

## AUTOLOGOUS REGENERATIVE THERAPY IN YOUR HANDS

Clinical studies have demonstrated the efficacy of therapies based on the autologous grafting of autologous adult mesenchymal stem cells to accelerate the healing and regeneration processes of the skin and mesenchymal tissues, as well as finding application in the treatment of autoimmune diseases for their immunomodulatory abilities.

Adipocyte-derived mesenchymal stem cells (ADSCs) are pluripotent adult progenitor cells derived from embryonic connective tissue. ADSCs are able to differentiate in an adipogenic, osteogenic, chondrogenic, myogenic cells, but also towards non-mesodermal cell lines (neuronal, endothelial, epithelial, etc.). They are also able to secrete a series of growth factors, such as VEGF (Vascular Endothelial Growth Factor), HGF (Hepatocyte Growth Factor), FGF-2 (Fibroblast Growth Factor-2) and IGF-1 (Insulin-like Growth Factor-1) which give them a regenerative and angiogenetic power.

One of the richest tissues in adult mesenchymal stem cells is adipose tissue. Adult mesenchymal stem cells of adipose origin (Adipose Derived Stem Cells - ADSCs) are today among the most widely used cells in Regenerative Medicine due to their characteristics and ease of availability. Adult mesenchymal stem cells of adipose origin (ADSCs) exert their regenerative and immunomodulatory capacity thanks to the paracrine effects through trophic factors that exhibit antifibrotic, antiapoptotic and pro-angiogenic activity. Furthermore, mesenchymal stem cells exert their regenerative capacity also thanks to their intrinsic ability to transform into mesenchymal and endothelial cells, promoting tissue repair.

ADSCs have the ability to modulate a wide range of immune system cells, such as B and T lymphocytes, neutrophils and natural killer cells. This immunomodulatory and anti-inflammatory capacity is being recently investigated in the field of allografts. Studies have shown that the association with ADSCs favors the engraftment of allografts.

Numerous studies have recognized adipose tissue as one with the highest concentration of adult mesenchymal stem cells (ADSCs), and in particular in its stromal component (SVF Stromal Vascular Fraction). Studies have shown a higher concentration of mesenchymal stem cells in adipose tissue (ADSCs) than in bone marrow (Marrow Stem Cells- MSCs). Comparing bone marrow mesenchymal stem cells (MSCs) with ADSCs, it was found that the latter have a higher concentration in the same volume of harvested tissue, are collected in a much less traumatic method and their concentration seems less influenced by the age of the patient.

The proven regenerative capacities of mesenchymal stem cells (ADSCs) are exploited in numerous medical specialties such as: reconstructive and aesthetic surgery, maxillofacial surgery, vulnology, orthopedics, vascular surgery, orthopedics, cardiology, urology, otolaryngology, proctology and dermatology and lately also the GYNECOLOGICAL field.

## AUTOLOGOUS REGENERATIVE MEDICINE IN GYNECOLOGY

At the genital level, as in all other tissues, aging causes a reduction in collagen, with aging of the tissues and thinning of the mucosa, which are further damaged by the lack of

estrogens. The resulting atrophy leads to a series of extremely disabling symptoms, called genitourinary syndrome, such as vaginal dryness, burning, dyspareunia, recurrent cystitis, vaginal infections, incompatible with a good quality of life.

Autologous mesenchymal stem cells (ADSCs) have the ability to differentiate according to the characteristics of the tissue in which they are implanted and, at the level of the vaginal mucosa, cause an increase in elastin and fibroblasts with consequent restoration of tissue turgor, elasticity and spontaneous lubrication. In addition, vascular growth factors (VEGF) improve microcirculation by improving skin and mucous trophism.

The possibility of grafting micro-fragmented adipose tissue rich in SVF cells and ADSCs at the level of the fourchette, where the mucosa is particularly thin and inelastic and where abrasions and bleeding during sexual intercourse are more frequent, creates the conditions for a significant improvement in life quality.

Autologous mesenchymal stem cells (ADSCs) can also be used in the treatment of perineal scars, outcomes of episiorraphy, removal of Bartholin's gland cysts, incision of abscesses and in all situations in which patients report situations of dryness resistant to common local therapies even in childbearing age.

They can also be used to increase the tone and thickness of the vaginal mucosa in wide vagina syndrome (vaginal laxity due to close deliveries, precipitous labor), which, contrary to vaginal atrophy, does not create pain on penetration, but less sensitivity during intercourse.

Women can be truly treated in the gynecology with their own mesenchymal cells at 360 degrees, to achieve a satisfying quality of life at all ages.

Finally, clinical studies demonstrate the effectiveness of homologous stem cells grafting in menopausal scleroatrophic lichen, a chronic inflammatory disease of the vulva that leads to scar tissue sclerosis.

The implantation of autologous stem cells stimulates fibroblasts, precursors of collagen and elastic fibers, resulting in tissue repair and resolution of the clinical picture and symptoms.

