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Editor's Invited Discussion

Invited Discussion on: "Nanofat Cell-Mediated Anti-Aging Therapy: Evidence-Based Analysis of Efficacy and an Update of Stem Cell Facelift" by Bishara Atiyeh, Fadi Ghieh, Ahmad Oneisi

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Since its introduction in 2013, the Nanofat method [1] has become the preferred method for the mechanical isolation of the stromal vascular fraction (SVF), as demonstrated by the steep increase in publications describing its clinical use [2]. Specifically, an increasing number of reports have described the use of mechanically isolated SVF such as Nanofat, in facial rejuvenation, necessitating an in-depth analysis comparing not only the reported clinical outcomes, but also a critical review of the applied methods. The initiative of Atiyeh et al. [3] is ,therefore, to be applauded. In their systematic review on the use of mechanically isolated SVF as an anti-aging therapeutic modality for treating facial aging, the authors included seven studies and concluded that '*more robust evidence with well-defined primary outcome end points and objective outcome measures are required'*. This conclusion supports previously postulated conclusions, denoting the difficulty in comparing previously published evidence [2]. This is not only due to the discrepancies between practices, the varying levels of evidence and the different methods for assessing clinical outcomes, but also points to a lack of systematic reporting on the used methodologies, and similar to many COVID trials [4], insufficient experimental design.

With the continuous outpour of evidence, systematic reviews increasingly offer clinicians a way to stay abreast of current evidence-based medicine. To this end, the Cochrane collaboration (https://www.cochrane.org/), prospective registries such as PROSPERO (https://www.crd.york.ac.uk/prospero/), and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [5, 6] offer invaluable tools to facilitate a correct and transparent design, execution and reporting of systematic reviews. Indeed, reviewing research evidence requires an equal, if not higher, level of rigor as was used in producing the research evidence in the first place. This immediately brings us to the Achilles heel of any systematic review (and meta-analysis), namely its design. Indeed, before one can start including relevant articles, extract and analyze the data, draw conclusions and report these, ample attention must be spent on designing a meticulous protocol, including a comprehensive search strategy and appreciation of the complexity of the subject one wants to analyze. With the currently available databases and search engines, one no longer needs to manually plow through the mazes of the global scientific literature, but can simply, by providing razor-sharp instructions, instruct the 'robot' to pool all relevant articles. This also means that, if you give the robot a wrong or incomplete command, the poor thing is inevitably going to underperform. This is also the case in the analysis performed by Atiyeh et al. [3], where the authors designed the following search strategy: *(nanofat OR nano fat OR nano-fat OR stem cell) AND (lipofill* OR lipotransfer OR fat graft* OR fat transplant*) AND (rejuvenat* OR cosmetic OR esthetic OR skin quality OR anti-aging)*. In our opinion, this search strategy contains a number of flaws. First, in addition to nanofat and variations, there is a multitude of other terms ascribed to methods for isolating the adipose SVF [2, 7, 8]. By not including sufficient synonyms and variations in their search strategy, the authors consciously excluded many irrelevant articles, but unconsciously missed relevant ones. Second, the fat grafting procedure seems irrelevant in respect to the scope of this review. Therefore, its inclusion in the search strategy should have been obviated. Third, by combining the previously mentioned terms using the Boolean operator AND, the authors potentially excluded relevant articles describing the clinical use of mechanically isolated SVF, but not describing 'fat grafting', 'anti-aging', and similar other terms. A potential explanation is that the initial search strategy only included '*nanofat OR nano fat OR nano-fat OR stem cell'* as *free text*-terms; without a suffix indicating how the term should be sought in the database, the engine searches indiscriminately for the terms, regardless of their relevance and positioning throughout the article. As a result, the authors were faced with an insurmountable number of results, most likely due to the inclusion of 'stem cell' as a search term. To overcome this, the authors possibly opted for the combination of several word blocks with the AND operator, reducing the results to a more manageable number, but simultaneously excluding relevant matches. The search strategy would have benefited greatly by adding the suffix [TIAB]—TItle and ABstract—which instructs the engine to search for words that are only used in the title and/or abstract of articles, thereby allowing to search for relevant articles describing the provided terms, rather than pooling articles were the terms are only marginally discussed. Indeed, by implementing available tools such as the [TIAB] suffix, researchers could more efficiently conduct the literature searches, and subsequently, systematic reviews [9].

