.1002/sctm.19 0202

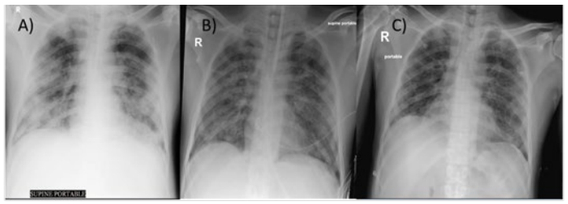
ABOUT RESISTANCE TO COVID-19

The UAE has announced the development of a breakthrough treatment for the new strain of coronavirus using stem cells from human blood. The therapy, developed by the Abu Dhabi Stem Cell Center, a research facility in the UAE; activation of stem cells taken from the blood of patients infected with the new coronavirus, turning them into microparticles, and inhalation, leading them directly into the lungs. All 73 patients who received this treatment recovered and had no side effects. Inhalation of activated stem cells regenerates lung cells and suppresses excessive immune response, which is expected to have a therapeutic effect.



[Source] https://news.tvasahi.co.jp/news_international/articles/000183130.html

Some patients recover with pulmonary fibrosis, a condition that affects daily lung function, makes them short of breath when they walk, makes exercise difficult, and makes them more susceptible to other bacterial and viral infections. Pulmonary fibrosis is a virtually untreatable disease, except for artificial lungs and stem cell transplants. In February 2020, a team of researchers from North Carolina State University published research in the international journal Nature Communications on "fog inhalation therapy," a non-invasive treatment for patients with pulmonary fibrosis that uses aerosol inhalation to deliver secretions from lung stem cells to effectively repair lung damage caused by pulmonary fibrosis.



[Source]
Stem cell nebulization therapy for COVID-19 infection: radiological and clinical outcomes. https://doi.org/10.1186/s43055 021 00492-3

Left : Male, age 47, chest X-ray

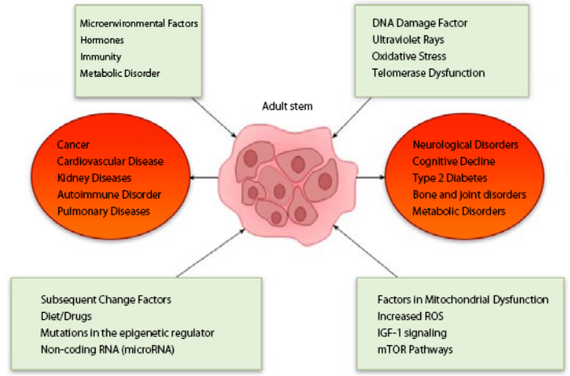
a) On the day of admission (April 17)
There is extensive diffusely organized consolidation in all lobes of the lung, almost invading most of the lower lobes.

b) Fourth day after treatment (April 21).

c) Day 13 after treatment (April 30).
X-ray showed partial disappearance of the lesion. (Day 13 after treatment (April 30).) Compared to the previous partial healing, there is mottled opacity predominantly in the middle and lower lungs on both sides.

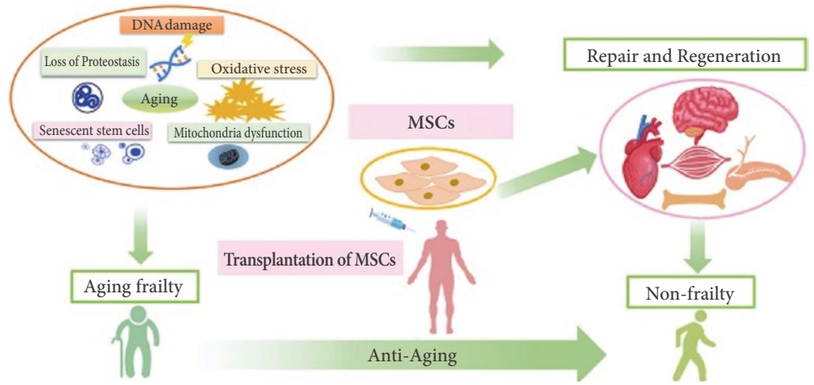
01 AGING FRAILTY

Pluripotent stem cells have remarkable self-renewal potential and can differentiate into multiple diverse cell types. There is slowly growing evidence that the aging process may have a negative effect on stem cells. As stem cells age, their regenerative capacity decreases and their ability to differentiate into various cell types is narrowly altered. Therefore, it has been suggested that aging-induced loss of stem cell function may play an important role in the pathophysiology of various aging-related diseases. Understanding the role of the aging process in stem cell function is important not only in understanding the pathophysiology of aging-related diseases, but also in developing effective stem cell-based therapies to treat future aging-related diseases. This review article focuses on the various aging-related disease-linked stem cell dysfunction. Next, we discuss some concepts regarding possible mechanisms that may contribute to aging-related stem cell dysfunction. Potential therapies for aging-related stem cell defects that are under development will be briefly discussed.



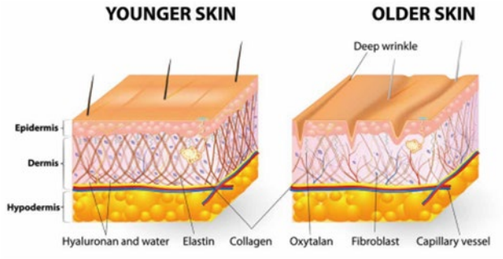
[Source] World J Exp Med. 2017 Feb 20; 7(1): 1–10.Effect of aging on stem cells. Abu Shufian Ishtiaq Ahmed,et al

Mesenchymal stem cells have been gradually revealed as an ideal cell source for solving organ problems. They have strong self-renewal and differentiation potential. They can also be easily harvested from many tissues and can be viable at the site of injury. Furthermore, due to its immunoprivileged status and anti-inflammatory properties, MSC-based therapy is expected to have systemic applications. Currently mesenchymal stem cells have been shown to improve frailty by promoting the function of several vital organs, including the brain, muscles, heart, and endocrine system. Mesenchymal stem cells can alleviate symptoms in frail patients, and no treatment-related serious adverse events have been reported.



[Source] Zhu Y, Ge J, Huang C, Liu H, Jiang H. Application of mesenchymal stem cell therapy for aging frailty: from mechanisms to therapeutics. Theranostics 2021; 11(12):5675 5685. doi:10.7150/thno.46436.

02 FACE REJUVENATION



Regeneration

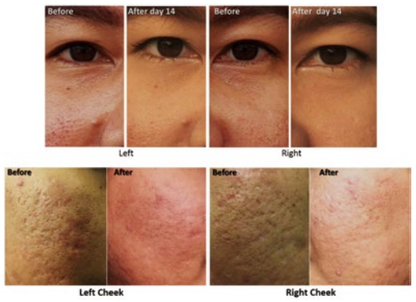
- Anti-inflammation
- Immunoregulation
- Promotes keratinocyte and fibroblast growth
- Vascular Regeneration
- Neural Regeneration

Rejuvenation

- Immunomodulation
- Collagen synthesis
- Elastin synthesis
- Reduction of reactive oxygen species
- Inhibition of MMP activation
- Hair regeneration

Aging skin is largely associated with loss of function and structural degeneration. With aging, the skin naturally loses its collagen content and elastic fibers which go haywire to maintain the skin structure. In addition, aging skin shows increased oxidative activity, increased production of matrix metalloproteinases (MMPs) production, which are generally involved in matrix degradation. Furthermore, exposure to ultraviolet radiation is known to promote premature skin aging, or photoaging. Adipose-derived mesenchymal stem cells secrete multiple growth factors necessary for efficient re-epithelialization and skin regeneration, therefore multiple efforts have been focused on anti-aging treatment of the skin. Recently, researchers have observed histological and structural changes in aged facial skin after injection of expanded adipose-derived mesenchymal stem cells. When adipose-derived mesenchymal stem cells were administered, the superficial layers of the dermis elastic fibers increased and the network of collagen and reticular fibers was modified and more well-organized. Subsequently, adipose-derived mesenchymal stem cells solar injection has gained elasticity in photodamaged skin. The results of this study are as follows. In sun-aged skin, the normal elastin matrix is lost. but treatment via adipose-derived mesenchymal stem cells through adipose-derived mesenchymal stem cells has been successful in restoring inhibition of the precursor molecules involved in new elastin production.

【Example of treatment-1】

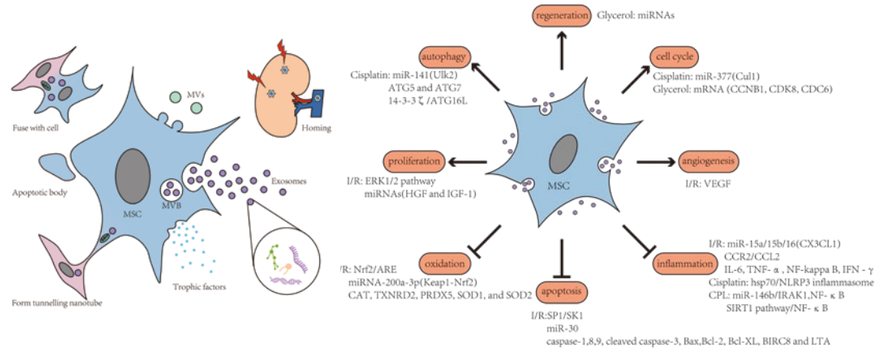


【Left】
After MSCs-containing RGFÒ solution injection, atrophic acne scars were reduced and wrinkles around the eyes disappeared on the 14th day.

[Source]
Phonchai,R.,Naigowit,P.,Ubonsaen,B.,Nilubol,S.,Brameld,S.and Noisa,P.(2020) Improvement of Atrophic Acne Scar and Skin Complexity by Combination of Aqueous Human Placenta Extract and Mesenchymal Stem Cell Mesotherapy. Journal of Cosmetics,Dermatological Sciences and Applications,10,1-7.doi:10.4236/jcdsa.2020.101001.

03 KIDNEY FAILURE

While mesenchymal stem cells reach damaged kidney tissue, kidney regeneration was shown to be accelerated. In addition, the injected cells were shown to partially differentiate into endothelial cells and smooth muscle cells, contributing to angiogenesis, vasculogenesis, and endothelial repair. Furthermore, mesenchymal stem cells have been suggested to protect the kidney by suppressing inflammatory cytokines. The restorative role of these mesenchymal stem cells may be multifactorial, providing cytokines that inhibit apoptosis, promote proliferation, and suppress inflammatory responses.