### **IMPIANTO TISSUTALE SEFFI e microSEFFI (Superficial Enhanced Fluid Fat Injection)**

Since 2015 Gennai et al. have published several studies (see bibliographical references) on new tissue grafting techniques SEFFI and MicroSEFFI.

These techniques aim to graft the autologous adipose tissue, including the stromal fraction (SVF) and the adipose tissue derived stem cells (ADSC) contained in it, in order to obtain a trophic improvement of the tissues and a restoration of the lost volumes. The authors demonstrated that the adipose tissue can be harvested through special cannulas with very small side ports, in order to select small cell clusters, therefore it is not necessary to carry out manipulations to fluidify the tissue.

SEFFI and MicroSEFFI are now among the most used techniques for tissue regeneration and volume restoration for the rejuvenation of the face and other areas of the body. These techniques are considered minimally invasive surgical procedures, so they require surgical experience, particularly in liposuction, and adequate facilities.

In light of these evidences, Autologous Regenerative Therapy has always been only in the hands of plastic surgeons and has not been open to aesthetic doctors, gynecologists or dermatologists.

Dr. Gennai firmly believes that Autologous Regenerative Therapy must also be performed by doctors without specific training in liposuction surgery. For this reason he has developed, standardized and patented \* a special guide, that is a device designed for harvesting fat tissue in a safe, easy, effective way, even without any surgical liposuction skills.

From this original idea, SEFFILINE has developed SEFFIGYN®, a medical device where all the components necessary for the treatment to be found in the box and for single use, to allow doctors to perform regenerative autologous treatments in their offices in a safely, easily and effectively.

\*Brevetto italiano

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## WHY SEFFIGYN®

In order to perform a correct Regenerative Therapy, the need arises to have a SAFE, SIMPLE, RAPID, EFFECTIVE and STANDARDIZED method to harvest the naturally micro-fragmented adipose tissue containing the stromal component (SVF) and adult mesenchymal stem cells (ADSCs).

This method must have the following characteristics:

- the least traumatic tissue harvesting both for the tissue and the patient.
- Harvesting the adipose tissue in the most superficial plane possible, to obtain a tissue richer in mesenchymal stem cells (ADSCs). This procedure must be standardized also in the hands of colleagues without specific experience in collecting superficial adipose tissue.
- Collecting cell clusters containing adipocytes, stromal tissue (SVF) and mesenchymal stem cells (ADSCs) of reduced size, to favor their engraftment in the recipient site.
- Minimal manipulation of the harvested tissue, to maintain the highest cellular vitality and without using tissue fragmentation devices, to make the method faster and less expensive.
- Being as little traumatic as possible for the patient and minimizing the complications of blood sampling such as hematomas, seromas, infections and skin irregularities.
- The tissue to be implanted must be sufficiently fluid to be grafted with thin needles or cannulas to cause as little trauma as possible to the vulvo-vaginal region.

- The procedure must be able to be performed by doctors even without specific experience in liposuction procedures.
- The method should be performable by doctors without a specific experience in liposuction procedures.

**SEFFIGYN®** meets all these needs:

1. It is the only guided medical device. Its guide (Italian patent) allows doctors without specific experience in superficial liposuction to perform tissue harvesting in a standardized, simple, safe and effective way.
2. It is the only medical device containing all the components needed for the entire procedure, from harvesting to grafting. This is why the procedure can be carried out on an outpatient basis in a simple, standardized, safe and effective way.
3. The device is sterile and disposable, to make the procedure safe for the patient and the physician.
4. The micro harvesting cannula allows to collect micro fragmented adipose tissue as it makes the tissue fluid. This method follows the philosophy of the SEFFI technique (Superficial Enhanced Fluid Fat Injection described above), which involves taking cellular micro clusters that do not require subsequent substantial manipulations before grafting, while maintaining the biological characteristics of the harvested tissue. This makes the method not only simple and standardized, but also quick and effective.

The SEFFIGYN® device is designed for use in public and private hospitals in an outpatient setting for the following indications in the gynecological field:

- Genitourinary Syndrome,
- Outcomes of episiorraphy and perineal surgical scars,
- Wide vagina syndrome (postpartum vaginal laxity),
- Lichen sclerosus.

## BIBLIOGRAPHY

1. Gennai A., Bernardini F. P.; Superficial enhanced fluid fat injection (SEFFI and MicroSEFFI) in facial rejuvenation; CellR4 2017; 5 (1): e2239
2. Gennai A., Zia S., Roda B., Maggio A., Bonsi L., Alviano F., Zattoni A., Reschiglian P., Bernardini F.P., SEFFI (Superficial Enhanced Fluid Fat Injection) for aesthetic and clinical regenerative treatments, Global Journal of Dermatology & Venereology, 2020, 8, 32-40
3. Martina Rossi; Barbara Roda; Silvia Zia; Ilaria Vigliotta; Chiara Zannini; Francesco Alviano; Laura Bonsi, Andrea Zattoni, Pierluigi Reschiglian, and Alessandro Gennai; Characterization of the Tissue and Stromal Cell Components of Micro-Superficial Enhanced Fluid Fat Injection (Micro-SEFFI) for Facial Aging Treatment; Aesthetic Surgery Journal 2018, 1–12
4. Francesco P. Bernardini, Alessandro Gennai; Superficial Enhanced Fluid Fat Injection for Volume Restoration and Skin Regeneration of the Periocular Aesthetic Unit. An Improved Fat Grafting Technique to enhance the beauty of the eye; JAMA Plastic Facial Surgery January- February 2016 Vol. 18 n. 1
5. Francesco P. Bernardini, Alessandro Gennai, Luigi Izzo, Alessandra Zambelli, Erica Repaci, Ilaria Baldelli, G. Fraternali-Orcioni, Morris E. Hartstein, Pier Luigi Santi, and Rodolfo Quarto; Superficial

