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Comparison of the infrapatellar and subcutaneous adipose tissue biopsy material as a source of mesenchymal stromal cells for regenerative medicine in traumatology

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Objective. The use of infrapatellar fat pad (IPFP) as the source of mesenchymal stem cells (MSCs), show an age-independent proliferation and differentiation potential. In addition, the pronounced chondrogenic potential of IPFP-ASCs makes them promising candidates for research for use in other methods of regenerative therapy. Methods. A direct immunohistochemical study was carried out in serial paraffin sections of the biopsy material of Hoffa's fat pad and subcutaneous fat tissue, using monoclonal antibodies. The minimum criteria established by the International Society for Cell Therapy to ensure the identity of MSCs use CD73 and CD105 as positive markers and CD34, CD31, CD45 as a negative. Results. According to the results of histological, immunohistochemical, morphometric and statistical studies, it was found that in the biopsy of both tissues the relative number of cells with an immunoprofile CD105+, CD73+, CD34-, CD31-, CD45- in the standard field of view ($\times 200$) was: 7.25 (5.42; 8.89) %, and 11.11 (8.46; 13.45) %, no statistically significant difference was found between comparison groups ($p > 0,05$). Conclusion. The therapeutic effect of mesenchymal stem cells of subcutaneous adipose tissue has been proven, thus the identification of similar cells in the biopsy material and relatively similar density in the Hoffa body makes it an important source of adipose-derived stem cells that can be used for regenerative engineering tissue.

Мета. Використання інфрапателлярного жирового тіла, як джерела мезенхімальних стовбурових клітин демонструє незалежний від віку потенціал проліферації та диференціації. Крім того, виражений хондрогенний потенціал стовбурових клітин жирового тільця Гоффа робить їх перспективними кандидатами для дослідження та використання в різних методах регенеративної терапії. Методи. Проводили пряме імуногістохімічне дослідження серійних парафінових зрізів біопсійного матеріалу жирового тільця Гоффа та підшкірної жирової клітковини з використанням моноклональних антитіл. Мінімальні критерії, встановлені Міжнародним товариством клітинної терапії для забезпечення ідентичності мезенхімальних стовбурових клітин, використовують CD73 і CD105 як позитивні маркери та CD34, CD31, CD45 як негативні. Результати. За результатами гістологічних, імуногістохімічних, морфометричних та статистичних досліджень встановлено, що в біоптаті обох тканин відносна кількість клітин з імунопрофілем CD105+, CD73+, CD34-, CD31-, CD45- у стандартному полі зору ($\times 200$) була: 7,25 (5,42; 8,89) % та 11,11 (8,46; 13,45) %, статистично значущої різниці між групами не виявлено ($p > 0,05$). Висновок. Терапевтичний ефект мезенхімальних стовбурових клітин підшкірної жирової клітковини доведено, тому ідентифікація подібних клітин у біопсійному матеріалі та у відносно однаковій щільності в тільці Гоффа робить його важливим джерелом стовбурових клітин жирового походження, які можна використовувати для регенерації та тканинної інженерії. Ключові слова. Стромальна васкулярна фракція, мезенхімальні стромальні клітини, остеогенез, хондрогенез.

Keywords. Stromal vascular fraction, mesenchymal stromal cells, osteogenesis, chondrogenesis

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Introduction

In young patients, joint cartilage damage typically occurs due to injuries and early degenerative processes, which can lead to pain syndrome and significant structural changes, as seen in the terminal stage of arthritis. This significantly affects all aspects of the patient's activities. Joint cartilage defects are incapable of self-renewal, and when they reach a critical level, irreversible changes occur [1]. The ideal treatment for such patients would be the regeneration of hyaline cartilage using tissue engineering.

