ISSN 0030-5987. Orthopaedics, traumatology and prosthetics. 2023. № 3

УДК 611.018.4.26:616.71-018.3:576.31

DOI: http://dx.doi.org/10.15674/0030-59872023351-56

Morphological comparison of the stromal-vascular fraction of the subcutaneous fat cell and infrapatellar fat pad

S. O. Maslennikov, Yu. M. Avramenko, M. L. Golovakha

Zaporizhzhya State Medical and Pharmaceutical University. Ukraine

The use of regenerative technologies is widespread in modern medicine. Adipose-derived stem cells (ADSCs) in the stromal-vascular fraction (SVF) appear to be most attractive for use in cell therapy. The objective of this study is to identify morphological differences in the stromal-vascular fraction from the subcutaneous tissue and infrapatelar fat pad (IFP). Material and methods. Data analysis of 15 patients was carried out. The average age of the patients was (44.0 ± 3.8) years with a body mass index of (20.1 ± 1.6) kg/m². Aspirates of subcutaneous adipose tissue were obtained from the anterior abdominal wall of 8 women without signs of obesity and comorbid metabolic diseases, as well as the resected IFP from 7 patients during therapeutic and diagnostic arthroscopy, also without signs of obesity. We conducted a comparative characterization of the composition of the obtained histological samples. Morphometric measurements of the diameter of adipocytes were performed. Parametric and non-parametric criteria of statistical analysis were applied. Parametric criteria were used to determine the average diameter of adipocytes and the standard deviation. Non-parametric ones were used to test the hypothesis about the normality of the distribution of the studied indicators according to the Shapiro–Wilk test. The results. SVF, which were obtained in our study from subcutaneous adipose tissue and from IFP, differ in composition, namely, the amount of the stromal component, which is visually much more pronounced in the SVF of IFP, the size of adipocytes, which in SVF from subcutaneous adipose tissue of a statistically larger diameter. Conclusions. It was morphologically proven that SVF of IFP is a promising source of adipose mesenchymal stem cells for regenerative medicine, especially for cartilage regeneration.

Використання регенеративних технологій широко поширене в сучасній медицині. Стовбурові клітини жирового походження (ADSCs) у стромально-васкулярній фракції (SVF) здаються найбільш привабливими для використання в клітинній терапії. Мета цього дослідження виявлення морфологічних відмінностей стромально-васкулярної фракції з підшкірної клітковини та тільця Гоффа. Матеріал і методи. Проведено аналіз даних 15 пацієнток. Середній вік хворих становив (44,0 ± 3,8) роки з індексом маси тіла (20,1 ± 1,6) кг/м². Отримали аспірати підшкірної жирової клітковини з передньої черевної стінки 8 жінок без ознак ожиріння та коморбідних метаболічних захворювань, а також резековане тільце Гоффа 7 пацієнток під час виконання лікувально-діагностичної артроскопії, також без ознак ожиріння. Провели порівняльну характеристику складу отриманих гістологічних зразків. Виконали морфометричні вимірювання діаметра адипоцитів. Застосовано параметричні та непараметричні критерії статистичного аналізу. За допомогою параметричних критеріїв визначали середній діаметр адипоцитів і стандартне відхилення. Непараметричні використовували для перевірки гіпотези про нормальність розподілу досліджуваних показників за критерієм Шапіро–Уїлка. Результати. SVF, які були отримані в нашому дослідженні з підшкірної жирової клітковини та з жирового тільця Гоффа, відрізняються за складом, а саме кількістю стромального компонента, який візуально значно вираженіший у SVF жирові подушечки Гоффа, розміром адипоцитів, які у SVF із підшкірної жирової тканини статистично більшого діаметра. Висновки. Морфологічно було доведено, що SVF інфрапателярного жирового тільця Гоффа є перспективним джерелом жирових мезенхімальних стовбурових клітин для регенеративної медицини, зокрема для регенерації хряща. Ключові слова. Стромальна васкулярна фракція, мезенхімальні стромальні клітини, остеогенез, хондрогенез.

