Comment

Comment on Celentano et al. Suitability of a Progenitor Cell-Enriching Device for In Vitro Applications. Coatings 2021, 11, 146

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The intent of this letter is to comment on an article entitled “Suitability of a Progenitor Cell-Enriching Device for In Vitro Applications” [1] recently published in your journal, as we are afraid it could lead to disorientation and confusion among the scientific community.

Despite reading this article carefully, we have not fully understood the rationale of this paper, as the authors aimed to test a Class 2a Medical Device, whose use is only addressed to disaggregate autologous tissue sample (i.e., full thickness skin), to “evaluate the potential of the device for an in vitro cell model” by processing instead a single-cellular suspension.

It is of concern that the authors have not described carefully how the device works and, specifically, have not criticized that the obtained outcomes are achieved only due to the fact that the device has been used inadequately and could not work with a single cell suspension.

The device consists of:

• A grid with hexagonal holes, each hole is equipped at the edges with micro-blades
• A rotating helix.

Once activated, the rotating helix repeatedly drives the tissue sample onto the blades, which micro-fragments the tissue into smaller particles named micrografts.

We think it is adequate to discuss, point by point, the reasons why this article lacks scientific rationale:

• As already mentioned, the authors claimed that the aim of the study was to “evaluate the potential of the device for an in vitro cell model”, even though the intended use of the device is to disaggregate mechanically tissue sample in a micrograft suspension.
• As reported in Sections 2.3 and 2.4 the authors processed a cellular suspension, already separated by trypsinization, with the Rigeneracons device.

It is difficult to understand the authors’ experimental design/scope in doing so, as the only capacity of the device is to disaggregate tridimensional samples of tissue and, in this case, the authors wrongly used an already processed cellular suspension. The outcome is, of course, that the cells were under stress/processed twice, as well as the device, since there is nothing to process.

As the author stated, “the results obtained were not suggestive of any further space for this device in an in vitro context” and this is absolutely unacceptable as the device has been used in at least 16 publications for in vitro experiments (Table 1) with successful results.

Therefore, the author should report in the title their real setting, which is not an in vitro context (as this is too broad a description, and has already been proven to be possible with the device), but a single cell suspension.

• The Rigeneracons device allows us to disaggregate autologous human tissues (not cells), and is used with a specific amount of saline; both conditions were not respected...
Table 1. List of the papers which suggest the suitability of the device for in vitro context.

| Author(s)                                                                 | Year | Journal                                                                 | PMID/PMCID                                                                 |
|--------------------------------------------------------------------------|------|-------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Xie Y, Lampinen M, Takala J, Sikorski V, Solymani R, Tarkia M, Lalowski M, Mervaa A, Kupari M, Zheng Z, Hu S, Harjula A, Kankuri, E; AADC consortium. Epicardial transplantation of atrial appendage micrograft patch salvages myocardium after infarction. | 2020 | J Heart Lung Transplant. 2020;39(7):707-718. doi: 10.1016/j.healun.2020.03.023. Epub 7 April 2020. PMID: 3234944. |   |
| Dai Fre E, Busato A, Manucci S, Vurro F, De Francesco F, Riccio V, Solito S, Biswas R, Bernardi P, Riccio M, Sbarbati A. In Vitro Characterization of Adipose Stem Cells Non-Enzymatically Extracted From the Thigh and Abdomen. | 2020 | Int J Mol Sci. 27 April 2020;21(9):3081. doi: 10.3390/ijms21093081. PMID: 32349299; PMCID: PMC7247667. |   |
| Senesi L, De Francesco F, Farinelli L, Manzotti S, Gagliardi G, Papalia GF, Riccio M, Gigante A. Mechanical and Enzymatic Procedures to Isolate the Stromal Vascular Fraction From Adipose Tissue: Preliminary Results. | 2020 | Front Cell Dev Biol. 7 June 2019;7:88. doi: 10.3389/fcell.2019.00088. PMID: 31231649; PMCID: PMC6565890. |   |
| Balli M, Chui JS, Athanasouli P, Abreu de Oliveira WA, El Laithy Y, Sampaoleisi M, Llius F. Activator Protein-1 Transcriptional Activity Drives Soluble Micrograft-Mediated Cell Migration and Promotes the Matrix Remodeling Machinery: Stem Cells Int. 31 December 2019;2019:6461580. doi: 10.1155/2019/6461580. PMID: 32082384; PMCID: PMC7012246. | 2020 |   |   |
| Balli M, Vitali F, Janiszewski A, Caluwé E, Cortés-Calabuig A, Carpentier S, Duaen R, Ronzoni F, Marcelis L, Bosio FM, Bellazzi R, Luttun A, De Angelis MGC, Cecarelli G, Llius F, Sampaoleisei M. Autologous micrograft accelerates endogenous wound healing response through ERK-induced cell migration. Cell Death Differ. 25 October 2019. doi: 10.1038/s41418-019-0433-3. | 2020 |   |   |
| Kawakami S, Shiota M, Kon K, Shimogishi M, Kasugai S. The Effect of Dissociated Soft Tissue on Osteogenesis: A Preliminary In Vitro Study. Int J Oral Maxillofac Implants. May/June 2019;34(3):651–657. doi: 10.11607/jomi.7021. | 2019 |   |   |
| Viganò M, Tessaro I, Trovato L, Colombini A, Scala M, Magi A, Toto A, Peretti G, de Girolamo L. Rationale and pre-clinical evidences for the use of autologous cartilage micrografts in cartilage repair. J Orthop Surg Res. 6 November 2019;13(1):279. doi: 10.1186/s13018-018-0983-y. | 2019 |   |   |
| Francesco De Francesco, Silvia Manucci, Giaima Conti, Elena Dai Prè, Andrea Sbarbati and Michele Riccio. A Non-Enzymatic Method to Obtain a Fat Tissue Derivative Highly Enriched in Adipose Stem Cells (ASCs) from Human Lipoaspirates: Preliminary Results. Int. J. Mol. Sci. 2018, 19, 2061; doi:10.3390/ijms19072061. | 2018 |   |   |
| Noda S, Sumita Y, Ohba S, Yamamoto H, Asahina I. Soft tissue engineering with micrornized-gingival connective tissues. J Cell Physiol. 2018; 233:249–258. | 2018 |   |   |
| Rodríguez Y Baena R, D’Aquino R, Graziano A, Trovato L, Aloise AC, Ceccarelli G, Cusella G, Pelegrine AA, Lupi SM. Autologous Periosteum-Derived Micrografts and PLGA/HA Enhance the Bone Formation in Sinus Lift Augmentation. Front Cell Dev Biol. 27 September 2017:5:87. doi: 10.3389/fcell.2017.00087. eCollection 2017. | 2017 |   |   |
| Gentile P, Sciolgi MG, Bielli A, Orlandi A, Cervelli V. Stem cells from human hair follicles: first mechanical isolation for immediate autologous clinical use in androgenetic alopecia and hair loss. Stem Cell Investig 2017;4:58. | 2017 |   |   |
| Jimi Shiro, Kimura Masahiko, De Francesco Francesco, Riccio Michele, Hara Shuuji, Ohjimi Hiroyuki. Acceleration Mechanisms of Skin Wound Healing by Autologous Micrograft in Mice. Int. J. Mol. Sci. 2017, 18, 1675; doi:10.3390/ijms18081675. | 2017 |   |   |
| Ceccareli G, Gentile P, Marcarelli M, Balli M, Ronzoni FL, Benedetti L, Cusella De Angelis MG.In Vitro and In Vivo Studies of Alar-Nasal Cartilage Using Autologous Micro-Grafts: The Use of the Rigenera®® Protocol in the Treatment of an Osteochondral Lesion of the Nose. Pharmaceuticals (Basel). 13 June 2017;10(2). pii: E53. doi: 10.3390/ph10020053. | 2017 |   |   |
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| Purpura V, Bondioli E, Graziano A, Trovato L, Melandri D, Gheti M, Marchesini A, Cusella De Angelis MG, Benedetti L, Ceccarelli G, Riccio M. Tissue Characterization After a New Disaggregation Method for Skin Micro-Grafts Generation. J Vis Exp. 4 March 2016;109:e53579 doi: 10.3791/53579. | 2016 |   |   |
| Trovato L, Monti M, Del Fante C, Cervio M, Lampinen M, Ambrosio L, Redi CA, Perotti C, Kankuri E, Ambrosio G, Rodriguez Y Baena R, Pirozzi G, Graziano A. A New Medical device rigeneracons allows to obtain viable micro-grafts from mechanical disaggregation of human tissues. J Cell Physiol, 2015;230:2299-303. | 2015 |   |   |