Adipose-derived stem cells (ADSCs) are utilized in the treatment of joint cartilage injuries because they are easily collected and possess a high regenerative potential for cartilage restoration. Sources of ADSCs include the infrapatellar fat pad (IPFP) and subcutaneous fat (SCF), which act as reservoirs of mesenchymal stem cells (MSCs). The IPFP is a rich source of MSCs. A large amount of adipose tissue can also be obtained from subcutaneous fat removed via liposuction. Mesenchymal stem cells from the infrapatellar fat pad (IPFP-MSCs) are a type of ADSC [2].

The infrapatellar fat pad (IFP), also known as Hoffa's fat pad, is the intracapsular/extrasynovial tissue in the anterior compartment of the knee, which is densely innervated and has a well-developed vascular network [3]. Primarily serving a biomechanical function, it acts as a pressure cushion during knee articulation. Additionally, the relatively high density of nerves in the IFP may indicate its role as a mechanoreceptor/proprioceptor [4].

The primary advantage of IPFP-ASCs compared to other sources of MSCs is that these cells demonstrate age-independent potential for proliferation and differentiation, whereas others typically exhibit age-related declines in these properties [5].

The *objective* of this study is to compare the expression of markers used to identify stromal stem cells in the obtained biopsy material of the infrapatellar fat pad and abdominal subcutaneous adipose tissue to determine their characteristics.

Materials and methods

The materials of the study were reviewed and approved by the Bioethics Committee at Zaporizhzhia State Medical University (Protocol No. 8 dated December 26, 2022). All patients involved in the study were informed about the surgical intervention plan and signed informed consent forms.

For the study, data analysis was conducted on 30 patients (both male and female) who underwent surgical or combined treatment for knee osteoarthritis.

A histological examination of the biopsy material from Hoffa's fat pad and abdominal subcutaneous tissue was conducted. Macroscopic examination and standard tissue processing with hematoxylin-eosin staining were performed. Microscopy was conducted using a Carl Zeiss Scope.A1 microscope (Germany) with a Progres Gryphax Jenoptik 60N-C1"1.0x426114 camera (Germany), connected to a computer. The digital analysis program Progres Gryphax 1.1.4.2 (Jenoptik Optical System, Germany) was used for obtaining microphotographs and further image analysis.

Immunohistochemical analysis was performed on serial paraffin sections of biopsy material from Hoffa's fat pad (n = 15) and abdominal subcutaneous tissue (n = 15), using monoclonal antibodies: CD105 (105 Endoglin, clone EP274 («Bio SB», USA)), CD31 (Mo a-Hu CD31 Endothelial Cell Marker Ab-1, clone JC/70A («DAKO», Denmark)), CD73 (Ecto-5'-nucleotidase (NT5E) clone RM431 («Bio SB», USA)), CD34 (clone QBEND/10 («Thermo scientific», USA)), CD45 (Leucocyte common antigen, clone PD7/26/16 + 2B11 («Thermo scientific», USA)).

For immunohistochemical analysis, paraffin blocks containing tissue fragments were sliced into 4µm sections, deparaffinized, and rehydrated. High-temperature antigen retrieval was performed by heating in Tris-EDTA buffer (pH = 9.0) on a water bath to unmask antigens. Endogenous peroxidase activity was suppressed with a 3 % hydrogen peroxide solution, and a blocking serum was applied. Incubation with primary antibodies was conducted according to the instructions provided by the manufacturers. DAKO EnVision+System detection systems with diaminobenzidine (DAB) (DAKO, USA) were used for visualizing the IHC reaction. Afterward, sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted with a Canadian balsam.

Mesenchymal stem cell populations were identified using parallel determination of multiple positive and negative markers, considering minimal criteria established by the International Society for Cellular Therapy to ensure MSC identity [6], and taking into account the characteristics of adipose tissue-derived MSCs, which are typically classified as CD34-negative [7]. Evaluation was performed in 5 standardized fields of view using a Carl Zeiss Scope.A1 microscope (Germany) with a Progres Gryphax Jenoptik 60N-C1"1.0x426114 camera (Germany) at a magnification of ×200 in each case under investigation. Digital images of microscopic specimens were obtained, and the relative quantity (%) of cells expressing CD105, CD73, CD34, CD31, and CD45, as well as cells with the immunophenotype CD105+, CD73+,

CD34–, CD31–, CD45–, was calculated in each standard field of view ($\times 200$ magnification).