Another shortcoming in their design lies in the use of a single database. PubMed is without a doubt the most widely used database. Experienced researchers nevertheless further expand their search via Embase, Web of Science, Cochrane Central, and/or Google Scholar—although google scholar's search algorithm is still controversial—in which publications are indexed slightly differently. Also, the geographical coverage differs between search engines, with Embase more comprehensively covering Europe, and indexing with less delay. Finally, for reviews considering the inclusion of conference proceedings, the Embase and Web of Science databases are essential. Although using these different search engines seems arduous, they are complementary due to the aforementioned differences, and embracing them will most likely result in a more complete search with a substantially lower chance of missing relevant articles [10]. While one will certainly face a large number of duplicates—i.e., articles that are indexed in multiple databases and are retrieved by the designed search strategy—current reference management software and platforms are capable of automatically excluding duplicates. Thereafter, a manual control is strongly advised—which is significantly facilitated after automatic deduplication. In our previous work [2], we retrieved 7885 results using Embase, PubMed, Web of Science, and Cochrane

Central Register of Controlled Trials databases. Following automatic deduplication 4613 remained, which was reduced to 4,505 after manual deduplication.

We further question the motivation for excluding articles before 2013. The authors clearly indicated that they wanted to evaluate the progress of evidence since the introduction of the landmark paper on Nanofat [1], which coincided with the authors first review of the literature titled 'Stem Cell Facelift: Between Reality and Fiction' [11]. However, Condé-Green and colleagues had already shown in 2010 that centrifugation resulted in a pellet enriched in SVF cells [12]; centrifugation could therefore be considered as a mechanical SVF isolation method. Regardless, systematic reviews, and more specifically, meta-analyses, benefit from including large patient populations, thereby allowing for more rigorous comparisons and conclusions. Furthermore, the higher total number of included patients facilitates subgroup analysis when sufficient objects are available —e.g., compare treatment outcomes before and after the introduction of a novel surgical technique. Therefore, excluding papers published before a specific date should be obviated, as this often introduces unnecessary bias.

To their credit, the authors managed to include most relevant reports. Surprisingly, however, two other comparative clinical studies, to which the authors refer in their introduction and discussion, were not incorporated in their overview [13, 14], while another two were not discussed at all [15, 16]. It would have been understandable if the preliminary report by Cohen et al. [16] was not included due to the topical nature of the treatment —topical adipose-derived stem cells (ADSC) therapy is defined as an exclusion criterium. However, Cohen and colleagues mechanically isolated the SVF and not ADSCs (see infra), and their work should have therefore been included. Regarding the other three articles [13, 14, 15], we could not justify their exclusion based on the reported inclusion or exclusion criteria. In fact, some were surprisingly similar to other papers that were in fact included in the systematic review. On the other hand, it is not clear why one article reporting on enzymatically isolated SVF cells was not excluded [17]. It is important for the reader, who is not familiar with all the modalities of regenerative surgical techniques, that a rigorous distinction is maintained between the isolated use of ADSCs and the SVF, with the latter being a collection of numerous cell types including resident and circulating immune cells such as macrophages, endothelial lineage cells and fibroblasts, pre-adipocytes, and ADSCs, but not adipocytes. ADSCs can never be isolated through simple mechanical processing, and always require additional experimental steps for their selection. Therefore, authors using mechanical SVF isolation methods should refrain from claiming to isolate ADSCs; enrichment/concentration is not the same as isolating. SVF on the other hand can be obtained through simple mechanical processing. Unless justified by clearly described methodological processes, authors are encouraged to refrain from using ADSC and SVF interchangeably.

Regarding the discussion offered by the authors, we would like to address three things.