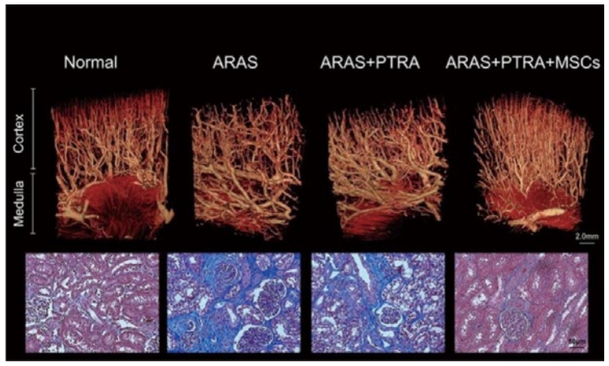


MESENCHYMAL STEM CELLS / MESENCHYMAL STEM CELL-EV FUNCTIONS AND PATHWAYS

Mesenchymal stem cells can be placed specifically at the site of kidney injury. Mesenchymal stem cells secrete growth factors, chemokines, cytokines, and other trophic factors that act on peripheral cells. and cytokines to peripheral cells, form tunneling nanotubes, and deliver EV's to secretion, and fusion with cells to deliver them to intracellular structures and mitochondria. Mesenchymal stem cells-EVs alleviate AKI by inhibiting oxidation, apoptosis, and inflammation, and alleviate AKI by regulating angiogenesis, cell cycle, regeneration, autophagy, and proliferation.

[Source] Huang, Y., Yang, L. Mesenchymal stem cells and extracellular vesicles in therapy against kidney diseases. Stem Cell Res Ther12, 219 (2021). https://doi.org/10.1186/s13287-021-02289-7

STENOSIS - RENAL MICROVASCULAR LOSS AND FIBROSIS WERE REDUCED IN ANIMALS RECEIVING MESENCHYMAL STEM CELL THERAPY



▪Above: Improved microvascular structure in pigs with atherosclerotic renal artery stenosis undergoing percutaneous transluminal renal angioplasty (PTRA). A representative micro-computed tomographic 3D image of a kidney segment, four weeks early of adipose tissue-derived mesenchymal stem cell (MSC) intra-adrenal infusion.

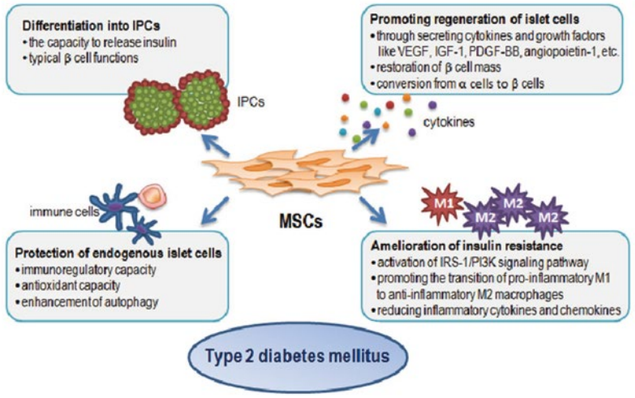
▪Bottom: ARAS + PTRA + pig MSCs Representative renal trichrome showing reduced fibrosis polychrome staining (×40, blue)

[Source] Alfonso Eirin and Lilach O Lerman* Mesenchymal stem cell treatment for chronic renal failure, Stem Cell Research & Therapy 2014, 5:83 http://stemcellres.com/content/5/4/83

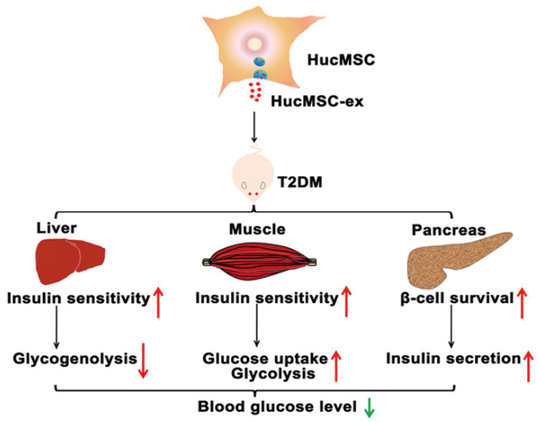
04 TYPE-2 DIABETES

Type 2 diabetes(T2DM) is caused by a combination of insulin resistance and dysfunction of insulin-producing pancreatic beta cells. Peripheral strategies that improve insulin resistance and simultaneously promote β-cell regeneration may be an ideal treatment for T2DM. The identification of stem cells that differentiate into insulin-producing cells (IPCs) and have the potential to promote pancreatic regeneration and ameliorate insulin resistance, providing an alternative to islet cell transplantation. MSCs promote islet β-cell regeneration, protect endogenous islet β-cells from apoptosis, and provide a niche microenvironment by secretion of paracrine factors and deposition of extracellular matrix which is known to improve insulin resistance in peripheral tissues.

MOLECULAR MECHANISMS OF ACTION OF MESENCHYMAL STEM CELLS



[Source] Mesenchymal stem cell therapy intype 2 diabetes mellitus. Zang et al. DiabetolMetabSyndr(2017) 9:36 DOI 10.1186/s13098-017-0233-1

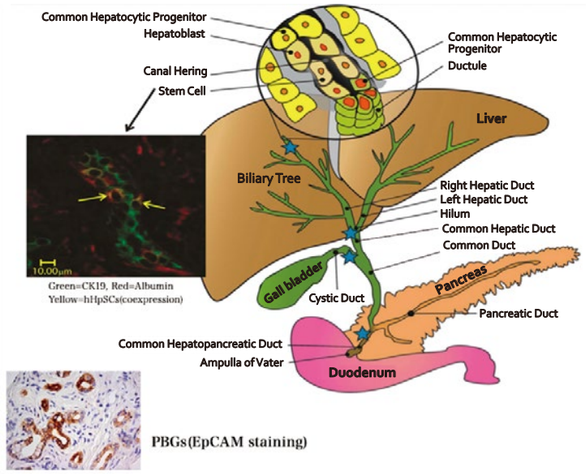


Researchers have established a rat model of type 2 diabetes mellitus and injected intravenously with mesenchymal stem cell exosomes from the human umbilical cord. Exosomes restores phosphorylation of insulin receptor substrate 1 and protein kinase B (tyrosine site), accelerates the secretion of glucose transporter protein 4 and membrane transport in muscles, maintains glucose homeostasis in vivo and glycogen storage in the liver has increased. In addition, β-cell apoptosis was inhibited and insulin secretory function was restored.

[Source] Human Mesenchymal Stem Cell Derived Exosomes Alleviate Type 2 Diabetes Mellitus by Reversing Peripheral Insulin Resistance and Relieving β Cell Destruction Yaoxiang Sun, Hui Shi, Siqi Yin, Cheng Ji, Xu Zhang, Bin Zhang, Peipei Wu, Yinghong Shi, Fei Mao, Yongmin Yan, Wenrong Xu, and Hui Qian ACS Nano 2018 12 (8), 7613 7628 DOI: 10.1021/acsnano.7b07643

05 LIVER FAILURE

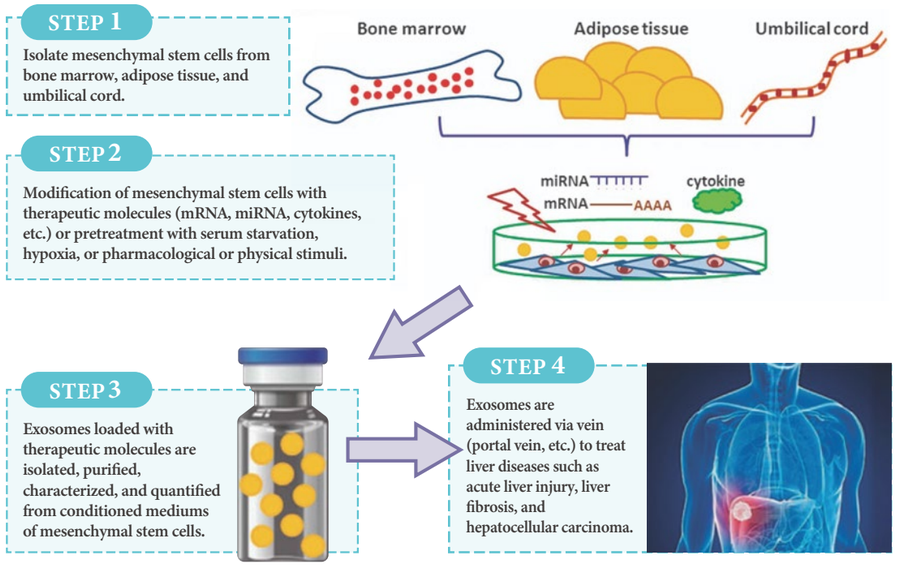
The use of stem cell/progenitor cell therapy to repair damaged organs is moving into clinical programs. Liver and pancreas, organs sharing endodermal stem cell clusters, biliary stem cells (hBTSCs) are concentrated in the biliary trunk. The effects of stem cells (fetal liver stem/progenitor cells) transplanted into the veins of patients with various liver diseases have been clinically conducted over the years. Immunosuppression was not necessary. All control subjects given the given criteria died within one year or had impaired liver function. Subjects transplanted with 100-150 million liver stem/progenitor cells showed improved liver function and survival over several years. The evaluation of the safety and efficacy of transplantation is still in its infancy.



