

Discussion: Nanofat Cell Aggregates: A Nearly Constitutive Stromal Cell Inoculum for Regenerative Site-Specific Therapies

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The importance of this article cannot be overestimated.¹ Regenerative capacities of adipose tissue have now become well recognized, and each week several articles are being published on the use of fat and/or its derivatives for reconstructive and regenerative purposes.

The most important component of adipose tissue with regard to regenerative capacities is the stromal vascular fraction, which mainly consists of pericytes/endothelial cells, adventitial cells, mesenchymal stem cells, preadipocytes, fibroblasts, macrophages (types I and II), vascular smooth muscle cells, T-lymphocytes, and miscellaneous native blood-derived cells.² Until now, the gold standard for isolating this stromal vascular fraction from the mature adipocytes has been collagenase enzymatic separation, as this delivers the “purest” form of stromal vascular fraction. Although mechanical processing methods for stromal vascular fraction have been around for several years,³⁻⁵ most of these mechanical methods seem to produce a lower cell yield than enzymatic methods.^{4,6} Apart from this apparent superiority, the collagenase isolation method suffers from very restrictive regulations in most countries, preventing its widespread clinical use.

The authors draw attention to the nanofat method as a mechanical dissociation method that has yielded undeniable clinical results not only by the authors of the original nanofat article of 2013, but also by several other authors.^{1,5,7-9} Intrigued by this fact, they resolve to explain the mechanism behind these clinical outcomes. For their research, the authors have reproduced the original nanofat processing method, with the exception of the procedure for washing the initial fat aspirate, for which they are using the “fat press.” However, it deserves mentioning that this very practical device by Tulip Medical Products (San Diego, Calif.) is not commercially available yet.

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It appears that the advantages of mechanical dissociation are both qualitative and quantitative. Adipose tissue that has been mechanically dissociated delivers stromal vascular fraction consisting of cell aggregates, which stands in contrast to enzymatically dissociated stromal vascular fraction, which does not have the extracellular matrix component and which is dissociated from its vasculature.¹⁰ The extracellular matrix component is now recognized as of great importance to the actual secretory bioactivity of the tissue,² which may be an essential contributor to the clinically observed activity of nanofat. It may even be that, in nanofat, the cells are not the most essential component; rather, this extracellular environment and the intercellular signaling proteins and exosomes are most important.^{10,11} This may partly explain the highly qualitative clinical results displayed in nanofat articles.^{5,7-9}

In assessing the quantitative properties of enzymatic versus mechanically dissociated stromal vascular fraction, the authors introduce the concept of “constitutive cell dose,” which is the minimum amount of cells that would need to be injected to treat a damaged tissue site.¹ This dose should approximate the number of native cells in a particular tissue block, the “constitutive cell burden” of the native tissue. Any treatment that does not deliver a dose of cells near the constitutive cell dose would likely be ineffective for tissue repair or regeneration.

The cell yield was measured differently in the nanofat samples and the enzymatically disaggregated samples because image cytometry does not allow accurate counting with cellular aggregates as one finds in nanofat, in contrast with the single cell layer generated by enzymatic digestion. This brings us also to one essential difference in clinical applications of these two methods of stromal vascular fraction segregation: aggregates as generated by nanofat processing

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are probably not suited for specific procedures such as intravenous delivery, which need separate cells as generated by collagenase separation. The remarkable outcome of this research is that enzymatic digestion, long considered the gold standard for stromal vascular fraction isolation, for a similar amount of donor adipose tissue delivers a cell yield only one-tenth of the cell yield obtained with nanofat processing,¹ which makes it much easier to obtain a sample containing a sufficient number of cells, approximating the constitutive cell dose.

When comparing this research¹ with other articles, one comes across a 2016 landmark article from the Irvine group.¹² In their research, Banyard et al. have observed a stable number of cells after nanofat processing, but also a decreased viability percentage. The article by Sesé et al. does not corroborate these findings, pointing out that “no current reliable method has been established to determine cell viability.” Banyard et al., however, have observed a three-fold up-regulation of the expression of the CD34 phenotypical marker for preadipocytes and transitional cells.¹² This means that both studies provide pieces of scientific explanation for the clinical results observed after nanofat treatments.

It is difficult to summarize this article by Dr. Sesé et al., as every sentence is a condensation of very in-depth scientific research and consideration, and this article demands the reader's full attention to grasp its true impact. The main conclusion of this article is that mechanically disrupted cell aggregates require 10 times less donor tissue and that the method is cost-effective. Nevertheless, the importance of this work also lies in opening perspectives for further research into the clinical outcomes and the mechanisms of action of different stromal vascular fraction isolation methods, comparing mechanically dissociated cell aggregates with enzymatically isolated cells.¹

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