Non-parametric statistical analysis parameters were used. The Shapiro-Wilk test was applied to check the hypothesis of normal distribution of the investigated parameters. The results of immunohistochemical analysis are presented as medians and interquartile range Me (Q1; Q3). The non-parametric Mann-Whitney U-test was used to compare two independent groups. Differences were considered significant at $p < 0.05$. Statistical analysis was performed using the Statistica® for Windows 13.0 software (StatSoft Inc., license №JPZ804I382130ARCN10-J).

Results and their discussion

Microscopically, the infrapatellar fat pad consists of lobular type white adipose tissue, with an average diameter of (1.15 ± 0.11) mm, characterized by thin connective septa. Adipocytes in the infrapatellar fat pad have significantly smaller cell volumes compared to subcutaneous adipose tissue [8]. Additionally, a considerable number of fibroblasts responsible for producing extracellular matrix, as well as immune cells such as macrophages, adipocytes, and lymphocytes, are present [9]. Typically, this type of adipose tissue, characterized by a significant prevalence of collagenous stroma, is typical for areas subjected to considerable mechanical stress [10].

Adipocytes in adipose abdominal tissue are formed in the process of differentiation of multipotent progenitor cells (mesenchymal stem cells — MSC) [11] these cells are key regulators of adipose tissue. They are involved in tissue homeostasis due to their powerful ability to differentiate into adipogenesis and angiogenesis. In addition, ASCs coordinate and maintain the local and systemic environment through immunomodulation and damage repair through their paracrine signaling and direct intercellular interactions [12, 13]. These cells are considered

useful for applications in regenerative medicine due to their availability and diverse functions in tissue remodeling and homeostasis [14].

According to the results of the study, it was found that the relative number of CD31+ cells in the biopsy material of IPFP was 30.84 (26.45; 36.99) %, subcutaneous fat tissue — 31.62 (25.39; 46.48) % (Table), no statistically significant difference was found ($p > 0,05$).

The relative number of CD105+ cells in the biopsy material of IPFP is 39.12 (29.73; 48.64) %, in the biopsy material of the subcutaneous adipose tissue — 34.57 (29.41; 40.00) % (Table), no statistically significant difference was found ($p > 0,05$).

The relative number of CD73+ cells in the biopsy material of IPFP was determined at the level of 62.91 (53.40, 74.14) %, which is statistically significant in 1.23 times more than in the biopsy material of subcutaneous fat tissue – 51.26 (36.05; 63.24) % (Table), ($p < 0,05$).

The relative number of CD45+ cells in the biopsy material of IPFP was determined at the level of 33.44 (27.59; 36.29) %, in the biopsy material of subcutaneous fat tissue — 26.05 (21.37; 31.00) % (Table), which is 1.28 times less than in the Hoff's fat body biopsy material, the difference is statistically significant. ($p < 0,05$).

The relative number of CD34+ cells in the biopsy material of IPFP was 73.86 (61.54; 85.48) %, in the biopsy material of subcutaneous adipose tissue — 88.52 (80.88; 89.92) % (Table), which is 1.2 times more, a statistically significant difference ($p < 0.05$).

According to the results of the study, it was found that in the biopsy material of Hoff's fat body and subcutaneous adipose tissue, the relative number of cells with an immunoprofile CD105+, CD73+, CD34–, CD31–, CD45– in the standard field of view ($\times 200$) was 7.25 (5.42; 8.89) %, and 11.11 (8.46; 13.45) % (Table, fig. 1–3), no statistically significant difference was found ($p > 0,05$).