PBGs(EpCAM staining)

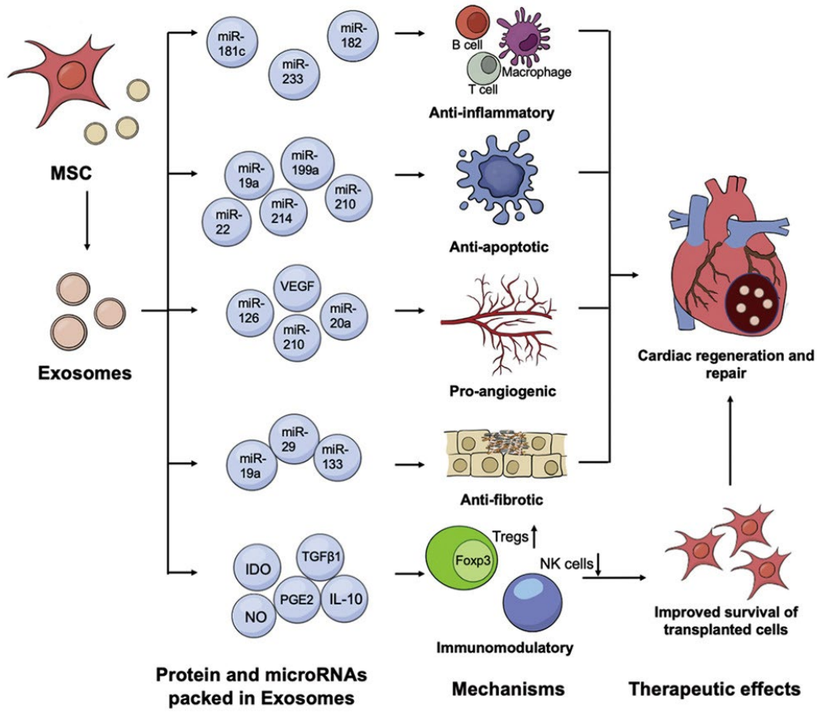
[Source] Stem Cells. 2013 Oct;31(10):2047-60. doi: 10.1002/stem.1457. Concise review: clinical programs of stem cell therapies for liver and pancreas.Lanzoni G1, Oikawa T

ATTRACTIVITY OF MESENCHYMAL STEM CELL-DERIVED EXOSOMES AS A TREATMENT FOR LIVER DISEASE



[Source] Mesenchymal stem cell derived exosomes as a new therapeutic strategy for liver diseases. Experimental & Molecular Medicine (2017) 49, e346; doi:10.1038/emm.2017.63; published online 16 June 2017

06 MAJOR ADVERSE CARDIAC AND CEREBROVASCULAR EVENTS (MACCE)



In the cardiovascular system, mesenchymal stem cells reduce inflammation levels, promote cardiomyocyte differentiation and angiogenesis around the infarcted area, induce apoptosis resistance, and inhibition of fibrosis, thus giving the tissue qualities that are ideal for cardiovascular repair and protection of the myocardium.

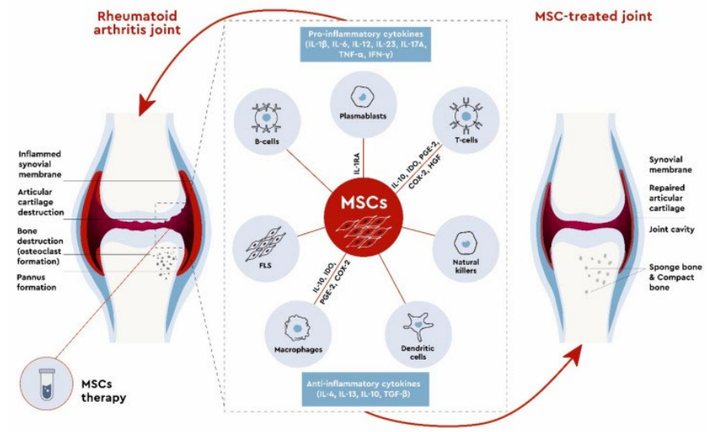
- Mesenchymal stem cells ameliorate cardiovascular disease through their immunomodulatory capacity, antifibrotic effects, and neovascular characteristics.
- Mesenchymal stem cells exert their therapeutic function in cardiovascular disease primarily through their paracrine activity.
- They exert epidemic control functions via innate and acquired immune systems.

[Source] Mesenchymal stromal cell derived exosomes in cardiacregeneration and repair https://doi.org/10.1016/j.stemcr. 2021.05.003

Adipose-derived mesenchymal stem cells are easy to obtain and proliferate and have attracted attention as a new adult stem cell source for the treatment of cardiovascular diseases. These cells have been shown to have the ability to differentiate into cardiomyocytes, vascular smooth muscle cells, and endothelial cells. Furthermore, adipose-derived mesenchymal stem cells secrete a series of paracrine factors that promote neovascularization, inhibit apoptosis, and suppress fibrosis, thereby contributing to myocardial regeneration.

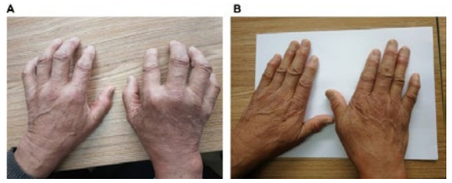
07 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic systemic disease that causes damage to joints, connective tissue, muscles, tendons, and fibrous tissue. Onset of an illness may last from several months to several years, with the presence of circulating autoantibodies, elevated levels of inflammatory cytokines, chemokines, and altered cellular metabolism. The advanced form is characterized by severe and debilitating chronic pain. MSCs infusion therapy is considered an entirely new therapy for RA due to its self-regenerative, tissue/organ regenerative, and their strong immunosuppressive properties. These properties enable the control of the inflammatory cell activity.

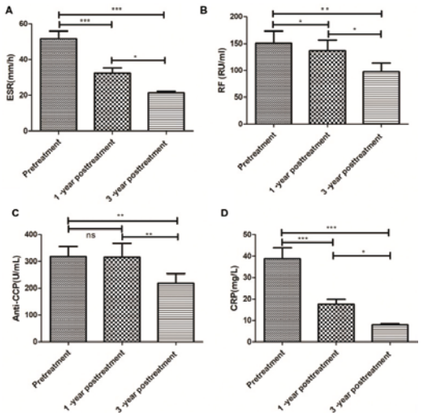


[Source] Mesenchymal Stem Cell-Based Therapy for Rheumatoid Arthritis. Madina Sarsenova,Assel Issabekova, Saule Abisheva, Kristina Rutskaya-Moroshan, Vyacheslav Ogay, and ArmanSaparov. doi: 10.3390/ijms222111592.

A study of 64 RA patients between the ages of 18 and 64 years was conducted overseas. For the treatment, a total sum of 4000 mL of MSC suspension product (20 million cells/20 mL) was injected intravenously. The joint function index (DAS28) improved significantly after 1 and 3 years of treatment.



A 68-year-old man was diagnosed with RA.
(A) The patient is unable to extend his hand straight.
(B) Three years after treatment and discontinuation of anti-rheumatic drugs, the hand is freely extending and the rheumatoid nodules around the joint are gradually softening and thinning.



Inflammatory and RA serological such as ESR, CRP, RF, and anti-CCP markers were shown to decrease at 1 and 3 years post-treatment compared to pre-treatment.

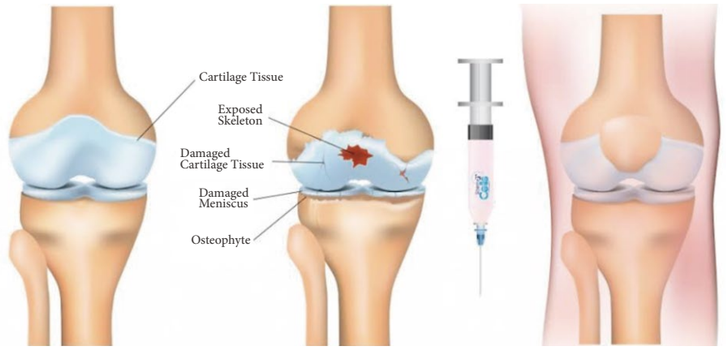
[Source] Efficacy and Safety of Umbilical Cord Mesenchymal Stem Cell Therapy for Rheumatoid Arthritis Patients: A Prospective Phase I/II Study. Liming Wang,Shigao Huang, Shimei Li, Ming Li, Jun Shi, Wen Bai, Qianyun Wang, Libo Zheng, and Yongjun Liu. doi: 10.2147/DDDT.S225613

08 OSTEOARTHRITIS

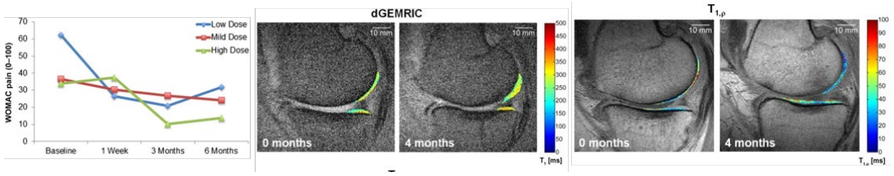
Osteoarthritis is one of the most common degenerative diseases of the elderly, characterized primarily by cartilage deterioration, synovial membrane inflammation, and subchondral bone remodeling. It has been reported that cartilage repair is achieved by taking a small amount of one's own tissue from a wound, extracting the cells that make up the cartilage, increasing them, and then putting them back into the joint. The efficacy of MSC-based cartilage regeneration therapy is being attributed to the paracrine activity of exosomes.

KNEE JOINTS

This disease is caused by the wear and tear (destruction) of the cartilage covering the surface of the bone due to the load placed on the knee joint. The destruction of cartilage causes inflammation, contracture, and deformation of the knee joint, resulting in severe pain. The knee may feel stiff and walking may become difficult.
Major causes include aging of articular cartilage, trauma, obesity, and predisposing factors (genes). Aging causes joint cartilage to lose elasticity with age, and overuse causes cartilage to wear away, resulting in joint deformity. Stem cell therapy is effective in the regeneration of worn out articular cartilage and meniscus. Osteoarthritis of the knee is often caused by these factors, so those who have been diagnosed with osteoarthritis of the knee have a high probability of improvement. However, if in addition to osteoarthritis of the knee, there is also a ligament tear of the meniscus, these will not improve.
Stem cells inhibit inflammation in joints and work to repair cartilage, thus regenerating intra-articular structures and significantly reducing pain.Stem cells are also effective in repairing ligaments and muscles, and are used by top athletes overseas.



In this overseas study, 18 patients with symptomatic severe knee arthritis were enrolled in a single intra-articular injection of autologous adipose-derived stem cells (MSCs). The study design consisted of three consecutive groups (6 participants in each) with progressively increasing doses: low dose (2 million MSCs), medium dose (10 million MSCs), and high dose (50 million MSCs). After 6 months of follow-up, the procedure was found to be safe and no serious adverse events were reported. Patients receiving MSCs experienced significant improvements in pain levels and function.