Enhanced Fluid Fat Injection (SEFFI) to Correct Volume Defects and Skin Aging of the Face and Periocular Region; *Aesthetic Surgery Journal* 2015, 1–12

6. Pignata G, Gennai A, Bernardini F; Rejuvenation of the centre of the face: a new paradigm Endoscopic lifting with fat grafting; *Plsr Aesthet Res* 2018;5:23
7. Tabanella G., Ferlosio A., Orlandi A., Gennai A., Adipose-derived mesenchymal stem cells transplantation for socket preservation: a clinical report, *EC Dental Science, Case Report*, 2019
8. Gennai A, Zambelli A, Repaci E, et al., Skin Rejuvenation and Volume Enhancement with the Micro Superficial Enhanced Fluid Fat Injection (M-SEFFI) for Skin Aging of the Periocular and Perioral Regions. *Aesthet Surg J.* 2017;37(1):14-23.
9. Gennai A, Bernardini F-P, Rejuvenation faciale par lifting endoscopique avec le petite incision incision vertical associée à une injection superficielle de graisse: un repositionnement endoscopique, une regeneration tissulaire et une restauration volumetrique (La technique R3); *J. Med. Esth. Et Chir. Derm.* Vol. XXXXIV, 174, juin 2017, 87-95
10. Gennai A, Bernardini F-P, R3 facial rejuvenation through minimal incisions vertical endoscopic lifting (MIVEL) and superficial enhanced fluid fat injection (SEFFI): endoscopic repositioning, tissue regeneration volume restoration; *Aesthetic Medicine*, vol. 1, No. 2, July- Sept 2015
11. Veronica Boero, Massimiliano Brambilla, Elisa Sipio, Carlo A Liverani, Mirella Di Martino, Benedetta Agnoli, Giada Libutti, F M Cribiù, Alessandro Del Gobbo, Enrico Ragni, Giorgio Bolis: Vulvar lichen sclerosus: A new regenerative approach through fat grafting *Gynecol Oncol*, 2015 Dec;139(3):471-5. doi: 10.1016/j.ygyno.2015.10.014. Epub 2015 Oct 21.
12. Serena Tamburino, Giuseppe AG Lombardo, Maria Stella Tarico, Rosario Emanuele Perrotta The Role of Nanofat Grafting in Vulvar Lichen Sclerosus: A Preliminary Report *Archives of Plastic Surgery* Vol. 43 / No. 1 / January 2016
13. Samer Jabbour, MD; Elio Kechichian, MD; Barbara Hersant, MD; Philippe Levan, MD; Lena El Hachem, MD; Warren Noel, MD; and Marwan Nasr, MD Labia Majora Augmentation: A Systematic Review of the Literature *Aesthetic Surgery Journal* 2017, Vol 37(10) 1157–1164
14. Almadori, Aurora MSc; Hansen, Esther DClínPsy; Boyle, Deborah FRCOG; Zenner, Nicole MRCOG; Swale, Victoria FRCP; Reid, Wendy FRCOG; Maclane, Allan FRCP; Butler, Peter E.M. FRCS(Plast) Fat Grafting Improves Fibrosis and Scarring in Vulvar Lichen Sclerosus: Results From a Prospective Cohort Study *Journal of Lower Genital Tract Disease: July 2020 - Volume 24 - Issue 3 - p 305-310*
15. Juan Monreal: Safety and Efficacy of Stromal Vascular Fraction Enriched Fat Grafting Therapy for Vulvar Lichen Sclerosus CUREUS DOI: 10.7759/cureus.7096
16. Goldstein AT, King M, Runels C, Gloth M, Pfau R: Intradermal injection of autologous platelet- rich plasma for the treatment of vulvar lichen sclerosus. *J Am Acad Dermatol.* 2017, 76:158- 160. 10.1016/j.jaad.2016.07.037
17. Barbara Hersant, Samer Jabbour, Warren Noel, Laurent Benadiba Simone La Padula, Mounia SidAhmed-Mezi, Jean Paul Meningaud Labia Majora Augmentation Combined With Minimal Labia Minora Resection: A Safe and Global Approach to the External Female Genitalia *Ann Plast Surg* 2018 Apr;80(4):323-327.
18. Singer NG, Caplan AI, Mesenchymal stem cells: mechanisms of inflammation. *Annu Rev Pathol* 2011; 6:457–478
19. Delarosa O, Dalemans W, Lombardo E, Mesenchymal stem cells as therapeutic agents of inflammatory and autoimmune diseases. *Curr Opin Biotechnol* 2012; 23:1–5.
20. Krampera M, Glennie S, Dyson J et al., Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigenspecific T cells to their cognate peptide. *Blood* 2003; 101:3722–3729
21. Delarosa O, Sánchez-Correa B, Morgado S et al. Human adipose-derived stem cells impair natural killer cell function and exhibit low susceptibility to natural killer-mediated lysis. *Stem Cells Dev* 2012; 21:1333–1343.