Keywords. Stromal vascular fraction, MSC, osteogenesis, chondrogenesis

Introduction

The use of regenerative technologies, namely, cell therapy, is becoming widespread in modern medicine. In recent decades, the field of orthopedics has received a perspective and a new impetus for the development of new regenerative technologies and surgical methods of their application. The use of stem cells in regenerative medicine is a particularly attractive area of research that has attracted great interest in recent years. Scientists know of four main sources of stem cells, including the following tissues: embryonic, fetal, adult, and differentiated somatic cells after genetic reprogramming, which are called induced pluripotent stem cells. In adults, adipose tissue-derived stem cells (ADSCs) are contained in the stromal-vascular fraction (SVF), isolated from adipose tissue, and are most advantageous for use in cell therapy and tissue engineering [1]. Compared to mesenchymal stem cells of other locations [2, 3], ADSCs can be relatively easily obtained in larger quantities with less discomfort and minimal damage to the donor site. Mesenchymal stromal cells (MSCs) are able to interact with the neighboring microenvironment, which leads to the generation of new committed progenitor cells. They also secrete exosomes containing growth factors, cytokines, chemokines and micro-RNAs, which are involved in the restoration of tissue defects and biological functions. Some literature sources report that the relative amount of stem cells and progenitor cells in uncultured SVF was up to 3 % of the total cell volume [4]. In comparison, the number of SVF cells that can be isolated from subcutaneous liposuction aspirates is approximately $0.5–2.0 \times 10^6$ cells per gram of adipose tissue [4, 5], with the percentage of stem cells ranging from 1 to 10%, which, most likely, depends on the donor and the place of tissue collection. Thus, about 0.5×10^4–2×10^5 stem cells can be isolated per gram of adipose tissue, depending on the patient. The effectiveness of using the regenerative potential of MSCs in orthopedics directly depends on their amount in the used drug [6].

The infra- and suprapatellar fat bodies (Hoff body) surrounding the knee are a potential autologous source of ADSCs for patients after total joint replacement. Administration of ADSCs obtained from the infrapatellar fat body of laboratory animals with severe osteoarthritis significantly reduced joint inflammation and the degree of cartilage degeneration, increased glycosaminoglycan production and induced endogenous chondrogenesis compared to the control group. Overall, infrapatellar SVF offers a potential autologous regenerative treatment for patients with degenerative knee osteoarthritis [7].

Among specialists in the orthopedic community, there is a thesis that Hoff's body, which is part of the knee joint, as a separate organ, is closely connected not only by anatomical-physiological and biomechanical connections (a single system of blood supply, innervation, connection with the synovial apparatus, etc.), but and histological and biochemical (tendency of stem cells to differentiate towards chondral tissue or to influence its condition due to mediators, growth factors and chemotactic regulation chains) [8]. That is why the null thesis was proposed, that Hoff's body should be determined by morphological and cytological characteristics, as well as by the tendency of poorly differentiated cells to develop specifically in the chondrogenic side of the diferon compared to the cells of the subcutaneous tissue.

Purpose: to determine morphological differences of the stromal-vascular fraction from the subcutaneous tissue and Hoff's body, which were obtained mechanically.

Material and methods

The research materials were reviewed and approved by the Bioethics Committee at Zaporizhia State Medical and Pharmaceutical University (protocol No. 8 dated 26.12.2022). All patients involved in the study were familiarized with the plan of surgical interventions and signed the informed consent.

The study involved selection and assessment of cases of 15 patients who underwent surgical or combined treatment for knee arthrosis.

To reduce the impact of potential confounding factors, we obtained aspirates of subcutaneous adipose tissue from the anterior abdominal wall of 8 women without signs of obesity and comorbid metabolic diseases, as well as the resected body of Hoff from 7 patients during therapeutic and diagnostic arthroscopy, also without signs of obesity. The average age of the patients was (44.0 ± 3.8) years with a body mass index of (20.1 ± 1.6) kg/m2.

Preparation of samples was carried out mechanically and by centrifugation in order to be as close as possible to clinical conditions and to protect cells from enzymatic effects. A 10 ml syringe with a 3 mm diameter almond-shaped cannula was used to perform microliposuction under negative pressure. Previously, the donor site (front wall of the abdominal cavity) was processed and anesthetized according to all principles of surgical intervention. The resulting liposuction aspirate was centrifuged at 1200 × g for 4 min. After fixing the classic layering of the fractions, the upper — oily and lower — vascular layers were removed, and the adipose tissue with mesenchymal cells was preserved as much as possible without mixing. Two 5 mL end-to-end Luer Lock syringes with an internal diameter of 2.4 mm were used for subsequent microfractionation. The fat fraction was crushed by pouring it through a connector until a uniform homogeneous mass was obtained. The obtained fraction was re-centrifuged at 2000 × g for 4 min. After removing the oil and the middle layer of adipose tissue, which consisted mostly of mature adipocytes, the lower layer, rich in mesenchymal cells, was removed. Resuspension was performed using autologous concentrated plasma (ACP).

Preparation of SVF from Hoff bodies followed a similar step except that the final material was less compared to abdominal fat and the collection was performed with a shaver that mechanically minced the tissue, so a second centrifugation was not appropriate and only one was performed at 2000 × g for 4 min. Collection of the lower fraction of enriched MSCs and resuspension was performed according to the described method.