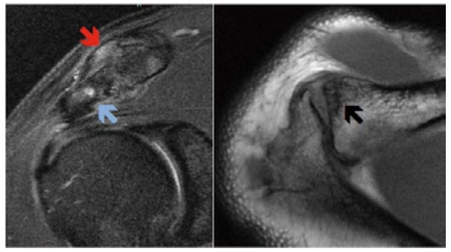


[Source] Adipose Mesenchymal Stromal Cell-Based Therapy for Severe Osteoarthritis of the Knee: A Phase I Dose-Escalation Trial. Stem Cells Transl Med. 2016 Jul; 5(7): 847–856.doi: 10.5966/sctm.2015-0245doi: 10.5966/sctm.2015-0245

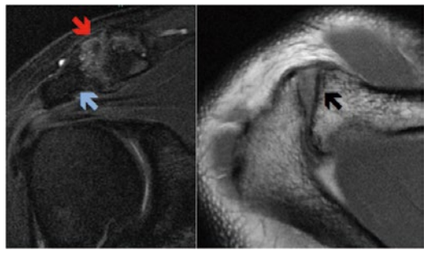
The average change in clinical results from baseline to 1 week, 3 months, and 6 months is summarized in the pictures. All clinical outcome parameters (pain, function, and mobility) were improved. The high-dose group showed the most significant improvement in pain. dGEMRIC and T1rho magnetic resonance imaging (MRI) of selected patients. The graph on the left shows dGEMRIC and T1rho values before and 4 months after cell therapy. Increases in dGEmRIC and decreases in T1rho are known to correspond to increases in glycosaminoglycan/proteoglycan content and thus cartilage shows an improved condition of cartilage.

SHOULDER JOINTS

In an international study, a 43-year-old patient presented with painful acromioclavicular joint osteoarthritis (AC joint OA) and underwent MSC therapy. Patients reported pain and functional improvement as assessed by a pain rating scale. Imaging at 12 months revealed subchondral edema, synovitis, and subchondral cysts were reduced and there was structural improvement.



Coronal and axial MRI of the shoulder before treatment showed thickening of the joint capsule and arthritis with evidence of AC arthritis with arthritis (red arrows), clavicle and acromion bone edema (blue arrows), and distal clavicle subcortical cyst formation (black arrows).



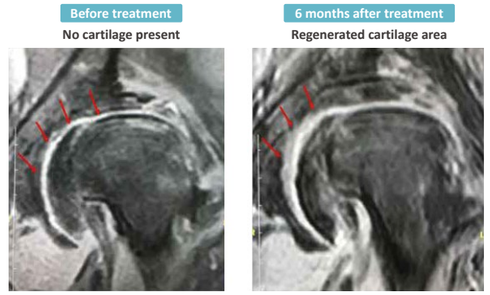
Coronal and axial MRI after treatment showed decreased joint effusion (red arrow), decreased subchondral bone edema (blue arrow), and significant resolution of subchondral cysts (black arrows).

[Source] Effect of autologous adipose-derived mesenchymal stem cell therapy in the treatment of acromioclavicular joint osteoarthritis. Julien Freitag, JamesWickham, Kiran Shah, and Abi Tenen. doi: 10.1136/bcr-2018-227865

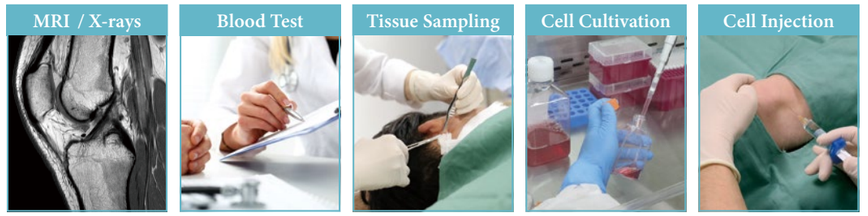
HIP JOINTS

This patient is a 59 year old woman. She had quite severe osteoarthritis of the hip, which was completely amenable to surgery, but she chose stem cell therapy because it was difficult for her to stay in the hospital. Six months after treatment, an MRI was taken and it clearly showed the regenerated cartilage.

[Source] Dr Iwata's blog
(https://riso-clinic.com/blog/post-740.html)

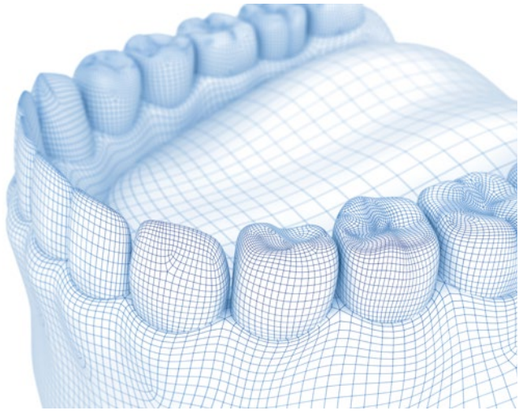


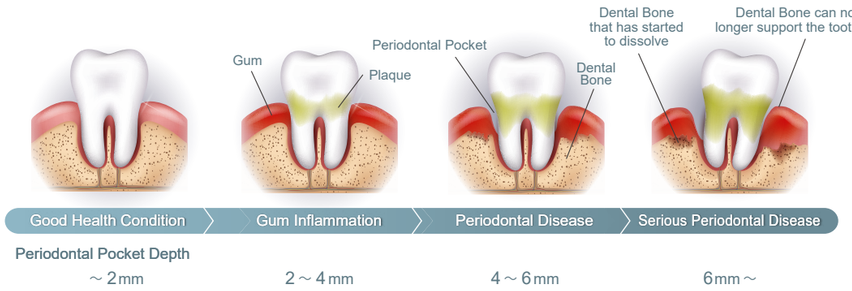
Examination Process ····· **Length of treatment : 1 to 6 months**



09 BONE RESORBABLE DENTAL DISEASE

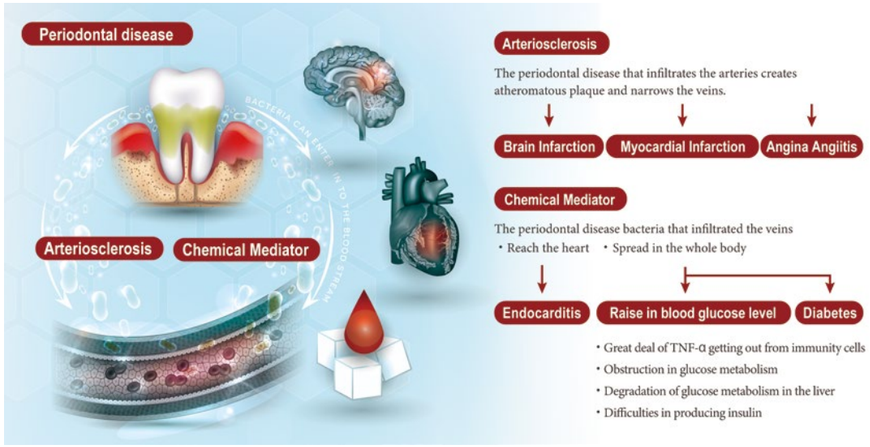
If the border between the teeth and gums (gingival sulcus) is not cleaned properly, a lot of bacteria can accumulate there and the gingival margin becomes inflamed and red. This is a situation known as periodontal disease.
The boundary between the teeth and gums, called periodontal pockets, deepens and the tooth support (alveolar bone) dissolves. Finally, the alveolar bone can no longer support the teeth. This is a condition called "bone-resorptive dental disease". Most periodontal diseases are painless and often progress unnoticed. Early detection and treatment of periodontal disease is the key to a healthy life span of the teeth.





CLOSE RELATIONSHIP WITH THE BODY

Recent studies have shown that people with periodontal disease are 2.8 times more likely to suffer a stroke than those without periodontal disease. It can be said that there is a close relationship between the body and dental health. (See below).



10 STEM CELL COSMETICS

STEM CELLS ARE CELLS THAT HAVE THE REMARKABLE ABILITY TO REPLACE OLD CELLS AND REPLENISH LOST CELLS WITH NEW ONES

Age-related skin changes result in the loss of baby-like skin, but the baby cells are still present with age. They are the stem cells. Stem cells are capable of regenerating new skin and reviving their own skin.

WE CULTIVATE THE BABY CELLS (STEM CELLS) THAT ALREADY EXIST IN YOUR BODY AND PROVIDE THEM AS COSMETICS

Nowadays, a wide variety of skin care products are available. The main purpose of using skincare is to "maintain healthy skin to prevent aging". Unfortunately, no matter how many good ingredients you use in your skincare regimen, you will not get the benefits of skincare if your metabolism is not working properly. The "Full Order Serum" is a new serum that focuses on the cellular level, transforming the aging cells to baby cells so that you can get the most out of the active ingredients in your skincare regimen.

Self-renewal versus external toxic environment, which prevails?

The body is already programmed at birth to recover and regenerate its own skin, but the external toxic environment prevails. Your own stem cells are harvested by taking a small piece of skin (about 5 mm) from behind the ear.

Stem cells are cultured and grown at our cell culture facilities and used as cosmetics. Our clinic has completed a training program on regenerative medicine with a well-developed facility environment and hygiene management system.
We also regularly receive appropriate advice from external culture institutions to ensure our culture techniques are always up-to-date. Serum and other biologically derived substances are not added to the culture process, it is safe and which means there are no ethical issues. Our CPC never use animal serum, which is conventionally used for cell culture, and provide safer, more secure, and higher quality stem cell culture. The cells are cultured under strict control in a cell culture room attached to the clinic.

RISKS OF STEM CELL TREATMENTS

A SAFE AND PURE STEM CELL PRODUCTION TECHNOLOGY

In order to assure the quality of the stem cells we provide to our patients, we have outsourced the testing of stem cell samples produced using the same process as in our certificate of quality. The testing is in accordance with GMP (Good Manufacturing Practice), GLP (Good Laboratory Practice) for regenerative medicine, and also includes three independently designed experiments. Equivalent tests are performed in our own laboratory. The results of the verification of the quality of stem cells provided to patients by a third-party reagent company are also described below.

