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SPECIAL ARTICLE

Adipose derived stem cells *versus* micro-fragmented adipose tissue in cartilage tissue regeneration and repair

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ABSTRACT

In recent years, the aim of regenerative medicine strategies is to develop therapeutic conservative approaches for the treatment of musculoskeletal injuries. Among these, the use of adult mesenchymal stem cells (MSCs) has represented the main instrument for the treatment of cartilage lesions in osteoarthritis, considered as chronic degenerative diseases with little chance of therapy. MSCs have shown promising cartilage repair from *in-vitro*, *in-vivo*, and clinical studies thanks to their self-renewing potential and multilineage differentiation capability, better if included in a 3D autologous scaffold. Adipose tissue could be considered an optimal source of stem cells called adipose derived stem cells (ADSCs) being a tissue easily available in large amounts and obtained through minus invasive procedures than others, but it has not to be underestimated the limits before implantation: *in-vitro* step variability, cell expansion, destruction of the original tissue with the loss of cells natural niche and of their long-term efficacy. To overcome this issue, lipoaspirate clusters, also called micro-fragmented adipose tissue (MFAT), provide stem cells in an intact stromal vascular niche with a high regenerative capacity. This review provides a comparative view of stem cells and lipoaspirate in terms of their sources, structure, properties and regenerative capacity. Moreover, are reviewed the most representative scientific papers showing *in-vitro*, *in-vivo*, and clinical results comparing ADSCs *versus* MFAT in the treatment of degenerative and inflammatory cartilage disease.

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During the last decades the focus on a therapeutic conservative approach for the treatment of musculoskeletal injuries is increasing[1] and regenerative medicine based on the use of adult stem cells has represented the main instrument. Only recently, lipoaspirate (clusters of fat obtained with liposuction), also known as micronized fat when reduced in dimensions with commercial devices, has been getting more attention in this rapidly advancing and evolving field of research. This paper seeks to provide a comparative view between stem cell and lipoaspirate in terms of their sources, regenerative capacity, and clinical application in osteoarthritis.

Due to the limited regenerative capacity, cartilage lesions are considered as chronic degenerative diseases with little chance of therapy. Some of the traditional treatments for cartilage injured and for osteoarthritis prevention include the intraarticular administration of hyaluronan or of

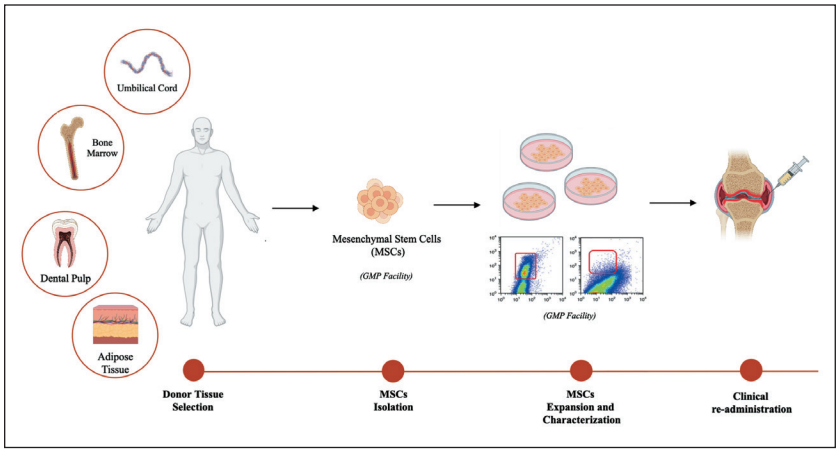

Figure 1.—Schematic representation of MSC isolation process. The process begins with the identification of donor tissue source and the isolation of MSCs from the selected one. MSCs are then subjected to an *in-vitro* expansion and characterization according to GMP safety and sterility criteria. The final step provides the re-administration of MSCs in the injured site of the donor as free cells therapy or as cells in a 3D scaffold.

PRP, bone marrow stimulation techniques, osteochondral grafting (mosaicplasty), autologous chondrocyte implantation (ACI), and matrix assisted autologous chondrocyte implantation (MACI).[2] In addition to the traditional treatments just mentioned, MSCs are showing promising clinical results for cartilage repair[3, 4] despite the difficulties related to the great number of processing steps (enzymatic digestion, cell expansion and differentiation) which increase the treatment variability. Indeed, in the studies published to date, cells were isolated from the originating tissue, expanded and differentiated *in vitro* before the transplantation as cell therapy into the damaged cartilage or before enriching a 3D scaffold as engineered tissue therapy.