22. Planat-Benard V, Silvestre JS, Cousin B, et al., Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation*. 2004;109:656 – 663.
23. Madonna R, Geng Y.J. and De Caterina R., Adipose Tissue-Derived Stem Cells: Characterization and Potential for Cardiovascular Repair; *Arterioscler Thromb Vasc Biol* 2009; -1728
24. Coleman S.R., Saboeiro A., Fat Grafting to the Breast Revisited: Safety and Efficacy *Plastic and Reconstructive Surgery* • March 2007.
25. Coleman S.R., Hand Rejuvenation with Structural Fat Grafting. *Plastic and Reconstructive Surgery*, 2002: 1731-44.
26. Madonna R, Geng Y.J. and De Caterina R., Adipose Tissue-Derived Stem Cells: Characterization and Potential for Cardiovascular Repair; *Arterioscler Thromb Vasc Biol* 2009; -1728
27. Caplan, A. I., Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J. Cell. Physiol.* 2007; 213(2):341–347.
28. Satija, N. K.; Singh, V. K.; Verma, Y. K.; Gupta, P., et al., Mesenchymal stem cell-based therapy: A new paradigm in regenerative medicine. *J. Cell. Mol. Med.* 2009; 13(11– 12):4385–402.
29. Garcia-Olmo D, Garcia-Arranz M, Garcia LG et al., Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int J Colorectal Dis* 2003;18:451–454.
30. F. de la Portilla et al., Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial *Int J Colorectal Dis* 2013; 28:313–323.
31. Salemi S, Rinaldi C, Manna F, Guarneri GF, Parodi PC, Reconstruction of lower leg skin ulcer with autologous adipose tissue and platelet-rich plasma. *J Plast Reconstr Aesthet Surg.* 2008 Dec; 61(12): 1565-7.
32. Cervelli V, Gentile P, De Angelis B et al., Application of enhanced stromal vascular fraction and fat grafting mixed with PRP in post-traumatic lower extremity ulcers. *Stem Cell Res.* 2011 Mar;6(2):103-11
33. Mingliang Sun, Yunfan He, Tao Zhou, Pan Zhang, Jianhua Gao, and Feng Lu, Adipose Extracellular Matrix/Stromal Vascular Fraction Gel Secretes Angiogenic Factors and Enhances Skin Wound Healing in a Murine Model *BioMed Research International*, Volume 2017, Article ID 3105780, 11 pages.
34. V. Falanga, Wound healing and its impairment in the diabetic foot, *Lancet*, vol. 366, no. 9498, pp. 1736–1743, 2005.
35. S. H. Lee, J. H. Lee, and K. H. Cho, Effects of human adipose-derived stem cells on cutaneous wound healing in nude mice, *Annals of Dermatology*, vol. 23, no. 2, pp. 150–155, 2011.
36. M. Isakson, C. de Blacam, D. Whelan, A. McArdle, and A. J. Clover, Mesenchymal stem cells and cutaneous wound healing: current evidence and future potential, *Stem Cells International*, vol. 2015, Article ID 831095, 12 pages, 2015.
37. L.Pan, J.Tang, H.Liu and B. Cheng, Sympathetic nerves: How do they affect angiogenesis, particularly during wound healing of soft tissues? *Clinical Hemorheology and Microcirculation*, vol. 62, no. 2, pp. 181–191, 2016.
38. Suzuki E, Fujita D, Takahashi M, et al., Adipose tissue-derived stem cells as a therapeutic tool for cardiovascular disease. *World J Cardiol.* 2015;7:454–65.
39. Bourin P, Bunnell BA, Casteilla L, et al., Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy.* 2013;15:641–8.
40. Gimble JM, Bunnell BA, Frazier T, et al., Adipose-derived stromal/stem cells. *Organogenesis.* 2013;9:3–10.