Examination Objectives	Test name	Sample used	Result
Infection, contamination	Sterility testing (GMP compliant, Japanese Pharmacopeia)	QC_AL_1	Negative
	Mycoplasma negativity test (GMP compliant, Japanese Pharmacopeia)	QC_AL_1	Negative
	Endotoxin test (GMP compliant, Japanese Pharmacopeia)	QC_AL_2	Below detection precision
	Human virus negativity test (GMP compliant)	QC_AL_4	Negative
Tumorigenesis	Soft agar colony formation test (regenerative medicine GLP)	QC_AL_4	Negative
Stem cell differentiation potential	FCM test（CD45-CD105+）	QC_AL_5	CD45- 99.3% CD105+ 92.6%
	Adipocyte Differentiation Test	QC_AL_5	Confirmation of lipid droplet staining

Adipose tissue from three sample donors were cultured in our laboratory and sent for examination. The sample is tissue taken from behind the ear, then the tissue is separated and broken down using special technology and a proprietary enzyme. The cells were made into single cells, incubated for one month, and those that reached the specified number were separated by MACS, and after the cell count was confirmed by cell counter, a part of them were provided to Takara Bio Inc., a third-party reagent company.

試験報告書一覧

2019年03月19日
タカラバイオ株式会社
品質管理部
田辺　雅茂

ご依頼いただいた下記試験検体に対する品質試験の試験報告書一覧を示します。
（受託ロット：Q18084）

ご依頼者：楯　晴菜　様
ご所属：STEMCELL株式会社
所在地：〒107-0062 東京都港区青山5-10-2 第二九曜ビル2F
TEL：03-6433-5769

試験検体
検体1：Lot No. QC_AL_1　　検体3：Lot No. QC_AL_3
検体2：Lot No. QC_AL_2　　検体4：Lot No. QC_AL_4

試験名	試験番号	使用検体	結果
無菌試験（日本薬局方　直接法　オプション：手法の適合性試験）	Q18084-01	検体1	手法は適切ではなかった
無菌試験（日本薬局方　直接法）	Q18084-02		判定しない
マイコプラズマ否定試験（日本薬局方参考情報　PCR法（7菌種の検査確認あり））	Q18084-03		PBSで2倍希釈したものを供試したとき、マイコプラズマ7菌種の検査はなく、陰性であった
エンドトキシン試験（日本薬局方　カイネティック－比濁法　オプション：反応干渉因子試験）	Q18084-04	検体2	希釈倍率10倍、50倍、100倍のいずれにおいても、反応干渉因子はなかった
エンドトキシン試験（日本薬局方　カイネティック－比濁法）	Q18084-05		1.0 EU/mL未満（希釈倍率：10倍）
細胞生存率試験	Q18084-06	検体4	生存率：65.2%
ヒトウイルス否定試験（リアルタイムRT-PCR法（GMP試験））	Q18084-07	検体3	陰性

以上

DOMESTIC AND INTERNATIONAL PAPER RESEARCH

"In current animal models, in which either human or animal cells (homologous models) are used, no evidence of tumor formation has been observed to date."

[Source] Risk of tumorigenicity in mesenchymal stromal cell based therapies Bridging scientific observations and regulatory viewpoints. DOI: 10.1016/j.jcyt.2013.03.005

"In conclusion, the systemic transplantation of hAdMSCs appears to be safe and does not induce tumor development."

[Source] Safety of Intravenous Infusion of Human Adipose Tissue Derived Mesenchymal Stem Cells in Animals and Humans. DOI: 10.1089/scd.2010.0466

"Our study provides a systematic examination for adverse events related to the use of MSCs. We did not identify any significant safety signals other than transient fever. Results from our systematic review should provide some assurance to investigators and health regulators that, with the present evidence, this innovative therapy appears safe."

[Source] Safety of Cell Therapy with Mesenchymal Stromal Cells (SafeCell): A Systematic Review and Meta-Analysis of Clinical Trials. DOI: 10.1371/journal.pone.0047559

"Between January 1998 and November 2008, 41 patients received 45 transplantations. We checked their records until their last visit. We telephoned or mailed the patients who had not visited the clinics recently to establish whether there were any abnormalities in the operated joints. Neither tumours nor infections were observed between 5 and 137 (mean 75) months of follow-up. Autologous BMSC transplantation is a safe procedure and will be widely used around the world."

[Source] Safety of autologous bone marrow derived mesenchymal stem cell transplantation for cartilage repair in 41 patients with 45 joints followed for up to 11 years and 5 months. DOI:10.1002/term.299

The advantages of MSC applications in tissue repair, i.e., their safety, relatively wide differentiation capacity, and high paracrine ability including EV release, make these cells an important material for further investigation and development of new approaches for cell-based therapies in future. However, more research studies on both preclinical and clinical levels have to be accomplished. New information related to MSCs will help to determine the efficiency of cells administered to the patients as a therapeutic approach. Additional studies would also be a major contribution to stem cell biology in general.

[Source] Challenges and Controversies in Human Mesenchymal Stem CellTherapy https://doi.org/10.1155/2019/9628536

Helene regenerative medicine is provided under the medical care of each specialist.

General Director, *Dr. Takaaki Matsuoka*

2003 Graduated from Keio University School of Medicine
Keio University Hospital, Department of Anesthesiology
2004 Department of Anesthesiology, Keiyukai Keiyu Hospital
2005 Joined Shobikai Medical Corporation
2009 Vice President of the General Administration of the Shomei Medical Association
2013 Opened Helene Clinic in Omotesando

< Academic Affiliations >
- Harvard Medical School PGA
- Member of the American Bored of Regenerative Medicine
- Executive MBA from Beijing University
- Board Certified Anesthesiologist, Japanese Society of Cosmetic Surgery
- Certified anesthesiologist by the Ministry of Health, Labor and Welfare

Born in 1977 in Kochi Prefecture, Japan.
Dr. Takaaki Matsuoka specializes in regenerative medicine, anesthesiology, and medical management.

In his previous position as a cosmetic surgeon in a renowned Japanese cosmetic surgery clinic group, he specialized in stem cell-based plastic surgery and performed over 3,000 stem cell plastic surgeries. In 2013, he opened the Helene Clinic in Omotesando, Tokyo, where he began to administer stem cells systemically. He was the first person to receive the Stem Cell IV Therapy under the Regenerative Medicine Act.
The safe and legal stem cell therapy has been requested by many patients and Helene has become well known as a leading clinic for stem cell therapy.
He is currently in the clinical field and treats patients with a focus on stem cell therapy.

Chairman of HELENE Medical Corporation

Dr. Nana Kobayashi [Surgeon]

March 2007 Graduated from Nihon University School of Medicine
April 2007 - March 2010 Nihon University Itabashi Hospital
Apr 2010 - Apr 2011 Gastroenterological Surgery, Showa Hospital
May 2011-March 2012 Gastrointestinal Surgery, Nihon University Nerima Ward Hikarigaoka Hospital
Apr 2013-Mar 2015 Gastroenterological Surgery, Sakura General Hospital
April 2015~ April 2021 Vice President, Jiyugaoka Medical Plaza, Gastroenterological Department
May 2021~Now

< Academic Affiliations >
- Japanese Society of Gastroenterological Surgery
- Japanese Society of Gastroenterology
- Japanese Society for Abdominal Emergency Medicine

Dentist
Dr. Mikuru Matsuoka, DDS

March 2004 Graduated from Iwate Medical University School of Dentistry
2005-2006 Tohoku University Hospital
2006-2009 Medical Corporation in Tokyo
2010-2014 Medical Corporation in Kanagawa Prefecture
2015 Opened Meguro Honmachi Dental Clinic
March 2018 Opened Meguro Honmachi Sakura Dental Clinic (branch clinic)

Cardiovascular Surgery
Dr. Keichiro Kuroki

1994 Graduated from Okayama University School of Medicine
1994 Cardiac Center Sakakibara Hospital
1997 National Iwakuni Hospital
2002 Fukuyama National Hospital
2007 Kawasaki Saiwai Hospital Aortic Center
2008 Nakagami Hospital
2009 Makiko Chuo Hospital
2011 Kosei General Hospital
2018 Hiroshima Varicose Veins Clinic

Orthopedics
Dr. Shinichiro Iwata

1996 Graduated from Keio University School of Medicine
-2001 Worked at Keio affiliated hospitals (Ashikaga Red Cross Hospital, Odawara City Hospital Ise Keio Hospital, Hino City Hospital)
2004 Studied at STANFORD University
2006 National Hospital Organization, Murayama Medical Center
2009 Established NPO, Institute of Team Medicine for Low Back Pain and Knee Pain
2016 Omotesando HELENE Clinic, Knee Joint Regenerative Medicine

< Academic Affiliations>
- Japanese Orthopaedic Association
- Japanese Knee Society

Cardiovascular Surgery
Dr. Takaaki Itohara

2003 Graduated from Shimane University School of Medicine
2003 Cardiovascular surgery at Kyoto University Hospital
2014 Specialized hospitals for cardiovascular surgery (Shin-Tokyo Hospital, Shinkoiwa Hospital, Yamato Naruo Hospital, etc.)
2014 Director of Tachikawa Varicose Vein Clinic
2018 Cardiovascular surgery at Kawasaki Sai Hospital
2022 Cardiovascular surgery at Imus Shinmatsudo Hospital
2023 Omotesando HELENE Clinic

Surgeon
Dr. Yoshio Shimizu

March 2009 Graduated from Fukui University School of Medicine
April 2009 University of Tsukuba Hospital, Initial
April 2011 Hitachinaka General Hospital, Hitachi, Ltd.
Oct 2011 University of Tsukuba Hospital, Gastroenterological Surgery, Mouth Surgery
April 2012 Tsukuba Reinikai, Tsukuba Gakuen Hospital
April 2013 Ryugasaki Saiseikai Hospital
April 2014 Mito Saiseikai Hospital
April 2015 University of Tsukuba Hospital
September 2019 Tachikawa Varicose Vein Clinic

Urologist
Dr. SHINICHIRO IWATA

2009 Graduated from Nippon Medical School
2009 Social Corporation Foundation, Ishinokai Sayama Hospital (currently Saitama Ishin-kai Hospital)
2011 Nippon Medical School Hospital
2011 Japan Medical Alliance Ebina General Hospital
2012 Nippon Medical School Hospital
2013 Heisei Tateishi Hospital, Naowakai Medical Corporation
2015 Nippon Medical School Hospital
2017 Gonohashi Ladies Clinic
2019 Opened the Yokohama Clinic for Varicose Veins of the Leg

< Qualifications>
- Specialist in Urology, Japanese Urological Association
- Specialist in Anti-Aging, Japanese Society of Anti-Aging Medicine

CELL CULTURE SPECIALIST

Technical Advisor

Professor *Ravindra Gupta*

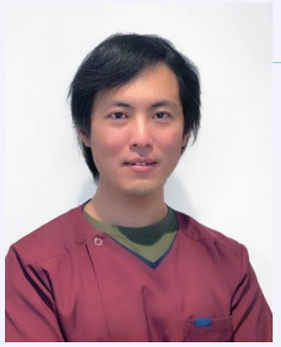
After graduating from medical school at Cambridge and Oxford University, he was granted a Fulbright Scholarship at Harvard University, where he completed a master's degree in public health. Returning to the UK, he did research in infectious diseases in Oxford and London (UCLH, Hospital for Tropical Diseases) where he completed a PHD on lentiviral antiretroviral drug evasion and innate immune response at UCL.