In recent times, the whole adipose niche, has gained remarkable attention thanks to its heterogeneous cellular population reservoir, including progenitor cells, pericytes, endothelial cells, pre-adipocytes, monocytes, macrophages, as well as an extracellular matrix (ECM).[5] The biological rationale behind the use of this niche derives from the hypothesis that cross talk among the different cellular populations can promote multiple repair and remodeling processes. Applications of the adipose niche structurally unchanged are possible through the use of fat specific micro fragmenting devices obtaining micro-fragmented adipose tissue (MFAT). The use of adipose niche as a tool to support regenerative medicine approach is favored by the cytokine release which can initiate the functionality restoring tissue process. This approach is giving good results in pre-clinical and clinical studies for the treatment of inflammatory musculoskeletal diseases.[6]

Stem cells

The therapeutic application of stem cells in tissue regeneration has always exhibited a promising potential. Stem cells present specific characteristics such as self-renewal (unlimited proliferative capacity), clonogenicity (ability to generate cells identical to the mother cell) and potential (differentiation capability in different cell lines).[7] They can be isolated from a large assortment of tissues among which bone marrow, peripheral blood, muscle, dental pulp, umbilical cord and adipose tissue, after extraction and expansion in GMP facilities they can be stocked and banked for future implantation with regenerative purposes as provided in Figure 1.

Based on the origin and biological properties, they can be categorized into embryonic stem cells and adult stem cells, the latter with reduced proliferative and differentiating capacity compared to the first one.[7]

Embryonic stem cells (ESCs) are appealing because of their pluripotency and self-renewal capacities making them a suitable choice for cell-based therapies. However, due to ethical implications and government restrictions on their investigation, mesenchymal stem cells (MSCs) derived from adult patients have become an alternative option with encouraging prospective. Infect, MSCs are characterized with high genomic

stability, self-renewing and have the capability of multilineage differentiation into bone, cartilage, fat and dermis, among others.[8] Despite the MSCs therapeutic potential has been evaluated through over 800 clinical trials on humans, the biological mechanisms involved in their promoting process of tissue regeneration is still not fully understood. One theory suggests that MSCs differentiate into tissue-specific cells through the environmental biological stimuli originating from the injured tissue, however, there has not been enough evidence to support it *in vivo*. Recently, a theory that the therapeutic potential of MSCs is primarily attributed to their paracrine activity is confirmed by a lot of studies which evidenced that stem cells can secrete trophic factors, including insulin-like growth factor (IGF-1), nerve growth factor (NGF) and epithelial growth factor (EGF), that modulate the surrounding environment and influence the behavior of nearby parenchymal cells which repair the tissues.[9]

The best source of stem cells should be easily harvested, abundant, expendable, and contain a high yield of cells.[10] Among the numerous sources of MSCs in human body, bone-marrow-derived stem cells (BMSCs) have been the primary source for tissue engineering applications for many years[11] and recently also dental pulp is considered a valuable source for orthopedic and maxillofacial reconstructions, with capacity to produce mineralized tissue, and other connective tissue, such as dentine, dental pulp, and periodontal ligament.[12]

More recent studies have proven that subcutaneous adipose tissue gives a clear advantage over other stem cell sources due to easy collection of a large volume that is simple to isolate, through liposuction that is minimally invasive with low patient discomfort. Moreover, one gram of adipose tissue yields ~5×10^3 stem cells, which is 100-fold higher than the number of mesenchymal stem cells in one gram of bone marrow,[13] with a superior proliferating ability in culture. These adipose-tissue-derived stem cells (ADSCs) are usually isolated with enzymatic digestion from the stromal vascular fraction of adipose tissue that also includes capillaries and microvessels whose external wall is composed of pericytes also known for their stemness.[14]