41. Nguyen A, Guo J, Banyard DA, et al., Stromal vascular fraction: a regenerative reality? Part 1: current concepts and review of the literature. *J Plast Reconstr Aesthetic Surg.* 2016;69:170–9.
42. Hass R, Kasper C, Böhm S, et al., Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue- derived MSC. *Cell Commun Signal.* 2011;9:12.
43. Katlin B. Stivers, Jason E. Beare, Paula M. Chilton, Stuart K. Williams, Christina L. Kaufman, and James B. Hoying, Adipose-derived cellular therapies in solid organ and vascularized- composite allotransplantation *Curr Opin Organ Transplant* 2017, 22:490–498
44. Wang RX, Yu CR, Dambuza IM, et al., Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat Med* 2014; 20:633 – 641.
45. Aarabi S, Bhatt KA, Shi Y, Paterno J, Chang EI, Loh SA, et al. (2007), Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. *Faseb J* 21: 3250–326
46. Liu S, Jiang L, Li H, Shi H, Luo H, Zhang Y, et al. (2014), Mesenchymal stem cells prevent hypertrophic scar formation via inflammatory regulation when undergoing apoptosis. *J Invest Dermatol* 134: 2648– 2657.
47. Sophie Domergue, Claire Bony, Marie Maumus, Karine Toupet, Eric Frouin, Valérie Rigau, Marie-Catherine Vozenin, Guy Magalon, Christian Jorgensen, Danièle Noël, Comparison between Stromal Vascular Fraction and Adipose Mesenchymal Stem Cells in Remodeling Hypertrophic Scars *PLOS ONE* | DOI:10.1371/journal.pone.0156161 May 26, 2016
48. Elizabeth Brett, MS; Elizabeth R. Zielins, MD; Monica Chin, BS; Michael Januszyk, MD; Charles P. Blackshear, MD; Michael Findlay, MD; Arash Momeni, MD; Geoffrey C. Gurtner, MD; Michael T. Longaker, MD, MBA; Derrick C. Wan, MD, Isolation of CD248-expressing stromal vascular fraction for targeted improvement of wound healing *Wound Rep Reg* (2017) 25 414– 422 VC 2017
49. Nie, C. et al. Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis. *Cell Transplant.* 20 (2), 205-216 (2011).
50. Shin, L., & Peterson, D. A., Human mesenchymal stem cell grafts enhance normal and impaired wound healing by recruiting existing endogenous tissue stem/progenitor cells. *Stem Cells Transl Med.* 2 (1), 33-42 (2013).
51. Kato, Y., Iwata, T., Washio, K., Yoshida, T., Kuroda, H., Morikawa, S., Hamada, M., Ikura, K., Kaibuchi, N., Yamato, M., Okano, T., Uchigata, Y., Creation and Transplantation of an Adiposederived Stem Cell (ASC) Sheet in a Diabetic Wound-healing Model. *J. Vis. Exp.* (126), e54539, doi:10.3791/54539 (2017).
52. Hea Gu, MD; Jae Sun Lee, MS; Deok-Woo Kim, MD; Eul-Sik Yoon, MD, PhD; Eun-Sang Dhong, MD, PhD, Neovascular potential of adipose-derived stromal cells (ASCs) from diabetic patients *Wound Rep Reg* (2012) 20 243–252 © 2012
53. Slavkovsky, R. et al. Zucker diabetic fatty rat: a new model of impaired cutaneous wound repair with type II diabetes mellitus and obesity. *Wound Repair Regen.* 19 (4), 515-525 (2011).
54. Michael H. Carstens, Arturo Gómez, Ronald Cortés, Elizabeth Turner, Cecilia Pérez Marlon Ocon, Diego Correa, Non-reconstructable peripheral vascular disease of the lower extremity in ten patients treated with adipose-derived stromal vascular fraction cells, *Stem Cell Research* 18 (2017) 14–21
55. Bura, A., Planat-Benard, V., Bourin, P., Silvestre, J.-S., Gross, F., Grolleau, J.-L., Saint- Lebese, B., Peyrafitte, J.-A., Fleury, S., Gadelorge, M., Taurand, M., Dupuis-Coronas, S., Leobon, B., Casteilla, L., 2014. Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. *PubMed — NCBI. Cytotherapy* 16, 245–257.
56. Rehman, J., Traktuev, D., Li, J., Merfeld-Clauss, S., Temm-Grove, C.J., Bovenkerk, J.E., Pell, C.L., Johnstone, B.H., Considine, R.V., March, K.L., 2004. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109, 1292–1298.
57. Rennert, R.C., Sorkin, M., Januszyk, M., Duscher, D., Kosaraju, R., Chung, M.T., Lennon, J., Radiya-Dixit, A., Raghvendra, S., Maan, Z.N., Hu, M.S., Rajadas, J., Rodrigues, M., Gurtner, G.C., 2014.

Diabetes impairs the angiogenic potential of adipose-derived stem cells by selectively depleting cellular subpopulations. *Stem Cell Res. Ther.* 5, 79.