in the study as the authors did not follow the manufactures guidelines/IFU (instructions for use). Consequently, the authors must state clearly that the device has been used without following the IFU to generate these “data”, which cannot be discussed regarding its relevance, nor can the clinical efficacy of the subjected medical device, as it has not been correctly used.

- The author claimed to separate progenitor cells from a population of fibroblasts, however it is mandatory to specify that the device is able to select the tissue cluster of a specific dimension containing progenitor cells due to hole size, as the device only act as a filter right after the tissue disaggregation. Again, it is difficult to understand the rationale perpetuated by the authors in using the Rigeneracons device only as a filter to collect progenitor cells; they could simply use a filter or a strainer. Even here, the scientific rationale in selecting progenitor cells from a single cell suspension using the Rigeneracons device is completely absent.

- A wide section of the article is written around the relevance of the metal debris found after the device’s use. Again, the device has not been used following the IFU, without any tissue sample, which generates more friction than the metal parts. There is no point in discussing/researching/publishing such information, as it is confusing and impolite toward the scientific community.
The author attempted to discuss the outcome that this metal debris would have in a clinical setting; again, this is extremely impolite as this is an “in vitro” addressed paper, and should therefore report the effect of this debris in their in vitro setting, specifying that this is the result of the incorrect use of the device. In any case, the effects of this debris, for the in vitro setting, is not as detrimental as the authors would like to report; Picture 1 shows acceptable cellular viability.

Despite the authors attempt to generate data against the use of the Rigenera technology, we would point out that the debris is generated by failure to follow the IFU, and from medical-classified materials.

Lastly, the device has been approved by several regulatory agencies, along with all the necessary reports, as a biocompatibility test with more than acceptable results; this has not been reported in the paper.

Considering all the above, it is difficult to understand how such a paper was even accepted into your journal as, ultimately, the authors have only processed an enzyme-dissociated cell suspension with a medical device, the scope of use of which is completely different, and proceeded to blame this for the later malfunction, even suggesting an improvement.

It is worth mentioning, since the authors have not, that Rigeneracons has almost 15 years of preclinical data and clinical data gained from patients’ treatments, ranging from myocardial regeneration, to wound healing, to cartilage and bone regeneration, and side effects have never been reported, Please check below the complete bibliography [2–61].

We ultimately suspect that the authors tried to make a more appealing paper supporting the possible selection of progenitor cells within the 2D cell in vitro by using the title “Suitability of a Progenitor Cell-Enriching Device for In Vitro Applications”; however, the entire paper has no link and scope to what is reported in the title.

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