Table

The relative number of cells expressing CD105+, CD31–, CD73+, CD34–, CD45– and cells with an immunohistochemical profile of CD105+, CD73+, CD34–, CD31–, CD45– in the biopsy material of IPFP (Biopsy Hoffa) and abdominal subcutaneous fat tissue (Biopsy Abd)

| CD marker | Biopsy Hoffa | Biopsy Abd |
|------------------------------------|----------------------|-----------------------|
| CD31– | 30,84 (26,45; 36,99) | 31,62 (25,39; 46,48) |
| CD105+ | 39,12 (29,73; 48,64) | 34,57 (29,41; 40,00) |
| CD73+ | 62,91 (53,40; 74,14) | 51,26 (36,05; 63,24)* |
| CD45– | 33,44 (27,59; 36,29) | 26,05 (21,37; 31,00)* |
| CD34– | 73,86 (61,54; 85,48) | 88,52 (80,88; 89,92)* |
| CD105+, CD73+, CD34–, CD31–, CD45– | 7,25 (5,42; 8,89) | 11,11 (8,46; 13,45) |

Note. * — statistically significant difference ($p < 0,05$).

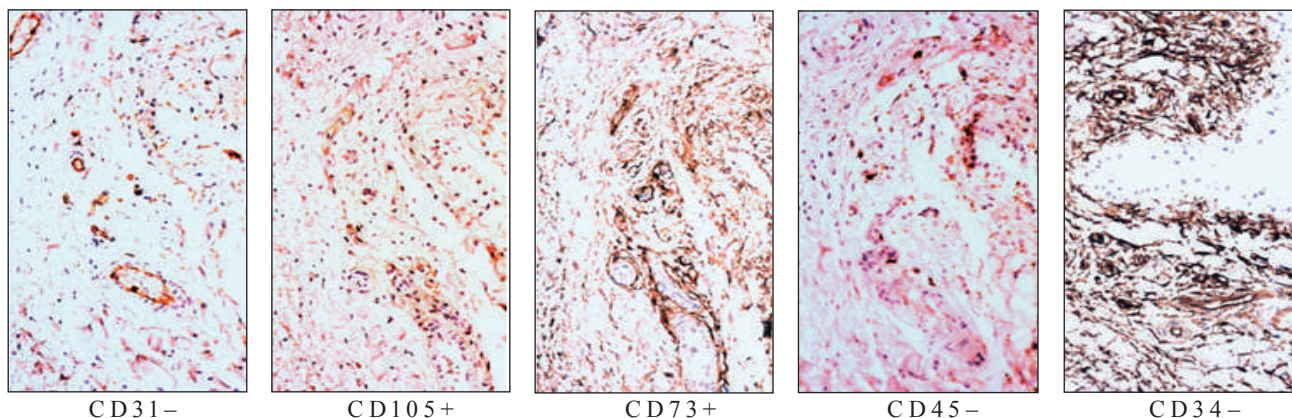


Fig. 1. Expression of CD31, CD105, CD73, CD45, CD34 in the biopsy material of Hoff's infrapatellar fat body in the standard field of view ($\times 200$ magnification)