First, the statement that '*nanofat is not fat at all and does not meet the true dimensions of nano sizes*' has already frequently been made. This remark is irrelevant as very often a name does not cover entirely the subject. For instance, a split thickness skin graft does not contain the entire dermis, nor any dermis-associated fat cells, but is considered skin. Nanofat consists of SVF, which contains up to 90% of the cellular content of adipose tissue, as such, the term 'fat' seems appropriate. Also, the 'true dimensions' argument is a non-issue. Indeed, Nanofat is not 1,000,000,000 times smaller than a normal fat particle, but it is surely smaller than microfat, a term used to describe milli- to micro-meter-sized fat particles used for autologous fat grafting. Whether controversial or not, the term 'Nanofat' is recognizable, and has attracted additional attention toward the regenerative potential of adipose tissue, both clinically and experimentally.

Second, the main conclusion in the current review deserves to be underlined. Indeed, the call for standardization supports previously formulated need for standardization, and is, therefore, well received. As highlighted by the authors, there is a considerable discrepancy between nearly all methods of mechanical SVF isolation, which impedes a proper comparison of the outcomes. Similar to fat grafting [18], all steps from the use of different harvesting cannulas, harvesting techniques (syringe or vacuum), processing (decanting, rinsing, filtering, centrifugating,…), and therapeutic application have their influence on the end product and its potency. In this regard, our team has initiated an in-depth analysis of various markers and will introduce minimal information guidelines in near future. However, standardization is also required when reporting clinical outcomes. Indeed, Atiyeh et al. [3] correctly referred to 'the diversity of reported metrics and outcomes' hampering comparison. As such, standardized reporting criteria for the evaluation of regenerative surgical procedures, such as nanofat facial therapy, are urgently needed.

Finally, we agree with the remark that 'SVF dose effect on outcome is presently poorly understood'. While the clinical effects of Nanofat are evident [2, 19], further research is indeed warranted. In this regard, it deserves to draw attention to recent work by Sesé and colleagues, who hypothesized that *'the therapeutic dose required to treat any damaged tissue must be at least near the constitutive cell burden present in the injury site'* [20]. Defining this dose as the *'constitutive cell dose'*, they showed that Nanofat contains 70% of its native cell mass, and as such, offers a *near* constitutive cell dose to induce local regeneration. Based on this hypothesis, future studies can perform dose-response studies, evaluating regenerative alterations through clinical and histological parameters among others.

In summary, the current systematic review offers a valuable contribution by providing an overview of the literature regarding the mechanical methods for SVF isolation [3]. The authors have highlighted a number of considerable shortcomings of the currently existing studies, mainly emphasizing the need for standardized reporting, an essential evolution in our discipline and beyond.

Publisher's Note

Declarations

Conflict of interest The authors declare that they do not have any conflicts of interest.

Human and Animal Rights This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent For this type of study informed consent is not required.

References

1. Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, Declercq H (2013) Nanofat grafting: Basic research and clinical applications. Plast Reconstr Surg 132(4):1017–1026. https://doi.org/10.1097/PRS.0b013e31829fe1b0

2. Ghiasloo M, Lobato RC, Díaz JM, Singh K, Verpaele A, Tonnard P (2020) Expanding clinical indications of mechanically isolated stromal vascular fraction: A systematic review. Aesthet Surg J 40(9):NP546–NP560. https://doi.org/10.1093/asj/sjaa111

3. Atiyeh B, Ghieh F, Oneisi A (2021) Nanofat Cell-Mediated Anti-Aging Therapy: Evidence-Based Analysis of Efficacy and an Update of Stem Cell Facelift. Aesthet Plast Surg. https://doi.org/10.1007/s00266-021-02353-w

4. Pearson H (2021) How COVID broke the evidence pipeline. Nature 593(7858):182–185. https://doi.org/10.1038/d41586-021-01246-x

5. Liberati A, Altman DG, Tetzlaff J et al (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. https://doi.org/10.1136/bmj.b2700

6. Page MJ, McKenzie JE, Bossuyt PM, The PRISMA et al (2020) statement: An updated guideline for reporting systematic reviews. BMJ 2021:372. https://doi.org/10.1136/bmj.n71