Morphological materials and methods

To determine the composition and histological features of the stromal-vascular fraction obtained from the subcutaneous tissue and the Hoff body, the obtained material was examined by standard histological methods. The obtained microsamples were stained with hematoxylin and eosin. Microscopy was performed with a Scope, A1 «Carl Zeiss» (Germany) device with a Progres Gryphax Jenoptik 60N-C1"1.0x426114 camera (Germany) connected to a personal computer.

Digital analysis program Progres Gryphax 1.1.4.2 (Jenoptik Optical System, Germany) was used. A comparative characterization of the composition of the obtained histological samples was carried out. We performed morphometric measurements of the diameter of adipocytes in 5 fields of view of a microscope with magnification: eyepiece × 10, lens × 20 in 10 samples from each comparison group.

Statistical processing of findings

Statistical processing of findings was carried out using a personal computer. Analyzed using the statistical software Statistica® for Windows 13.0 (StatSoft Inc., license No. JRR709H998119TE-A). Parametric and non-parametric criteria of statistical analysis were applied. Parametric criteria were used to determine the average diameter of adipocytes and the standard deviation. Non-parametric ones were used to test the hypothesis about the normality of the distribution of the studied indicators according to the Shapiro–Wilk test. For all types of analysis, differences were considered significant at $p < 0.05$.

Results and their discussion

White adipose tissue is widely distributed throughout the body and is organized in the so-called «depots». Most of them are subcutaneous, intra-abdominal or visceral. This tissue consists of fat cells, which are also called adipocytes or lipocytes. These are large spherical cells with a diameter of 80–120 μm with a characteristic large lipid vacuole that occupies most of the cytoplasm and displaces the oval or flattened nucleus to the periphery. In conventional hematoxylin-eosin sections, these cells appear empty due to fat dissolution during tissue processing [9]. In the subcutaneous tissue, adipocytes create complexes that are separated by connective tissue partitions [10]. These fibrous septa are continuous with the overlying dermis and contain arterial, venous, and lymphatic vessels and nerves. Each particle is equipped with an arteriole, which divides into branches and capillaries that feed the microparticles and surround individual adipocytes. Postcapillary venules drain into septal veins. There are no capillary connections between neighboring microparticles [9].

The obtained stromal-vascular fraction of subcutaneous adipose tissue was heterogeneous in its composition, consisting of numerous white single-chambered adipocytes, of (107.71 ± 28.22) μm in size, a large number of capillaries located at the point of contact of neighboring adipocytes, reticular fibers surrounding them, a variable number of fibroblasts, individual mast cells, and more pronounced islets of connective tissue between adipocytes with numerous vessels of the microcirculatory bed: arterioles, capillaries, and venules (Fig. 1).

The infrapatellar fat pad (IFP) or Hoff's fat pad is the largest soft tissue structure in the knee joint [11]. It is located between the patellar tendon, femoral condyles and tibial plateau, under the patella. Behind it is covered by a synovial membrane and is closely connected with the articular cartilage, which makes it intracapsular and extrasynovial. IFP shows an extensive network of vascular anastomoses, well innervated, consisting of lobules of white fibrous adipose tissue; for the most part, it is characterized by a significant prevalence of collagenous stroma, characteristic of areas that have a significant mechanical load. Adipocytes in IFP have a significantly smaller cell volume than in subcutaneous adipose tissue [12], in addition, a significant number of fibroblasts, which are responsible for the production of extracellular matrix, immune cells, such as macrophages, mast cells and lymphocytes [13].

According to the results of a microscopic study, the mechanically obtained stromal-vascular fraction of Hoff's fat pad was heterogeneous, consisted of fragments of connective tissue, a large number of vessels of various diameters — from medium-sized arteries to capillaries, venules and veins of medium diameter, groups of preserved white single-chambered adipocytes, with cell diameter of (67.04 ± 10.34) µm, fragments of the synovial membrane, in some samples with signs of proliferation with an array of underlying dense unformed connective tissue, nerve fibers of small and medium caliber, in different numbers of immune cells: mainly lymphocytes, mast cells, macrophages, which had the appearance of focal clusters in the stromal component and perivascular spaces, most often in the subsynovial sections (Fig. 2).

The differentiation capacity of adipose stem cells that have been isolated from different adipose tissue «depots» has been the subject of controversial debate over the last decade, with numerous scientific studies reporting mixed results [14, 15].