58. Sumi, M., Sata, M., Toya, N., Yanaga, K., Ohki, T., Nagai, R., 2007. Transplantation of adipose stromal cells, but not mature adipocytes, augments ischemia-induced angiogenesis. *Life Sci.* 80, 559–565.
59. Agnes S. Klar, Jakub Zimoch, and Thomas Biedermann, *Skin Tissue Engineering: Application of Adipose-Derived Stem Cells Hindawi BioMed Research International Volume 2017*, Article ID 9747010, 12 pages <https://doi.org/10.1155/2017/9747010>
60. B. Puissant, C. Barreau, P. Bourin et al., Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells, *British Journal of Haematology*, vol. 129, no. 1, pp. 118–129, 2005.
61. L. Cai, B. H. Johnstone, T. G. Cook et al., IFATS collection: human adipose tissue-derived stem cells induce angiogenesis and nerve sprouting following myocardial infarction, in conjunction with potent preservation of cardiac function, *STEM CELLS*, vol. 27, no. 1, pp. 230–237, 2009.
62. W.C.Gao, X.Qiao, S.L.Ma, and L. Cui, Adipose-derived stem cells accelerate neovascularization in ischaemic diabetic skin ap via expression of hypoxia-inducible factor- 1, *Journal of Cellular and Molecular Medicine*, vol. 15, no. 12, pp. 2575–2585, 2011.
63. Adas Darinskas, Mindaugas Paskevicius, Gintaras Apanavicius, Gintaris Vilkevicius, Liutauras Labanauskas, Thomas E. Ichim and Rytis Rimdeika, Stromal vascular fraction cells for the treatment of critical limb ischemia: a pilot study. *J Transl Med* (2017) 15:143 DOI 10.1186/s12967-017-1243-3
64. Stolzing A, Jones E, McGonagle D, Scutt A., Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. *Mech Ageing Dev.* 2008;129:163–73.
65. Han SK, Kim HR, Kim WK, The treatment of diabetic foot ulcers with uncultured, processed lipoaspirate cells: a pilot study. *Wound Repair Regen.* 2010;18:342–8.
66. Compagna R, Amato B, Massa S, Amato M, Grande R, Butrico L, de Franciscis S, Serra R. Cell therapy in patients with critical limb ischemia. *Stem Cells Int.* 2015;2015:931420.
67. Dong-Sic Chae, Seongho Han, Mina Son & Sung-Whan Kim, Stromal vascular fraction shows robust wound healing through high chemotactic and epithelialization property *Cytotherapy*, 2017; 19: 543–554
68. Smith AN, Willis E, Chan VT, Muffley LA, Isik FF, Gibran NS, et al. Mesenchymal stem cells induce dermal fibroblast responses to injury. *Exp Cell Res* 2010;316(1):48–54.
69. Luigi Clauser, MD, DMD Antonio Lucchi, MD, Ilaria Tocco-Tussardi, MD, Chiara Gardin, PhD, Barbara Zavan, PhD, Autologous Fat Transfer for Facial Augmentation and Regeneration Role of Mesenchymal Stem Cells *Atlas Oral Maxillofacial Surg Clin N Am* 26 (2018) 25–32
70. Luigi Clauser., Letizia Ferroni., Chiara Gardin, Riccardo Tieghi, Manlio Galie, Giovanni Elia, Adriano Piattelli, Paolo Pinton, Erierto Bressan, Barbara Zavan, Selective Augmentation of Stem Cell Populations in Structural Fat Grafts for Maxillofacial Surgery *PLoS ONE* 9(11): e110796. doi:10.1371/journal.pone.0110796
71. Luigi C. Clauser, MD, DMD, PhD Giuseppe Consorti, MD Giovanni Elia, MD Manlio Galié, MD, DMD, Riccardo Tieghi, MD, Three-Dimensional Volumetric Restoration by Structural Fat Grafting *Craniofacial Trauma Reconstruction* 2014;7:63–70
72. Ogura F, Wakao S, Kuroda Y, et al., Human adipose tissue possesses a unique population of pluripotent stem cells with nontumorigenic and low telomerase activities: potential implications in regenerative medicine. *Stem Cells Dev.* 2014;23(7):717-728.
73. Lendeckel S, Jodicke A, Christophis P, et al. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J Craniofacial Surg.* 2004;32(6):370-373.