In 2011, he set up a research group at UCL to work on the genetics and biology of HIV resistance and reservoir genetics and biology. He was promoted to full professor in 2016.

2019- Ravindra Gupta has been the Professor of Clinical Microbiology at the Cambridge Institute for Therapeutic Immunology and Infectious Diseases.
2020- Ravindra Gupta was named as one of the 100 Most influential people by TIME.
2022- Ravindra Gupta is a recipient of the Translational Microbiology Award and has been named one of the most influential researchers in the world.

Professor Gupta leads the Gupta Laboratory which has extensively studied HIV drug resistance at both the molecular and population levels. He has contributed to the assessment of the global scale of drug resistance. The group's work has also extended to the study of HIV reservoirs in cells, especially macrophages. This understanding is relevant both to the anatomical compartment in which HIV viral replication occurs in macrophages and to the design of strategies to treat HIV. The Gupta Lab is located in the United Kingdom and Durban, South Africa, and collaborates with the African Institute of Health Research.

In March 2019, Gupta led a team for an investigation of HIV-positive patients with Hodgkin lymphoma who have progressed after "unrelated" stem cell transplantation. It was reported that this London Patient's patients' HIV remission was demonstrated.



Cell Culture Specialist

Hiroaki Suita

[Education]
Tokyo Denki University, Graduate School of Advanced Science and Technology, B.S., Ph.

[Career]
・Advantech Corporation
Worked as a researcher on cancer cells, (CAR-)T cells, mesenchymal stem cells, and iPS cells in culture, cell staining, FACS analysis, Western blotting, gene transfer, and flow cytometry.
・DNAFORM Corporation (Genetic Analysis Technology Promotion Department)

Cell Culture Specialist

Arumosu

[Education]
Graduate School of Life Sciences, Inner Mongolia University

[Career]
・Technical Assistant, Institute of Medical Science, The University of Tokyo
・Academic Staff, Institute of Industrial Science, The University of Tokyo

FACILITY OF OMOTESANDO CELL CULTURE LABORATORY

Cell cultures are operated with the following hygiene controls based on strict management.

OMOTESANDO CELL CULTURE LABORATORY AND REGULATORY FACILITIES (CPC)

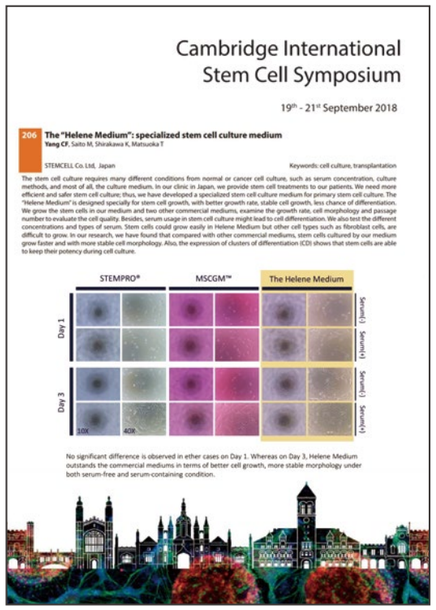
The clean bench is supplied with clean air of ISO class 5 (class 100). For cell proliferation and management, the number of micro particles in the Cell Processing Center (CPC) must be reduced by a fairly high standard and create a sterile space, that is to say, a bacteria-free space. Also, to guarantee high safety, tests to detect pathogens such as viruses are carried out when receiving cells.

In addition, to prevent mixing up samples, we have introduced strict quality management and established a cell culture operation management system, with traceability system (barcode) and historical control of all processes.

HELENE MEDIUM

HELENE MEDIUM is the medium for mesenchymal stem cells developed by our clinic. The Results of an experiment comparing HELENE MEDIUM with two other well-known media were published in CISCS. The results showed that HELENE MEDIUM was able to culture more cells than the others two mediums in the same period of time. We have consistently used non-animal culture media (animal-free) since the beginning.

HELENE MEDIUM ▶



CELL CULTURE EQUIPMENT AND DEVICES

01 **Adipose tissue cell sample collection**

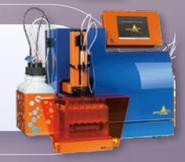
We have established a management system with a strict quality control of cultured cells.

02 **Perform cell culture for one month using our HELENE MEDIUM**

From the beginning, we have consistently used a non-animal culture medium (animal-free).

03 **autoMACS® Pro Separator Fully automated magnetic cell separator**

- 12 sorting steps
- Uses 50 nm MACS® microbeads
- Pioneer in automated cell sorting, with backed by thousands of publications
- Superparamagnetic nanoparticles, specific antibody binding
- Non-toxic, biodegradable

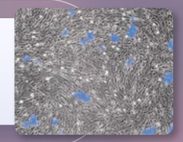


04 **Countess® II FL Automatic cell counter**

The number of cells is measured using an inspection device called a cell counter.

05 **Patented AI Image Cell Activity Identification System**

We use a patented AI image recognition technology to determine cell activity rates.



06 **Cryopreservation Thermo Scientific TM BioCane 73 Canister and Cane System**

The product is stored in a dedicated tank containing liquid nitrogen at minus 196°C. This allows for long-term storage.

CELLULAR REGULATION FACILITY (CPC)

HELENE CLINIC'S PROPRIETARY MANUFACTURING TECHNIQUES AND BLENDED THERAPIES

HEXOSOME™ MANUFACTURING TECHNOLOGY

Fat collection

Fat harvested from 5 mm skin depth

Stem Cell Culture

We use Helene medium, which was developed by our clinic exclusively for MSC culture and makes it possible to culture several times more MSCs than normal media.

Stem cell culture supernatant centrifugation

HEXOSOME™ is purified from culture supernatant by further advanced centrifugation to extract only small particles less than 100 nm in diameter.

Targeting

Using passive exosome culture technology, exosomes are cultured with active substances needed by the target cells. This enables exosomes to precisely reach and repair damaged areas.

Observation

Exosomes are measured using an optical microscope, NanoSight (LM10). The exosomes were successfully visualized by reflecting a laser beam.

The 2019 ISEV's official journal, the Journal of Extracellular Vesicles, defines the treatment of MSC-derived exosomes. However, regarding the standards and characterization of MSC exosomes for therapeutic purposes performed at our clinic, we follow the methods presented by the Japanese Society for Regenerative Medicine.

The definition of MSC cells (ISCT: International Society for Cell Therapy)

1. Glued to plastic culture containers and maintained in standard culture methods
2. With CD105,CD73,CD90 markers and no CD45,CD34,CD14orCD11b,CD79alpha or CD19, HLA-DR surface molecules.
3. Can differentiate into osteocytes, adipocytes, and chondrocytes in vitro.

Points to consider

1. cell source of MSCs (umbilical cord tissue, adipose tissue, etc.)
2. age of donor, cell passages
3. differences in the individuals sampled
4. whether it is your own or another's
5. method of MSC isolation
6. heterogeneity of MSCs
7. culture conditions such as Preconditioning and hypoxia

Definition of exosomes, etc. (defined by ISEV in MISEV2018)

Evaluate whether the target is isolated by surface markers and size of the target
Cell surface markers CD63, CD81, diameter around 100 nm, etc.

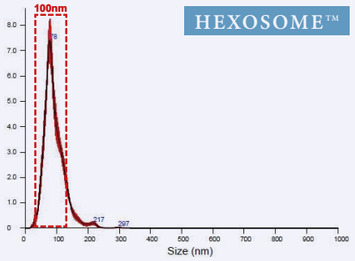
Confirmation of physiological substances and activity

Protein concentration
miRNA/RNA profile
Inhibition of T-cell activity
Anti Fibrosis
Checking anti-inflammatory effects, etc.

Safety evaluation (example)

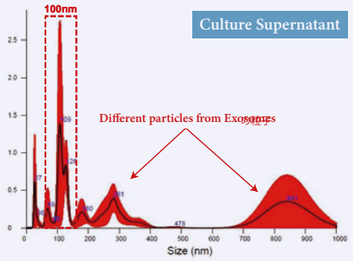
Viral and bacterial testing
Endotoxin testing
Mycoplasma testing
etc.

HEXOSOME™



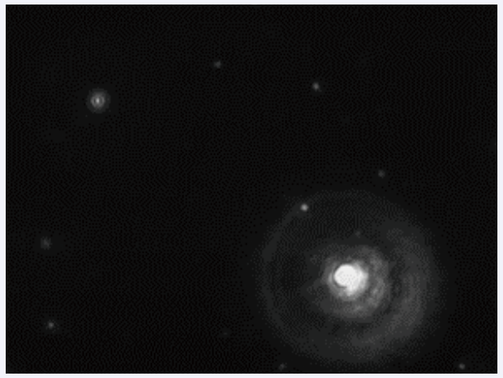
Averaged FTLA Concentration/Size for Experiment:

Culture Supernatant



Averaged FTLA Concentration/Size for Experiment:

HEXOSOME™ and culture supernatants produced at our clinic were measured by NanoSight (LM10) The graph displays cell diameter (horizontal axis) and particle count (vertical axis). The peak around 100 nm (steep peak) is HEXOSOME. Although exosomes are detected in both The culture supernatant also contains peaks at 300 nm and 800 nm, which are called exosomes (MV Micro Vesicles) and are particles with different functions from exosomes.