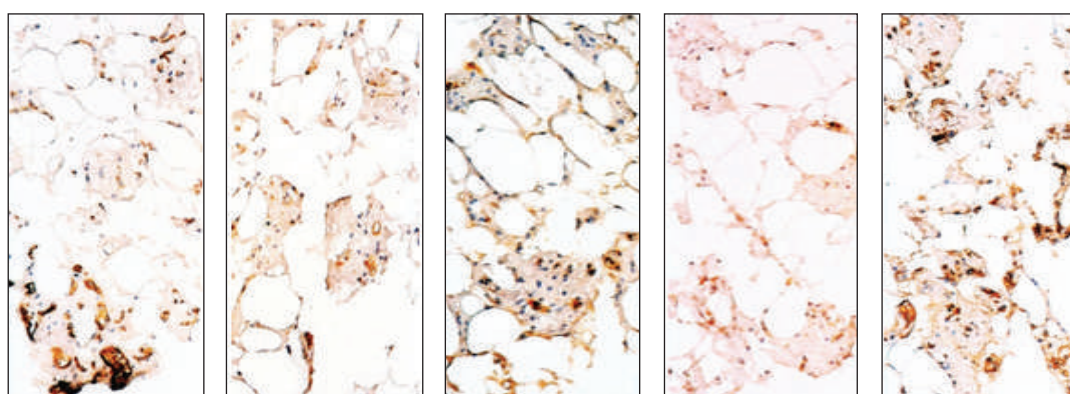


Fig. 2. Expression of CD31, CD105, CD73, CD45, CD34 in the biopsy material of subcutaneous adipose tissue in the standard field of view ($\times 200$ magnification)

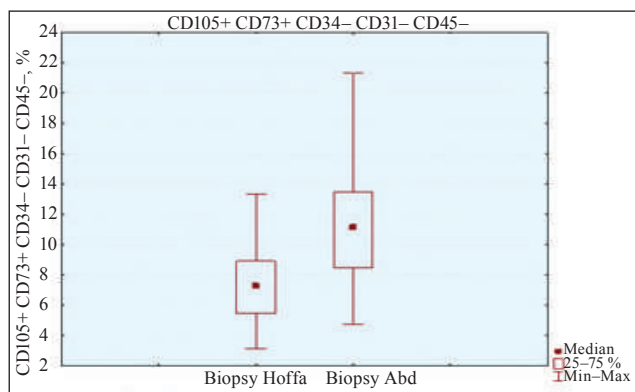


Fig. 3. Comparison of the relative number of cells with the profile CD105+, CD73+, CD34-, CD31-, CD45- in the standard field of view ($\times 200$) in the biopsy material of IPFP (Biopsy Hoffa) and abdominal subcutaneous fat tissue (Biopsy Abd)

According to the conducted histological, immunohistochemical and statistical research, IPFP, like SC, is characterized by almost the same relative number of CD31- and CD105+ cells, which indicates a very well-developed vascular system of both tissues. Although the relative number of cells with immunophenotype CD105+, CD73+, CD34-, CD31-, CD45- was lower in the biopsy material of Hoff's infrapatellar adipose body than in the biopsy material of subcutaneous

adipose tissue, this difference was not statistically significant.

A statistically significant difference ($p < 0,05$) was established in the relative number of CD73+ and CD45- immunopositive cells in the biopsy material of Hoff's infrapatellar fat body, which is explained by the greater diversity of the cellular composition in the IPFP and the greater number of inflammatory cells, due to various traumatic and non-traumatic changes in the knee joints of donors. In the biopsy material of subcutaneous adipose tissue, a statistically significant ($p < 0,05$) higher relative number of CD34- cells was determined, reflecting a higher number of adipocytes in SCF compared to IPFP.

The structure and content of adipose tissue varies depending on its location [15], as does the regenerative potential of collected MSCs [16]. In previous studies, IPFP demonstrated increased chondrogenic potential compared to subcutaneous abdominal fat in matched patient samples [17]. IPFP also differs in that when it is collected, the synovial membrane also enters the sample. Previous studies revealed significant MSC activity of IPFP-derived cells even in patients with severe osteoarthritis [18]. Taking into account the data listed above, it can be argued that Hoff's infrapatellar fat pad is an important and promising

source of stem cells for the restoration of cartilage tissue.

Several studies investigating the potential of stem cells isolated from Hoff's infrapatellar fat pad have concluded that the anatomical region where the cells are isolated influences the characteristics of ASCs, and the level of chondrogenic differentiation potential of IPFP-ASCs is higher due to the close contact of IPFPs with the synovial membrane and fluid, suggesting that IPFP can be considered as a high-quality resource for restorative therapy.

Conclusions

The number of mesenchymal stem cells with an immunohistochemical profile of CD105+, CD73+, CD34-, CD31-, CD45- in the biopsy material of Hoff's fat body and the biopsy material of abdominal subcutaneous adipose tissue does not differ statistically significantly ($p > 0.05$).