74. Rossi M. AF, Ricci F., Vignoli F., Marchionni C., Valente S., Zannini C., Tazzari P. L., Vignoli M., Bartoletti E., Bonsi L., In vitro multilineage potential and immunomodulatory properties of adipose derived stromal/stem cells obtained from nanofat lipoaspirates. *CellR4*. 2016; 4 (6):e2212.
75. Michalek J, Moster R, Lukac L, et al., Autologous adipose tissue-derived stromal vascular fraction cells application in patients with osteoarthritis. *Cell transplantation*. 2015.
76. Gotoh M, Yamamoto T, Kato M, et al., Regenerative treatment of male stress urinary incontinence by periurethral injection of autologous adipose-derived regenerative cells: 1- year outcomes in 11 patients. *Int J Urol*. 2014;21(3):294-300.
77. Granel B, Daumas A, Jouve E, et al., Safety, tolerability and potential efficacy of injection of autologous adipose-derived stromal vascular fraction in the fingers of patients with systemic sclerosis: an open-label phase I trial. *Ann Rheum Dis*. 2015;74(12):2175-2182.
78. Jo CH, Lee YG, Shin WH, et al., Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem Cells*. 2014;32(5):1254-1266.
79. Koh YG, Choi YJ, Kwon SK, Kim YS, Yeo JE. Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(5):1308-1316.
80. Koh YG, Choi YJ, Kwon OR, Kim YS. Second-Look Arthroscopic Evaluation of Cartilage Lesions After Mesenchymal Stem Cell Implantation in Osteoarthritic Knees. *Am J Sports Med*. 2014;42(7):1628-1637.
81. Maroesjka Spiekman Joris A. van Dongen, Joep C. Willemsen, Delia L. Hoppe, Berend van der Lei and Martin C. Harmsen. The power of fat and its adipose-derived stromal cells: emerging concepts for fibrotic scar treatment *J Tissue Eng Regen Med* 2017; 11: 3220– 3235.
82. Mohsen Khosravi Maharlooei Mansooreh Bagheri, Zhabiz Solhjoui, Behnam Moein Jahromi, Majid Akrami, Lili Rohani , Ahmad Monabati , Ali Noorafshan, Gholamhossein Ranjbar Omrani, Adipose tissue derived mesenchymal stem cell (AD-MSC) promotes skin wound healing in diabetic rats diabetes research and clinical practice 93 (2011) 228–234
83. Jingwei Feng, Kazuhide Mineda, Szu-Hsien Wu, Takanobu Mashiko, Kentaro Doi, Shinichiro Kuno, Kahori Kinoshita, Koji Kanayama, Rintaro Asahi, Ataru Sunaga & Kotaro Yoshimura, An injectable non-cross-linked hyaluronic-acid gel containing therapeutic spheroids of human adipose-derived stem cells *Scientific RepoRts* | 7: 1548 | DOI:10.1038/s41598-017-01528-3
84. Bilgic S, Durusu M, Aliyev B, Akpancar S, Ersen O, Yasar SM, Ardic S., Comparison of two main treatment modalities for acute ankle sprain. *Pak J Med Sci*. 2015;31(6):1496–1499. doi: 10.12669/pjms.316.8210.
85. Caplan AI., Mesenchymal stem cells. *J Orthop Res*. 1991;9(5):641–650. doi: 10.1002/jor.1100090504.
86. Carter DR, Beaupre GS, Giori NJ, Helms JA., Mechanobiology of skeletal regeneration. *Clin Orthop Relat Res*. 1998;355(Suppl): S41–S55. doi: 10.1097/00003086-199810001-00006.
87. Lendeckel S, Jödicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J Cranio-Maxillofac Surg*. 2004;32(6):370–373. doi: 10.1016/j.jcms.2004.06.002.
88. Pak J. Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with adipose-tissue derived stem cells: a case series. *J Med Case Rep*. 2011;7(5):296. doi: 10.1186/1752-1947-5-296.
89. Buckwalter JA. Articular cartilage injuries. *Clin Orthop Relat Res*. 2002;402(1):21–37.
90. Pak J, Chang JJ, Lee JH, Lee SH. Safety reporting on implantation of autologous adipose tissue-derived stem cells with platelet-rich plasma into human articular joints. *BMC Musculoskelet Disord*. 2013;14:337. doi: 10.1186/1471-2474-14-337