< HEXOSOME™ produced at our clinic are flowing>

HEXOSOME™ TARGETING TECHNOLOGY

Stem cells can reach the site of injury by receiving signals (SDF-1). Upon receiving a signal, the stem cell decides which active substance (cytokine, growth factor, etc.) to load into the exosome, marking the "street address" of the target location. After exosomes leave the stem cell, they follow their address to the injured area, release cytokines and growth factors and repair the damage. But that only happens when stem cells are injected into the body. Can this happen if only exosomes are injected?

We use passive exosome culture technology to culture exosomes, together with active substances needed by the target cells, and exosomes alone back to the site of injury so that they can precisely reach the site of injury and alleviate the lesion.

Incubation with exosomes

Just by incubating exosomes with an active substance, this substance spreads throughout exosomes, following a gradient concentration. The loading efficiency depends on the hydrophobicity of the substance molecule.

Incubation with donor cells

When the donor cells are exposed to an effective substance, exosomes containing the substance are secreted.

HEXOSOME™ BLEND THERAPY & LINEUP

We are the first clinic in Japan to use a mixture of mesenchymal stem cells and highly purified quantitative exosomes for treatment. In addition, we have received two certifications related to mesenchymal stem cell exosomes from the Japanese government .

- **【Regenerative medicine provision plan】** Autologous adipose-derived stem cells for anti-immunosenescence / stem cell exosome administration therapy

- **【Regenerative medicine provision plan】** Autologous adipose-derived stem cells for diabetes / stem cell exosome administration therapy

In-patient care ・**Autologous adipose-derived MSCs** ・**Exosome blend therapy**

- Exosomes themselves are purified cell-secreted components, and there is no risk that the local condition of the administered cells will affect the therapeutic effect, as is the case with cell therapy.
- Exosomes should be easy to quantify (number of particles)
- Good migration to the target organization
- Ability to administer multiple doses compared to cell therapy
- Theoretically low potential for mass embolization due to their smaller size than cells

Prescription UC Exosomes

- UC Exosomes 200 billion particles
- UC exosomes 400 billion particles
- UC exosomes 600 billion particles

护肤产品 ・**Serum** ・**Face Masks**

- HEXOSOME™ Serum
- HEXOSOME™ Face Masks

BEGINNER'S GUIDE

1 CONTACT

Please prepare documents that show symptoms of concern and current health condition (e.g., health checkup report or MRI within the last 3 months). When you are ready, please contact us with your photo identification (passport, etc.).

2 RESERVATION

After your inquiry, our staff will confirm the date of your visit and make an appointment for you. The clinic will notify you of the completion of your appointment.

3 FIRST VISIT

First, you will be given a checklist to fill out and the doctor will explain about stem cell therapy. Based on the materials you brought with you, we will tailor a treatment.

4 TISSUE COLLECT

We will collect about 5 mm cells from the blood and the skin behind the ears. The required time is around 20 minutes, under local anesthesia. After harvesting the cells, the nurse will dress the wound and give you medication.

5 PAYMENT

Our clinic adopts free medical examinations. Payment will occur at the time of cell collection. We are asking for your bill after the collection of cells.
Union Pay, Alipay, WeChat Pay, Line Payment, settlement by overseas transfer is possible.

OVERSEAS REMITTANCE

■ BANK NAME :	MIZUHO BANK,LTD
■ SWIFT CODE/(BIC CODE) :	MHCBJPJT
■ BRANCH :	HAMAMATSUCHO BRANCH
■ BENEFICIARY ACCOUNT NO :	148-1604172
■ BENEFICIARY NAME :	STEMCELL.CO
■ BRANCH ADDRESS :	2-4-1,HAMAMATSUCHO MINATOKU, TOKYO JAPAN 105-6101

6 CELL CULTURE

We will begin cultivating stem cells at our laboratory. The cultivating period is about 4 weeks.

Reservation on the administration day will be taken after payment of cell collection fee.

7 TREATMENT

Before treatment, the doctor and staff will report the results of the examination to you. After that, you will have a final confirmation of the treatment, the administration method, and then you will actually see the stem cells in front of your eyes.

* In order to maintain stem cell activity, please be sure to come to the clinic on the specified date and time.

8 INJECTION

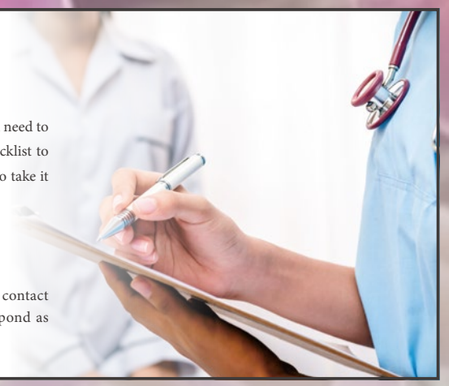
Stem cells are administered to the area of concern by subcutaneous injection or intravenous infusion. The procedure takes about 30 minutes to 1 hour. At the time of administration, the doctor checks the wound.

9 END OF TREATMENT

You can return home from the same day.
Before you go home, our staff will inform you of the precautions you need to take after the treatment. After three months, you will receive a checklist to fill out and a form with the notes mentioned above. Please be sure to take it home with you.

10 AFTER GOING HOME

If you are feeling unwell or have any concerns, please feel free to contact us at any time. Our staff and medical staff and doctors will respond as soon as possible.



11 3rd or 6th month

Our staff will contact you and ask you to report the check sheet. Please report back to us with the check sheet you were given. The doctor may order an examination according to your condition.

12 NEXT TREATMENT

If you wish to come back for a follow-up visit or re-treatment, please contact us. We will respond to your request.

REGENERATIVE MEDICINE PROVISION PLANS ACCEPTED BY MINISTRY OF HEALTH, LABOR AND WELFARE

- Specific Processed Cells Manufacturer, Regenerative Medicine Plans-

In Japan, the institutes that provide regenerative medicine have a tough examination based on law.
Our hospital has all the plans needed for anti-ageing treatment of mesenchymal stem cells.

- Notification Of Manufacture Of Specified Cell Processed Products
- Provision Plan For Immune Senescence Of Stem Cells / Stem Cell Exosomes Administration
- Provision Plan For Type-2 Diabetes Of Stem Cells / Stem Cell Exosomes Administration
- Provision Plan For Subcutaneous Facial Injection Of Stem Cells
- Provision Plan For Macce / Atherosclerosis Reduction Of Stem Cells Administration
- Provision Plan For Osteoarthritis Of Stem Cell Administration
- Provision Plan For Dental Disease Of Stem Cell Injection
- Provision Plan For Facial External Use Of Stem Cells
- Provision Plan For Alopecia Of The Head Of The Stem Cell Injection

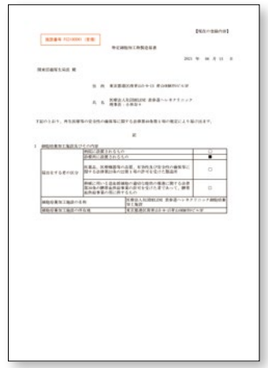
You can check the plan number and notification number
on the Ministry of Health, Labor and Welfare website.

http://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/iryou/saisei_iryou/

ひと、くらし、みらいのために
厚生労働省
Ministry of Health, Labour and Welfare

厚生労働省　再生医療 | 検索

REGENERATIVE MEDICINE PROVISION PLANS ACCEPTED BY MINISTRY OF HEALTH, LABOR AND WELFARE



Manufacture of Specific Cellular Products

Immune Senescence

Type-2 Diabetes

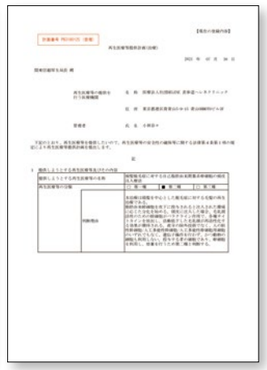
Subcutaneous Facial Injection

MACCE/ Arteriosclerosis reduction

Osteoarthritis

Dental Diseases

Facial External Use



Alopecia of the head

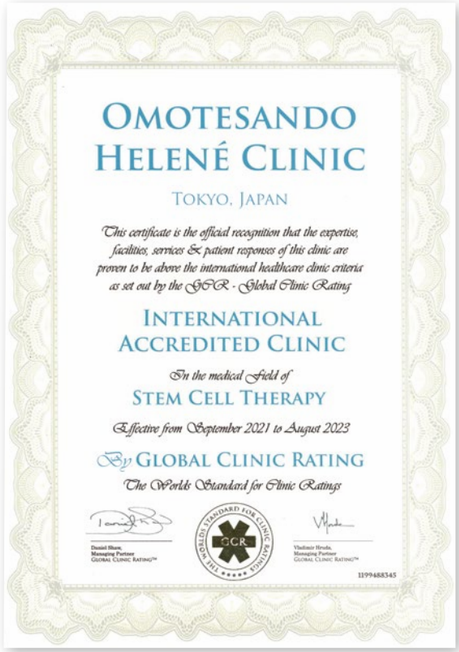
GCR CERTIFICATION CLINIC

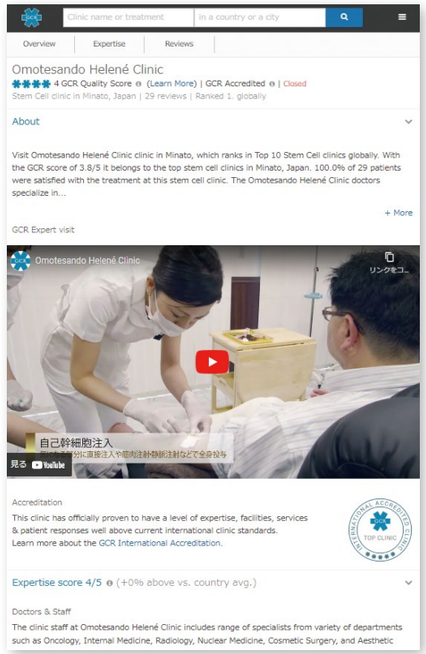
Our clinic is a GCR certified clinic.
In June 2017, we gained the GCR certification for the first stem cell therapy clinic in the world.