It has been demonstrated that ADSCs are recruited to damaged sites where they take part in the repair of the injured tissue thanks to their differentiating potential into several cell types, including adipogenic, osteogenic, chondrogenic and myogenic cells. Therefore, the injection of ADSCs can be considered as a promising tool to accelerate injured tissue repair through tissue regeneration, enhance tissue engineering, but also useful as clinical treatment for inflammatory and autoimmune pathologies. ADSCs from adipose tissue are obtained by enzymatic digestion (using collagenases) and may undergo prolonged *ex-vivo* expansion before reimplantation, with remarkable senescence and a decrease in multipotency, resulting in unsatisfactory clinical results. These steps should take place in strictly controlled conditions, in clean rooms with technical equipment under the supervision of specialized personnel, limiting its use in the clinical practice. Moreover, after the extraction procedure the variability of ADSCs is about 82%, so better has to be done.[15]

Micro-fragmented adipose tissue (MFAT)

Micro-fragmented adipose tissue should not be confused with stromal vascular fraction (SVF) which is generated through enzymatic or mechanical breakdown of the lipoaspirate resulting in complete destruction of original tissue structure to facilitate potential MSC isolation and cultivation. MFAT is adipose tissue reduced in size, in all its healthy structure, with a cluster diameter from 0.4 mm to bigger ones with various labelled methods. Besides the Coleman traditional technique, that exploits centrifugal force to separate oil and blood cells from liposuction fat,[16] have recently been developed a variety of dispositive to obtain smaller micro-fragmented fat, which retain the stromal vascular niche, ready to be used for implantation, *i.e.* Lipogems® technology (FDA-approved devices)[17] (Figure 2).

The better regenerative potential associated with MFAT smaller cluster size has been proven. The cells contained into the smaller MFAT clusters show better response to differentiating stimuli compared to the larger size ones, maybe due to the higher surface area in touch with the culture medium containing factors themselves.[18]

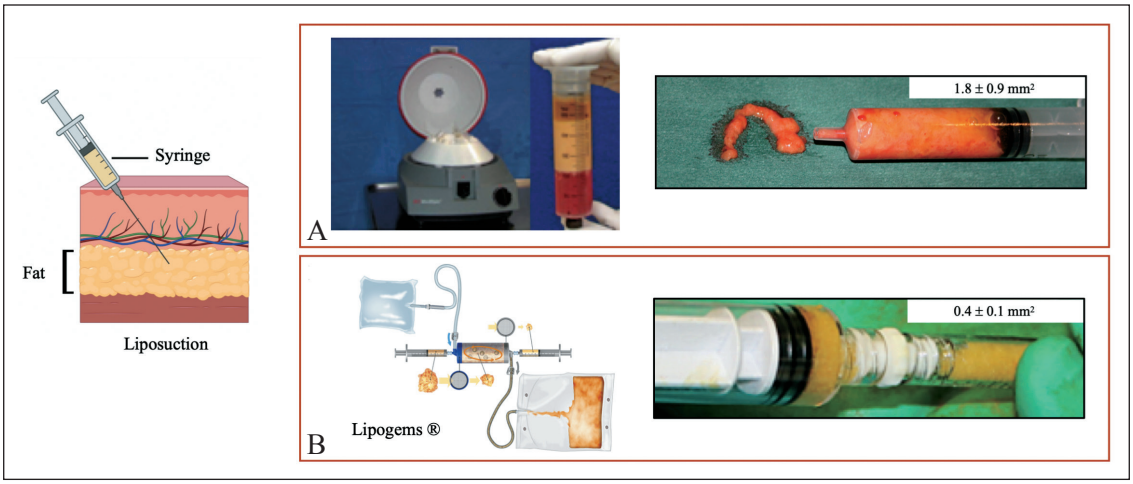

Figure 2.—Comparison between traditional technique and a recent dispositive used to obtain cleaned MFAT: A) Coleman traditional technique requires a centrifugation step process before to obtain lipoaspirate cleaned from oil residual and free of blood cells, with clusters dimension about 1.8 mm²; B) Lipogems® dispositive allow to micronize and wash lipoaspirate in a closed sterile dispositive obtaining micro-fragmented fat with a lower cluster dimension about 0.4 mm².