The relative number of CD73+ and CD45- immunopositive cells in the biopsy material of Hoff's infrapatellar fat body is statistically significant ($p < 0.05$) and in 1.23 and 1.28 times higher, respectively, compared to the biopsy material of abdominal subcutaneous fat tissue.

Biopsy material of IPFP is an important source of adipose-derived stem cells that can be used for regenerative tissue engineering.

Conflict of interest. The authors declare no conflict of interest.

References

- Hindle, P., Khan, N., Biant, L., & Péault, B. (2016). The Infrapatellar fat pad as a source of Perivascular stem cells with increased Chondrogenic potential for regenerative medicine. *Stem Cells Translational Medicine*, 6(1), 77-87. doi:10.5966/sctm.2016-0040
- Liao, H., Chang, C., Huang, C. F., & Chen, H. (2022). Potential of using infrapatellar-fat-Pad-Derived Mesenchymal stem cells for therapy in degenerative arthritis: Chondrogenesis, Exosomes, and transcription regulation. *Biomolecules*, 12(3), 386. doi:10.3390/biom12030386
- Fontanella, C. G., Carniel, E. L., Frigo, A., Macchi, V., Porzionato, A., Sarasin, G., ... Natali, A. N. (2017). Investigation of biomechanical response of Hoffa's fat pad and comparative characterization. *Journal of the Mechanical Behavior of Biomedical Materials*, 67, 1-9. doi:10.1016/j.jmbbm.2016.11.024
- Macchi, V., Porzionato, A., Sarasin, G., Petrelli, L., Guidolin, D., Rossato, M., ... De Caro, R. (2016). The Infrapatellar adipose body: A Histotopographic study. *Cells Tissues Organs*, 201(3), 220-231. doi:10.1159/000442876
- Huri, P. Y., Hamsici, S., Ergene, E., Huri, G., & Doral, M. N. (2018). Infrapatellar fat pad-derived stem cell-based regenerative strategies in orthopedic surgery. *Knee Surgery and Related Research*, 30(3), 179-186. doi:10.5792/ksrr.17.061
- Maslennikov, S., & Golovakha, M. (2023). The most commonly used cell surface markers for determining mesenchymal stromal cells in stromal vascular fraction and bone marrow autologous concentrate: a systematic review. *Journal of Biotech Research*, 14, 85-94.
- Safdar, B. K., Fischer, K., Möllmann, C., Solbach, C., Louwen, F., & Yuan, J. (2019). Subcutaneous and Visceral Adipose-Derived Mesenchymal Stem Cells: Commonality and Diversity. *Cells*, 8(10), 1288.
- Eymard, F., Pigenet, A., Citadelle, D., Tordjman, J., Foucher, L., Rose, C., Flouzat Lachaniette, C.-H., Rouault, C., Clement, K., Berenbaum, F., Chevalier, X., & Houard, X. (2017). Knee and hip intra-articular adipose tissues (IAATs) compared with autologous subcutaneous adipose tissue: a specific phenotype for a central player in osteoarthritis. *Annals of the Rheumatic Diseases*, 76(6), 1142-1148. https://doi.org/10.1136/annrheumdis-2016-210478
- Clockaerts, S., Bastiaansen-Jenniskens, Y. M., Runhaar, J., Van Osch, G. J. V. M., Van Offel, J. F., Verhaar, J. A. N., De Clerck, L. S., & Somville, J. (2010). The infrapatellar fat pad should be considered as an active osteoarthritic joint tissue: a narrative review. *Osteoarthritis and Cartilage*, 18(7), 876-882. https://doi.org/10.1016/j.joca.2010.03.014