91. Pak J, Lee JH, Park KS, Jeong BC, Lee SH. Regeneration of cartilage in human knee osteoarthritis with autologous adipose tissue-derived stem cells and autologous extracellular matrix. *BioRes Open Access*. 2016;5(1):192–200. doi: 10.1089/biores.2016.0024
92. Koh YG, Choi YJ. Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis. *Knee*. 2012;19(6):902–907. doi: 10.1016/j.knee.2012.04.001
93. Koh YG, Jo SB, Kwon OR, Suh DS, Lee SW, Park SH, Choi YJ. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. *Arthroscopy*. 2013;29(4):748–755. doi: 10.1016/j.arthro.2012.11.017.
94. Koh YG, Kwon OR, Kim YS, Choi YJ. Comparative outcomes of open-wedge high tibial osteotomy with platelet rich plasma alone or in combination with mesenchymal stem cell treatment: a prospective study. *Arthroscopy*. 2014;30(11):1453–1460. doi: 10.1016/j.arthro.2014.05.036
95. Kim YS, Choi YJ, Suh DS, Heo DB, Kim YI, Ryu JS, Koh YG. Mesenchymal stem cell implantation in osteoarthritic knees: is fibrin glue effective as a scaffold? *Am J Sports Med*. 2015;43(1):176– 185. doi: 10.1177/0363546514554190.
96. Bui KH-T, Duong TD, Nguyen NT, Nguyen TD, Le VT, Mai VT, Phan NL-C, Le DM, Ngoc NK, Pham PV. Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study. *Biomed Res Ther*. 2014;1(1):2–8. doi: 10.7603/s40730-014-0002-9.
97. Michalek J, Moster R, Lukac L, Proefrock K, Petrasovic M, Rybar J, Capkova M, Chaloupka A, Darinskas A, Michalek J Sr, Kristek J, Travnik J, Jabandzjev P, Cibulka M, Holec M, Jurik M, Skopalik J, Kristkova Z, Dudasova Z. Autologous adipose tissue-derived stromal vascular fraction cells application in patients with osteoarthritis. *Cell Transplant*. 2015. doi: 10.3727/096368915X686760
98. Fodor PB, Paulseth SG. Adipose derived stromal cell (ADSC) injections for pain management of osteoarthritis in the human knee joint. *Aesthet Surg J*. 2016;36(2):229–236. doi: 10.1093/asj/sjv135.
99. Brody LT, Thein JM. Nonoperative treatment for patellofemoral pain. *J Orthop Sports Phys Ther*. 1998;28(5):336–344. doi: 10.2519/jospt.1998.28.5.336
100. Pak J, Lee JH, Kartolo WA, Lee SH. Cartilage regeneration in human with adipose tissue- derived stem cells: current status in clinical implications. *Biomed Res Int*. 2016;2016:4702674. doi: 10.1155/2016/4702674
101. Englund M, Guermazi A, Gale D, Hunter DJ, Aliabadi P, Clancy M, Felson DT. Incidental meniscal findings on knee MRI in middle-aged and elderly persons. *N Engl J Med*. 2008;359(11):1108– 1115. doi: 10.1056/NEJMoa0800777
102. Pak J, Lee JH, Jeon JH, Lee SH. Complete resolution of a vascular necrosis of the human femoral head treated with adipose tissue-derived stem cells and platelet-rich plasma. *J Int Med Res*. 2014;42(6):1353–1362. doi: 10.1177/0300060514546940
103. Saxer F, Scherberich A, Todorov A, Studer P, Miot S, Schreiner S, Güven S, Tchang LA, Haug M, Heberer M, Schaefer DJ, Rikli D, Martin I, Jakob M. Implantation of stromal vascular fraction progenitors at bone fracture sites: from a rat model to a first-in-man study. *Stem Cells*. 2016;34(12):2956–2966. doi: 10.1002/stem.2478
104. Frazier TP, Gimble JM, Devay JW, Tucker HA, Chiu ES, Rowan BG. Body mass index affects proliferation and osteogenic differentiation of human subcutaneous adipose tissue- derived stem cells. *BMC Cell Biol*. 2013;14:34. doi: 10.1186/1471-2121-14-34
105. Jaewoo Pak, Jung Hun Lee, Kwang Seung Park, Moonhee Park, Lin-WooKang, and Sang Hee Lee, Current use of autologous adipose tissue-derived stromal vascular fraction cells for orthopedic applications *J Biomed Sci*. 2017; 24: 9.
106. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7(2):211–228. doi: 10.1089/107632701300062859.

107. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13(12):4279–4295. doi: 10.1091/mbc.E02-02-0105
108. Fandong Meng, Dongmei Zhou, Wei Li Adipose-derived stem cells as a potential weapon for diabetic foot ulcers *Int J Clin Exp Med* 2017;10(12):15967-15973
109. Konno M, Hamabe A, Hasegawa S, Ogawa H, Fukusumi T, Nishikawa S, Ohta K, Kano Y, Ozaki M, Noguchi Y, Sakai D, Kudoh T, Kawamoto K, Eguchi H, Satoh T, Tanemura M, Nagano H, Doki Y, Mori M and Ishii H. Adipose- derived mesenchymal stem cells and regenerative medicine. *Dev Growth Differ* 2013; 55: 309-318.
110. Nagata H, li M, Kohbayashi E, Hoshiga M, Hanafusa T and Asahi M. Cardiac adipose-de- rived stem cells exhibit high differentiation potential to cardiovascular cells in C57BL/6 mice. *Stem Cells Transl Med* 2016; 5: 141- 151.
111. Ribeiro CA, Fraga JS, Graos M, Neves NM, Reis RL, Gimble JM, Sousa N and Salgado AJ. The secretome of stem cells isolated from the adipose tissue and Wharton jelly acts differently on central nervous system derived cell populations. *Stem Cell Res Ther* 2012; 3: 18.
112. Kilroy GE, Foster SJ, Wu X, Ruiz J, Sherwood S, Heifetz A, Ludlow JW, Stricker DM, Potiny S, Green P, Halvorsen YD, Cheatham B, Storms RW and Gimble JM. Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro- inflammatory factors. *J Cell Physiol* 2007; 212: 702-709.
113. Nambu M, Kishimoto S, Nakamura S, Mizuno H, Yanagibayashi S, Yamamoto N, Azuma R, Nakamura S, Kiyosawa T, Ishihara M and Kanatani Y. Accelerated wound healing in healing impaired db/db mice by autologous adipose tissue-derived stromal cells combined with atelocollagen matrix. *Ann Plast Surg* 2009; 62: 317-321.
114. Bura A, Planat-Benard V, Bourin P, Silvestre JS, Gross F, Grolleau JL, Saint-Lebese B, Peyrafitte JA, Fleury S, Gadelorge M, Taurand M, Dupuis- Coronas S, Leobon B and Casteilla L. Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. *Cytotherapy* 2014; 16: 245- 257.
115. Jianming Guo, Alan Dardik, Kacey Fang, Ruixue Huang and Yongquan Gu, Meta-analysis on the treatment of diabetic foot ulcers with autologous stem cells *Stem Cell Research & Therapy* (2017) 8:228