In Figure 3 are provided stereomicroscopic and microscopic representative images of cluster structure obtained by liposuction and then processed through Coleman traditional method (A-D) or Lipogems® (Figure 3E-H). The small dimension of clusters obtained through the use of Lipogems® device can be observed in Figure 3E compared to Coleman technique in Figure 3A; morphometric quantification has highlighted a clusters area reduction of 77% without any damage to the tissue integrity as seen in Trichrome stained section (Figure 3G), no vitality loss as proven by perilipin staining (Figure 3H) together with an oil residual reduction (Figure 3F), relevant to avoid inflammatory reaction in the host.

Lipoaspirate being minimally manipulated, could be considered a source of autologous multipotent undifferentiated cells, and behave as a natural scaffold for mesenchymal cells that are easily trapped in the stromal vascular fraction,[19] significantly reducing the number of steps involved in the MSCs translational process.

The therapeutic potential advantage of MFAT compared to enzymatically derived cells also

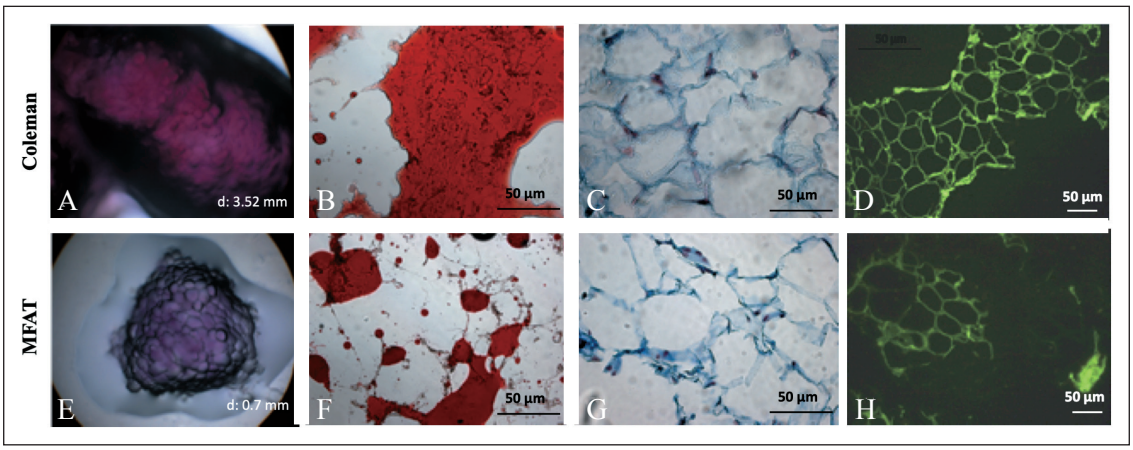

Figure 3.—Representative stereomicroscopic and microscopic images of MFAT compared to Coleman centrifuged liposuction clusters. Stereomicroscopic view of a representative cluster (A, E); microscopic images of paraffin embedded clusters (B-D and F-H) of sections stained with red oil (B, F), Masson's trichrome (C, G) and perilipin immunostaining (D, H).

seems to be related not only to stem cells but also to other residing cells: preadipocytes, dendritic cells, leucocytes, endothelial cells, fibroblasts, that could be co-players in the secretion of active paracrine factors. Other important cells to be considered are pericytes, present in the microvessels which remain intact after micro fragmentation process.[14] All these resident regenerative cells may perform their restoring action more indirectly, through the secretion of higher amounts of growth factors and cytokines. For this reason, MFAT could be considered as an alternative cell-based therapy able to release pro-healing growth factors and anti-inflammatory cytokines as well as healing-related peptides such as leptin and adiponectin. Another interesting study has also shown the ability of MFAT to secrete antibacterial factors, aspects that could be exploited in clinical practice. More interestingly, it has been demonstrated that if we simulate inflammation or infection, MFAT behaves like a vital organ, able to change the pattern of its secretion in response to the stimulus given.[20]

In addition to the advantages mentioned above, the use of lipoaspirate scaffold in place of ADSCs requires no processing steps prior to use, avoiding the hazard of infection or enzyme residual, and provides the reduction of time-consuming, costly, and laborious procedures that are required before implantation.

In-vitro and *in-vivo* results

Over the last ten years, hundreds of studies have provided fundamental information to the research and clinic community about the MSCs differentiation process to chondrocytes. Among them, several theories sustain that various growth factors and specific gene transcription factors control it through temporal and spatial activation or inhibition of cell signaling pathways, with the final objective to try to address chondrogenesis for cartilage repair and regeneration.