7. Trivisonno A, Alexander RW, Baldari S et al (2019) Intraoperative Strategies for Minimal Manipulation of Autologous Adipose Tissue for Cell- and Tissue-Based Therapies: Concise Review. Stem Cells Transl Med 8(12):1265–1271. https://doi.org/10.1002/sctm.19-0166

8. van Dongen JA, Tuin AJ, Spiekman M, Jansma J, van der Lei B, Harmsen MC (2018) Comparison of intraoperative procedures for isolation of clinical grade stromal vascular fraction for regenerative purposes: a systematic review. J Tissue Eng Regen Med 12(1):e261–e274. https://doi.org/10.1002/term.2407

9. Bramer WM, de Jonge GB, Rethlefsen ML, Mast F, Kleijnen J (2018) A systematic approach to searching: An efficient and complete method to develop literature searches. J Med Libr Assoc 106(4):531–541. https://doi.org/10.5195/jmla.2018.283

10. Bramer WM, Rethlefsen ML, Kleijnen J, Franco OH (2017) Optimal database combinations for literature searches in systematic reviews: A prospective exploratory study. Syst Rev 6(1):1–12. https://doi.org/10.1186/s13643-017-0644-y

11. Atiyeh BS, Ibrahim AE, Saad DA (2013) Stem cell facelift: Between reality and fiction. Aesthet Surg J 33(3):334–338. https://doi.org/10.1177/1090820X13478944

12. Condé-Green A, Baptista SL, De Amorin GNF et al (2010) Effects of centrifugation on cell composition and viability of aspirated adipose tissue processed for transplantation. Aesthet Surg J 30(2):249–255. https://doi.org/10.1177/1090820X10369512

13. Wei H, Gu SX, Liang YD et al (2017) Nanofat-derived stem cells with platelet-rich fibrin improve facial contour remodeling and skin rejuvenation after autologous structural fat transplantation. Oncotarget 8(40):68542–68556. https://doi.org/10.18632/oncotarget.19721

14. Rigotti G, Charles-De-Sá L, Gontijo-De-Amorim NF et al (2016) Expanded stem cells, Stromal-vascular fraction, and platelet-rich plasma enriched fat: Comparing results of different facial rejuvenation approaches in a clinical trial. Aesthet Surg J 36(3):261–270. https://doi.org/10.1093/asj/sjv231

15. Gontijo-De-Amorim NF, Charles-De-Sá L, Rigotti G (2017) Mechanical Supplementation with the Stromal Vascular Fraction Yields Improved Volume Retention in Facial Lipotransfer: A 1-Year Comparative Study. Aesthet Surg J 37(9):975–985. https://doi.org/10.1093/asj/sjx115

16. Cohen SR, Goodacre AK, Womack H et al (2020) Topical nanofat biocrème improves aesthetic outcomes of nonablative fractionated laser treatment: A preliminary report. Aesthet Surg J 40(8):892–899. https://doi.org/10.1093/asj/sjz240

17. Mailey B, Saba S, Baker J et al (2013) A comparison of cell-enriched fat transfer to conventional fat grafting after aesthetic procedures using a patient satisfaction survey. Ann Plast Surg 70(4):410–415. https://doi.org/10.1097/SAP.0b013e31827e5353

18. Zielins ER, Brett EA, Longaker MT, Wan DC (2016) Autologous Fat Grafting: The Science behind the Surgery. Aesthet Surg J 36(4):488–496. https://doi.org/10.1093/asj/sjw004

19. Menkes S, SidAhmed-Mezi M, Meningaud JP, Benadiba L, Magalon G, Hersant B (2021) Microfat and Nanofat Grafting in Genital Rejuvenation. Aesthet Surg J 41(9):1060–1067. https://doi.org/10.1093/asj/sjaa118

20. Sesé B, Sanmartín JM, Ortega B, Matas-Palau A, Llull R (2019) Nanofat Cell Aggregates: A Nearly Constitutive Stromal Cell Inoculum for Regenerative Site-Specific Therapies. Plast Reconstr Surg 144(5):1079–1088. https://doi.org/10.1097/PRS.0000000000006155