GCR is the abbreviation for Global Clinic Rating,
which means that our clinic has been officially proved to be above international standards.
https://gcr.org

From the viewpoint of a third party,
checking the quality, using the data collected by GCR
and aiming at the overall level of expertise, facilities, services and patient care.

We promise to offer better and better treatment so that each of our patients could visit with reassuring.



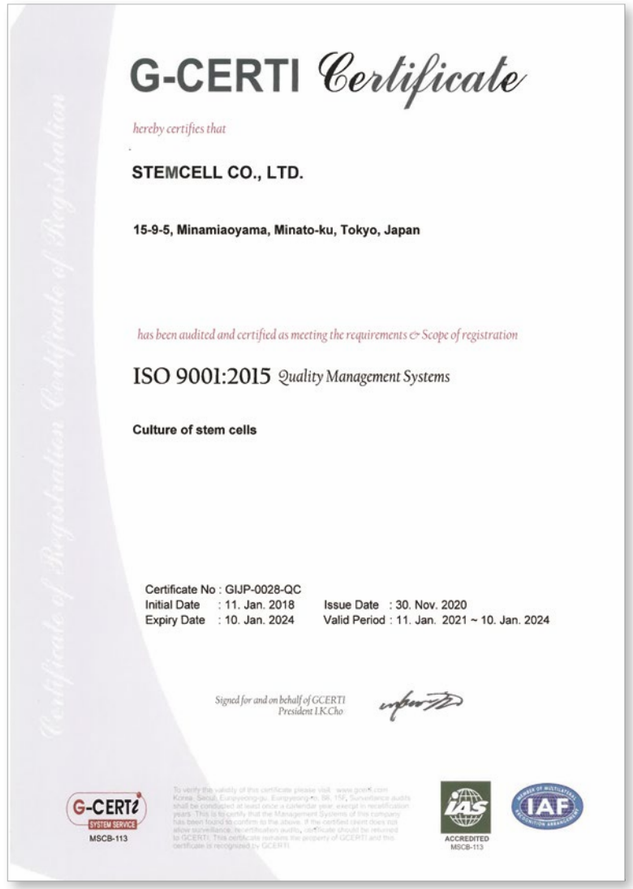


ISO 9001
CERTIFIED BY QUALITY MANAGEMENT SYSTEM

Our activities are carried out by non-government agencies located in the Geneva headquarters of Switzerland.
International Organization for Standardization
(ISO = International Standardization Agency) certification.

The ISO standard refers to the association between products and services.
The international standards set by the principle of "providing the same quality and the same level all over the world" are set and modified by 165 countries (including 2014) including Japan. Participate in the voting revision.

ISO 9001 is a specification for a quality management system that aims to continuously improve the quality of products and services provided to customers.



Q How long will it take to know the effect?

A At the earliest, some people will feel it the next day, generally around 2 weeks, and it takes about 3 to 6 months to see the change.

Q Is it necessary to restrict diet before blood collection?

A This blood test checks for items whose values do not change with diet, so dietary restrictions are not necessary.

Q Where are the stem cells?

A Stem cells are present throughout the body. As it may be uncomfortable for patients to obtain stem cells from abdominal fat, we collects adipose tissue from behind the ear using a skin punch.

Q Does it help with infertility? What about ovarian rejuvenation?

A This is not a treatment that works directly on the ovaries, so there is a great deal of uncertainty, but it has been shown to have a certain effect on ovarian rejuvenation & female infertility.

Q Can stem cells help with diabetes?

A Stem cell therapy has been reported in many studies to be highly effective in the treatment of type 2 diabetes. We have obtained a regenerative medicine provision plan for type 2 diabetes treatment, and we work in collaboration with diabetes specialists to provide treatment.

Q Can stem cells help with male function (erectile dysfunction/sustainability)?

A Stem cell therapy for male function, mainly spontaneous recovery, is said to be effective to some extent. In our experience, many of our patients have "felt the difference".

Q Can I still receive stem cell therapy when I am a senior?

A Because stem cells derived from fat are ingested, cultured, and administered, more stem cells can be increased and returned to the body in greater numbers. Therefore, stem cell therapy is a great way to revitalize the body as one gets older. In addition, many diseases occur with age from tissue loss. Stem cells are a good treatment in the sense that they can compensate for the loss of tissue.

Q If I have cancer, can I receive stem cell therapy?

A Stem cell therapy does not have the ability to cure cancer, but it can help reduce the burden on the body from cancer treatment and provide strength to fight cancer. For individuals with a history of cancer, it is necessary to consult with a doctor before receiving stem cell therapy.

Q What is the homing effect?

A Homing is the effect of moving to a remote location that requires a certain physiological effect. The "homing effect" is the effect of stem cells released into the body. Stem cells administered intravenously enter the peripheral blood circulatory system → reach site of injury via lymph and peripheral blood → adhere to vascular endothelium and infiltrate tissue → proliferate and differentiate into target cells.

Q Can stem cells help prevent disease?

A Even in the absence of symptoms, the condition may be systematically diseased. Finding the "unwell," stem cells work to reach and repair damaged tissue.The main effect of the treatment for those who are consciously healthy will be to eliminate "unwellness".

Q Are stem cells beneficial to health?

A While Western medicine treats with drugs and surgery, Oriental medicine tries to utilize the natural healing power of the body. Stem cells are a Western version of this Eastern medicine, which can be used to heal abnormalities in the body. Preventing and treating atherosclerosis can help your health.

Q Can I get a detailed explanation if I am going to receive stem cell therapy?

A When undergoing stem cell therapy, it is important that you receive a thorough explanation from your doctor, ask questions if you have any questions, and make sure you understand and are comfortable with the procedure. It is also important that the treatment be performed at a facility that has a lot of experience.

Q How long does stem cell therapy take?

A To undergo stem cell therapy, blood is drawn and fat tissue is removed, and only the stem cells present in the fat tissue are extracted and cultured. Culturing takes about 4 weeks, after which the stem cells can be administered. In the case of intravenous administration, the dose is slowly dropped over a period of 30 minutes.

Q Is the treatment painful?

A Cell collection is performed under local anesthesia, so please think of it as a dentist's treatment. The skin is about 5 mm in diameter, and after a few months, it is so blended in that it is hard to tell where the skin was removed. It is only an intravenous drip, so there is no pain.

Q How should I take care of the patient after the surgery?

A You can go home immediately after the surgery, the wound is small, and you can shower on the same day, allowing you to go about your daily life as normal. Our group takes care of any wound concerns or pain and follows up with phone calls, so you don't have to worry.

Q What is the serum medium for stem cells?

A Because of the risk of unknown infections with animal-derived media, we use animal-free serum-free medium.

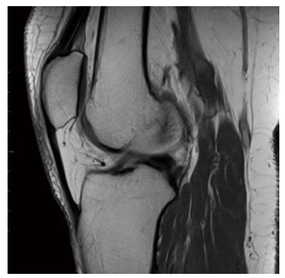
SUPPORT

JSRM (Medical facility registered to the Health Damage Compensation System)

Our clinic belongs to the Health Damage Compensation System of the Japanese Society for Regenerative Medicine.
The "Act on assuring safety of regenerative medicine" was enacted on November 27, 2013. It provides health damage insurance to the people who receive regenerative medicine treatment and the specialist providing regenerative medicine treatment using cells.

AFTER-CARE SUPPORT

We have a pre-treatment and post-treatment follow-up system. This survey is conducted in order to observe medical outcomes and observation of medical outcomes and efforts to improve the quality of medical care. Around 6 months after treatment, the physician or staff may contact you and request lab data or an MRI. We consider it as a part of treatment, and based on patient feedback, we will make treatment suggestions and schedule a return visit.



EMERGENCY RESPONSE

According to the statistics of adverse reactions to stem cell administration, IV administration of stem cells does not directly or indirectly cause sudden changes in the body. If there is a sudden change in the patient after administration, we will first check the patient's condition and contact our clinic after the patient has been examined by a local medical institution. Basically, stem cell therapy is a treatment that is unlikely to cause any abnormalities, but in the unlikely event of a sudden change, please contact our clinic and our specialized medical team will respond within 5 hours.

【 For inquiries, please contact 】

Stem Cell Transplant Coordinator
Website Consultation

Stem Cell Transplant Coordinator
LINE Consultation

Stem Cell Transplant Coordinator
WeChat Consultation

Tokyo Metro Ginza line, Hanzomon line, Chiyoda line Omotesando station B1 exit.
Go straight and turn left at Kotto Dori Street. Aoyama OHMOTO building 3F.

HELENE Medical Corporation
Omotesando **HELENE** Clinic

〒107- 0062
5-9-15 Minami Aoyama, Minato-ku,
Tokyo Aoyama OHMOTO Building 3F

[*Tel*] 03-3400-2277
[*Fax*] 03-3400-2276
[*Consultation hours*] 10 : 00 - 19 : 00
[*Closed days*] Wednesday · Sunday
[*Reservation Only*] ganxibao@helene.jp
[*Website*] https://stemcells.jp/

IF YOU COME WITH CHILDREN

If you will come with young children, please let us know in advance.
According to the nature of the treatment, you may be unable to bring children with you.
◆ If you come with a baby stroller, please use the B3 Exit of Omotesando Station.

IF YOU COME FROM A DISTANT PLACE

BY HIGH SPEED RAIL

◆ From Tokyo station (about 25 minutes), please take the Marunouchi line to Ogikubo direction and transfer at Akasaka Mitsuke station. Then please take the Ginza line to Shibuya direction and will stop at Omotesando station.

◆ From Shinagawa station (about 25 minutes), please take the JR Yamanote line and transfer at Shibuya station. Then please take the Ginza line to Asakusa and the next stop is Omotesando station.

BY AIRPLANE

◆ From Haneda airport (about 50 minutes), please take the Tokyo monorail and transfer at Hamamatsucho station. Take the JR Yamanote line to Shimbashi and transfer to Tokyo metro Ginza line to Shibuya, stopping at Omotesando station.

BY CAR

We are sorry that we do not have parking spaces. Please use the parking lots nearby. (There is no discount for the parking fee.)

- **Times Aoyama Dori First**
- **Aoyama Rise Square**
- **Mb Parking Minami Aoyama**
- **Meiji Yasuda Life Insurance Aoyama Palacio**