Several *in-vitro* studies have shown ADSCs paracrine effects through the production of cytokines and soluble factors that regulate immune cells functions, enhance microenvironment for tissue healing and carry out high strong immunosuppressive effects by reducing inflammatory cytokine production. It has been proven that also human adipose tissue, in the form of MFAT, is able to produce several factors involved in immunomodulatory processes, such as specific cytokines.[6] Important players are pericytes, contained in the lipoaspirate, that at the beginning detach and then turn into stem cells releasing a large number of cytokines that counteract the inflammation process, especially induced by macrophages.[14] Only later, more specialized cytokines with antibiotic, antifibrotic, angiogenic, and analgesic effects are secreted. Therefore, the implantation of lipoaspirate in the injured site might protect the tissue homeostatic mechanism through the immunomodulatory activity and the recruitment of heterogeneous cell population involved in the reparative/regenerative pathways.[20]

Based on *in-vitro* results, the intra-articular injection of lipoaspirate into the site of damage, may give rise to a fibrous tissue that provides mechanical support for the load on the damaged cartilage, induces resident chondrocytes to proliferate and produces ECM, and finally releases cells which could regenerate or repair the cartilage damaged. In this regard, in 2016 we have demonstrated the promotion of cartilage defect repair through micro-fragmented lipoaspirate clusters.[19] Our *in-vitro* studies have shown that lipoaspirate clusters can give rise to the spontaneous outgrowth of cells as shown in Figure 4. These cells, characterized by mesenchymal phe-

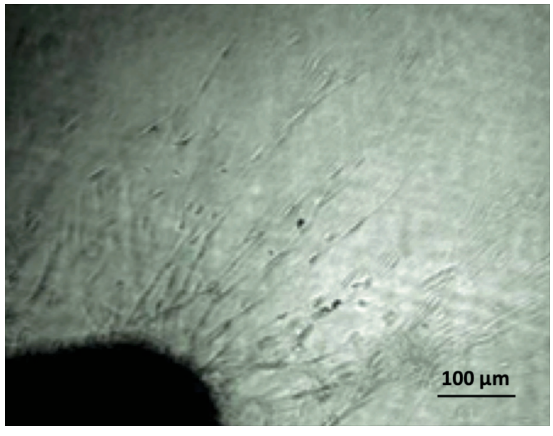

Figure 4.—Stem cells outgrowth from micro-fragmented lipoaspirate clusters. Representative phase contrast microscopy image, 10x magnification, shows a cluster of MFAT (in black) from which cell outgrowth can be seen as rays of sunlight.

notype, can induce primary chondrocytes proliferation and ECM production through their paracrine activities. Hence, due to its composition and properties, lipoaspirate shows a potential to be used as a natural scaffold and an alternative cell-based approach in addressing the treatment of cartilage defects.

To further confirm the beneficial potential effect of lipoaspirate, *in-vivo* studies, using intra-articular injection of ADSCs or MFAT in animal models, showed a good outcome on cartilage repair process that could be considered as a validation of *in-vitro* results for inflammatory musculoskeletal disorders treatments. Indeed, the object of Desando *et al.* study was to compare MFAT and ADSCs efficacy for the treatment of OA in rabbits. MFAT has demonstrated similar cartilage repair at one month compared to ADSCs, however, the first has shown a prolonged secretory activity and a higher CD-163 wound-healing macrophages positivity, which are considered an important cartilage repair mediator.[21]

It is important to remember how MFAT also finds other *in-vivo* applications such as prevention of radiation-induced atrophy, fibrosis and impaired wound healing, suggesting a remarkable new clinical practice during anticancer radiotherapies.[22] Other authors have suggested MFAT as 'Trojan horses' to improve the delivery of drugs and oncolytic viruses to treat intractable tumors or to overexpress neurotrophic factors, anti-inflammatory cytokines or angiogenic factors to facilitate the healing and recovery of tissues damaged by injury or disease.