- Macchi, V., Stocco, E., Stecco, C., Belluzzi, E., Favero, M., Porzionato, A., & De Caro, R. (2018). The infrapatellar fat pad and the synovial membrane: an anatomic-functional unit. *Journal of Anatomy*, 233(2), 146-154. https://doi.org/10.1111/joa.12820.
- Girousse, A., Gil-Ortega, M., Bourlier, V., Bergeaud, C., Sastourné-Arrey, Q., Moro, C., Barreau, C., Guissard, C., Vion, J., Arnaud, E., Pradère, J.-P., Juin, N., Casteilla, L., & Sengenès, C. (2019). The Release of Adipose Stromal Cells from Subcutaneous Adipose Tissue Regulates Ectopic Intramuscular Adipocyte Deposition. *Cell Reports*, 27(2), 323-333. e5. https://doi.org/10.1016/j.celrep.2019.03.038
- Louwen, F., Ritter, A., Kreis, N. N., & Yuan, J. (2018). Insight into the development of obesity: functional alterations of adipose-derived mesenchymal stem cells. *Obesity Reviews*, 19(7), 888-904. https://doi.org/10.1111/obr.12679
- Strong, A. L., Burrow, M. E., Gimble, J. M., & Bunnell, B. A. (2015). Concise Review: The Obesity Cancer Paradigm: Exploration of the Interactions and Crosstalk with Adipose Stem Cells. *STEM CELLS*, 33(2), 318-326. https://doi.org/10.1002/stem.1857
- Ritter, A., Friemel, A., Roth, S., Kreis, N.-N., Hooek, S. C., Safdar, B. K., Fischer, K., Möllmann, C., Solbach, C., Louwen, F., & Yuan, J. (2019). Subcutaneous and Visceral Adipose-Derived Mesenchymal Stem Cells: Commonality and Diversity. *Cells*, 8(10), 1288. https://doi.org/10.3390/cells8101288
- Russo, V., Yu, C., Belliveau, P., Hamilton, A., & Flynn, L. E. (2013). Comparison of Human Adipose-Derived Stem Cells Isolated from Subcutaneous, Omental, and Intrathoracic Adipose Tissue Depots for Regenerative Applications. *STEM CELLS Translational Medicine*, 3(2), 206-217. https://doi.org/10.5966/sctm.2013-0125
- Pires de Carvalho, P., Hamel, K. M., Duarte, R., King, A. G. S., Haque, M., Dietrich, M. A., Wu, X., Shah, F., Burk, D., Reis, R. L., Rood, J., Zhang, P., Lopez, M., Gimble, J. M., & Dasa, V. (2012). Comparison of infrapatellar and subcutaneous adipose tissue stromal vascular fraction and stromal/stem cells in osteoarthritic subjects. *Journal of Tissue Engineering and Regenerative Medicine*, 8(10), 757-762. https://doi.org/10.1002/term.1565
- Stanco, D., de Girolamo, L., Sansone, V., & Moretti, M. (2014). Donor-matched mesenchymal stem cells from knee infrapatellar and subcutaneous adipose tissue of osteoarthritic donors display differential chondrogenic and osteogenic commitment. *European Cells and Materials*, 27, 298-311. https://doi.org/10.22203/ecm.v027a21

18. Liu, Y., Buckley, C. T., Almeida, H. V., Mulhall, K. J., & Kelly, D. J. (2014). Infrapatellar Fat Pad-Derived Stem Cells Maintain Their Chondrogenic Capacity in Disease and Can be

Used to Engineer Cartilaginous Grafts of Clinically Relevant Dimensions. *Tissue Engineering Part A*, 20(21-22), 3050–3062. <https://doi.org/10.1089/ten.tea.2014.0035>

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ПОРІВНЯННЯ БІОПСІЙНОГО МАТЕРІАЛУ ІНФРАПАТЕЛЛЯРНОЇ ТА ПІДШКІРНОЇ ЖИРОВОЇ ТКАНИНИ ЯК ДЖЕРЕЛА МЕЗЕНХІМАЛЬНИХ СТРОМАЛЬНИХ КЛІТИН ДЛЯ РЕГЕНЕРАТИВНОЇ МЕДИЦИНИ В ТРАВМАТОЛОГІЇ

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