Clinical results

Both stem cells and lipoaspirate have enriched the field of tissue regeneration and numerous studies have contributed to the current state of the art. Before talking about musculoskeletal treatments, some important information can be extrapolated from other anatomical sites where they find clinical applications. ADSCs are used in skin diseases like keloids, for the treatment of burn,[23] chronic wounds[24] and for enhancing facial rejuvenation[25] through cell proliferation, angiogenesis promotion, antioxidant factors secretion or photoaging inhibition.[26] In ophthalmic field, these cells have proven to increase tear volume and corneal regeneration[27] and several clinical trials are ongoing for the treatment of myocardial infarction,[28] atherosclerosis, coronary diseases,[24] autoimmune diseases like systemic sclerosis and rheumatoid arthritis[29] and anal fistulas.[30]

On the other hand, the transplantation of MFAT has been employed in different medical fields both in human and veterinary medicine. Thanks to several post-application monitoring years, safety and efficacy of its use in patients treated in gynecological, aesthetic and orthopedic contexts have been demonstrated.[31] The use of MFAT in the plastic surgery field is widespread, in promoting healing of chronic leg and foot ulcers in diabetic patients, likewise in cosmetic ones, where it can be used individually or combined with other traditional surgical methods. Just recently, based on a pilot study MFAT has been shown to improve continence in patients with unsatisfactory results after sphincteroplasty[32] and in few pre-clinical studies, it has been pointed out how MFAT could be exploited as a drug-carrier for in loco treatment of solid tumors.[33]

A consistent number of clinical trials about inflammatory pathologies, such as osteoarthritis, are ongoing[34] to assess the immunomodulatory activities mediated by paracrine effects of ADSCs. Clinical results from more than 800 patients worldwide have proven that the intra-articular injection of MFAT-Lipogems® to treat degenerative and inflammatory musculoskeletal diseases resulted in a resolution of symptoms, knee function increase and pain reduction, with 100% safety of the procedure. Some patients who were candidates for prosthesis implantations, after intra-articular injection of lipoaspirate no longer needed it because of the complete or substantial resolution of their symptoms.[35] Moreover, intra-articular hip injections of Lipogems® are capable of relieving partially postoperative pain related to hip arthroscopy in the first 7 days after surgery.[36] The MFAT-Lipogems® benefits in musculoskeletal disease treatments have been widely demonstrated also in Veterinary Medicine. Indeed, several studies have shown its beneficial in the promotion of healing of damaged or degenerated ligaments, tendons and meniscal some species of animals.[37]

To date, studies assessment would provide proof that MFAT beneficial seems to be more advantageous compared to the only stem cells, not only due to the reduced time, cost and resources needed for its procurement and use.[38]

Conclusions

A critical point of all ADSCs or MFAT studies is related to their efficacy and clinical outcomes, that may depend on donor characteristics, processing techniques, site-specific requirements, as all factors that could alter the lipoaspirate properties and the yield and multilineage potential of processed cells. Lately Horinouchi *et al.*[39] have demonstrated that the donor age does not significantly affect the cell division or differentiation ability. This result was also confirmed by Schmitz *et al.*[40] who have demonstrated that key characteristics of uncultured ADSCs are independent of the subject's age, sex, body mass index (BMI) and ethnicity.

The use of either stem cells or Lipoaspirate is dependent on the kind of treatment and circumstances. The research interest could be also focused on the combination of the two regimes, utilizing Lipoaspirate as natural scaffold enriched with a high dose of stem cells for improving the treatments efficacy.

Ongoing research into the ADSCs and MFAT potential applications continues to obtain surprising results and grow in application fields that are currently little explored. However, methodology optimization and additional clinical studies are needed for a better comprehension in terms of efficacy and safety.

References

1. Notarnicola A, Covelli I, Moretti B. Musculoskeletal tissue injuries and tissue regeneration. Minerva Orthop 2022;73:1–3.

2. Talesa G, Manfreda F, Pace V, Ceccarini P, Antinolfi P, Rinonapoli G, *et al.* The treatment of knee cartilage lesions: state of the art. Acta Biomed 2022;93:e2022099.

3. Shenaq DS, Rastegar F, Petkovic D, Zhang BQ, He BC, Chen L, *et al.* Mesenchymal Progenitor Cells and Their Orthopedic Applications: Forging a Path towards Clinical Trials. Stem Cells Int 2010;2010:519028.

4. Cannas M, Sabbatini M. Osteoarthritis as age-related disease: from degenerative and inflammatory aspects to MSC therapy. Minerva Orthop 2022;73:493–5.

5. Lee YH, Petkova AP, Granneman JG. Identification of an adipogenic niche for adipose tissue remodeling and restoration. Cell Metab 2013;18:355–67.

6. Ceccarelli S, Pontecorvi P, Anastasiadou E, Napoli C, Marchese C. Immunomodulatory Effect of Adipose-Derived Stem Cells: The Cutting Edge of Clinical Application. Front Cell Dev Biol 2020;8:236.

7. Poliwoda S, Noor N, Downs E, Schaaf A, Cantwell A, Ganti L, *et al.* Stem cells: a comprehensive review of origins and emerging clinical roles in medical practice. Orthop Rev (Pavia) 2022;14:37498.

8. Ayala-Cuellar AP, Kang JH, Jeung EB, Choi KC. Roles of Mesenchymal Stem Cells in Tissue Regeneration and Immunomodulation. Biomol Ther (Seoul) 2019;27:25–33.

9. Fu Y, Karbaat L, Wu L, Leijten J, Both SK, Karperien M. Trophic Effects of Mesenchymal Stem Cells in Tissue Regeneration. Tissue Eng Part B Rev 2017;23:515–28.

10. De Ugarte DA, Ashjian PH, Elbarbary A, Hedrick MH. Future of fat as raw material for tissue regeneration. Ann Plast Surg 2003;50:215–9.

11. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al.* Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.

12. Ledesma-Martínez E, Mendoza-Núñez VM, Santiago-Osorio E. Mesenchymal Stem Cells Derived from Dental Pulp: A Review. Stem Cells Int 2016;2016:4709572.

13. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, *et al.* Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279–95.

14. Vezzani B, Shaw I, Lesme H, Yong L, Khan N, Tremolada C, *et al.* Higher Pericyte Content and Secretory Activity of Microfragmented Human Adipose Tissue Compared to Enzymatically Derived Stromal Vascular Fraction. Stem Cells Transl Med 2018;7:876–86.

15. Oedayrajsingh-Varma MJ, van Ham SM, Knippenberg M, Helder MN, Klein-Nulend J, Schouten TE, *et al.* Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. Cytotherapy 2006;8:166–77.

16. Coleman SR. Structural fat grafting: more than a permanent filler. Plast Reconstr Surg 2006;118(Suppl):108S–20S.

17. Tremolada C, Colombo V, Ventura C. Adipose Tissue and Mesenchymal Stem Cells: State of the Art and Lipogems® Technology Development. Curr Stem Cell Rep 2016;2:304–12.

18. Sawai S, Sowa Y, Kishida T, Inokawa H, Inafuku N, Tsuchida S, *et al.* Micronized Cellular Adipose Matrix Promotes the Therapeutic Effect of an Artificial Nerve Conduit in Peripheral Nerve Gap Injury. Plast Reconstr Surg 2023;151:959e–69e.

19. Bosetti M, Borrone A, Follenzi A, Messaggio F, Tremolada C, Cannas M. Human Lipoaspirate as Autologous Injectable Active Scaffold for One-Step Repair of Cartilage Defects. Cell Transplant 2016;25:1043–56.

20. Ceresa C, Borrone A, Fracchia L, Rinaldi M, Marchetti A, Tremolada C, *et al.* Lipoaspirate Shows In Vitro Potential for Wound Healing. Pharmaceutics 2022;14:447.

21. Desando G, Bartolotti I, Martini L, Giavaresi G, Nicoli Aldini N, Fini M, *et al.* Regenerative Features of Adipose Tissue for Osteoarthritis Treatment in a Rabbit Model: Enzymatic Digestion Versus Mechanical Disruption. Int J Mol Sci 2019;20:2636.

22. Sowa Y, Inafuku N, Kishida T, Mori M, Mazda O, Yoshimura K. Prophylactic Application of Human Adipose Tissue-Derived Products to Prevent Radiation Disorders. Plast Reconstr Surg 2023;151:1207–16.

23. Zahorec P, Sarkozyova N, Ferancikova N, Bukovcan P, Danisovic L, Bohac M, *et al.* Autologous mesenchymal stem cells application in post-burn scars treatment: a preliminary study. Cell Tissue Bank 2021;22:39–46.

24. Czerwiec K, Zawrzykraj M, Deptuła M, Skoniecka A, Tymińska A, Zieliński J, *et al.* Adipose-Derived Mesenchymal Stromal Cells in Basic Research and Clinical Applications. Int J Mol Sci 2023;24:3888.

25. Surowiecka A, Strużyna J. Adipose-Derived Stem Cells for Facial Rejuvenation. J Pers Med 2022;12:117.

26. He A, Zheng S, Luan W, Wang L, Qian L, Qi F, *et al.* Antiphotoaging Effect of Micronized Fat in Ultraviolet B-Induced Human Dermal Fibroblasts. Plast Reconstr Surg 2023;152:1023–33.

27. Musa M, Zeppieri M, Enaholo ES, Salati C, Parodi PC. Adipose Stem Cells in Modern-Day Ophthalmology. Clin Pract 2023;13:230–45.

28. Qayyum AA, Mathiasen AB, Mygind ND, Kühl JT, Jørgensen E, Helqvist S, *et al.* Adipose-Derived Stromal Cells for Treatment of Patients with Chronic Ischemic Heart Disease (MyStromalCell Trial): A Randomized Placebo-Controlled Study. Stem Cells Int 2017;2017:5237063.

29. Ueyama H, Okano T, Orita K, Mamoto K, Ii M, Sobajima S, *et al.* Local transplantation of adipose-derived stem cells has a significant therapeutic effect in a mouse model of rheumatoid arthritis. Sci Rep 2020;10:3076.

30. Cho YB, Park KJ, Yoon SN, Song KH, Kim DS, Jung SH, *et al.* Long-term results of adipose-derived stem cell therapy for the treatment of Crohn's fistula. Stem Cells Transl Med 2015;4:532–7.

31. Moccia F, Pentangelo P, Ceccaroni A, Raffone A, Losco L, Alfano C. Injection Treatments for Vulvovaginal Atrophy of Menopause: A Systematic Review. Aesthetic Plast Surg 2023. [Epub ahead of print]

32. Barisic G, Andjelkov K, Rosic J, Miladinov M, Koturstevuljevic J, Dinic T, *et al.* Application of nanofat for treatment of traumatic faecal incontinence after sphincteroplasty - A pilot study. Colorectal Dis 2022;24:1054–62.

33. Salagean A, Nechifor-Boila A, Bajwa N, Pastorello Y, Slevin M. Micro-Fragmented Adipose Tissue as a Natural Scaffold for Targeted Drug Delivery in Brain Cancer. Int J Mol Sci 2023;24:11530.

34. Şovrea AS, Boşca AB, Constantin AM, Dronca E, Ilea A. State of the art in human adipose stem cells and their role in therapy. Rom J Morphol Embryol 2019;60:7–31.

35. Shimozono Y, Fortier LA, Brown D, Kennedy JG. Adipose-Based Therapies for Knee Pain-Fat or Fiction. J Knee Surg 2019;32:55–64.

36. Aprato A, Ferrara A, Bistolfi A, Ferracini R, Artiaco S, Massè A. Intraoperative injection of microfragmented adipose tissue during hip arthroscopy as immediate postoperative pain reliever: a retrospective study. Minerva Orthop 2022;73:458–63.

37. Zeira O, Scaccia S, Pettinari L, Ghezzi E, Asiag N, Martinelli L, *et al.* Intra-Articular Administration of Autologous Micro-Fragmented Adipose Tissue in Dogs with Spontaneous Osteoarthritis: Safety, Feasibility, and Clinical Outcomes. Stem Cells Transl Med 2018;7:819–28.

38. Priano V, Priano F. Autologous adipose tissue enriched in stromal vascular fraction in knee osteoarthritis. Minerva Orthop 2022;73:416–25.

39. Horinouchi CD, Barisón MJ, Robert AW, Kuligovski C, Aguiar AM, Dallagiovanna B. Influence of donor age on the differentiation and division capacity of human adipose-derived stem cells. World J Stem Cells 2020;12:1640–51.

40. Schmitz C, Alt C, Azares AR, Pearce DA, Facile TR, Furia JP, *et al.* The Composition of Adipose-Derived Regenerative Cells Isolated from Lipoaspirate Using a Point of Care System Does Not Depend on the Subject's Individual Age, Sex, Body Mass Index and Ethnicity. Cells 2022;12:30.

Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions
All authors read and approved the final version of the manuscript.

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