SVFs obtained in our study mechanically from subcutaneous adipose tissue and Hoff fat pad differ in composition, namely the amount of the stromal component, which is visually significantly more pronounced in Hoff fat pad SVFs, the size of adipocytes, which in SVFs from subcutaneous adipose tissue were statistically larger diameter ($p < 0.005$). Thus, the thesis is confirmed that the composition of SVF depends on a number of factors, namely: the location of adipose tissue isolation, the treatment method, and the patient's own aabnormal status [16].

The different caliber of vessels in the two stromal-vascular fractions can play a significant role in the effectiveness of their use as a source of mesenchymal stem cells. Adipose tissue is known to be vascularized and has an extensive capillary network that surrounds each adipocyte. Therefore, angiogenic (differentiation) potential is its additional attribute.

Adipose tissue has advantages for obtaining adult stem cells. Although there are visually more large-diameter vessels in Hoff's fat pad, this tissue is also densely vascularized by the superior and inferior popliteal arteries. They are united by three horizontal anastomoses and have an irregular connection with the medial popliteal artery passing in the infrapatellar fold. In addition, there are many anastomoses with the vessels of the menisci and tendons of the patella anteriorly and with the tibial periosteum inferiorly. In this context, the «vascular stem cell theory» is the most relevant, it states that adipose stem cells exist in adipose tissue as a mixed population of «vascular stem cells» (VSCs), their differentiation potential is proportional to the angiogenic potential of the vasculature and fluctuates. VSCs are able to differentiate *in situ* into host tissue-specific cell types depending on their differentiation potential when isolated from the host tissue, and in general ADSCs are likely to be VSCs at different stages of differentiation [17].

The SVF of Hoff's fat pad also includes fragments of the synovial membrane, which is known to have a rich vascular supply. The literature suggests that synovial-derived mesenchymal stem cells originate from these perivascular cells. There is an opinion that the synovial membrane plays an active role in the immunomodulation of the knee joint, especially in the case of osteoarthritis [18]. The benefit of synovial cells corresponds to Kaplan's theory that MSCs

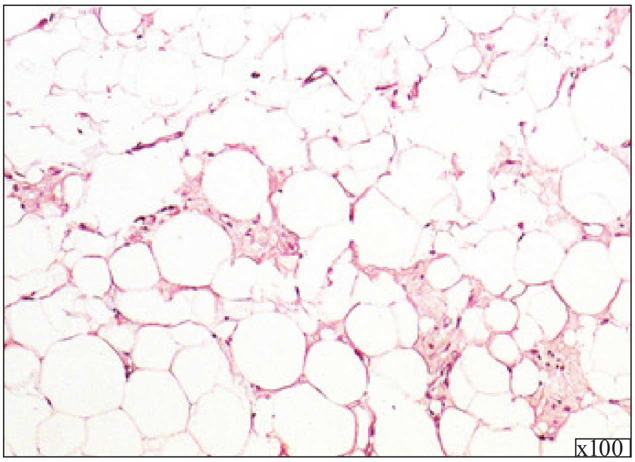

Fig. 1. Stromal-vascular fraction of subcutaneous adipose tissue. H&E stain

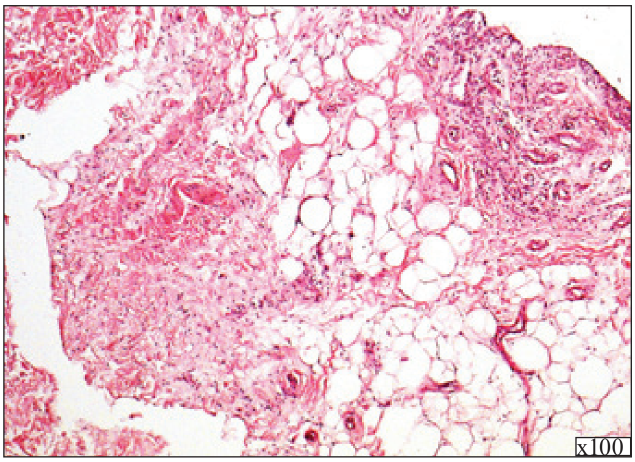

Fig. 2. Stromal-vascular fraction of Hoff's pad. H & E stain

work through growth factor and cytokine secretion rather than engraftment and differentiation [19].

The infrapatellar fat pad is believed to play an important role in the biomechanics of the knee and is also a source of stem cells for regeneration after knee injury. Infrapatellar fat pads-derived adipose stem cells (IPFP-ASCs) have an extended and age-independent differentiation capacity compared to other stem cells, making them a very promising candidate for stem cell-based regenerative therapy.

Conclusions

The stromal-vascular fraction of Hoff's infrapatellar fat pad differs in tissue and cellular composition from the stromal-vascular fraction of subcutaneous adipose tissue.

There is a very important common feature between the stromal-vascular fraction from Hoff's infrapatellar fat pad and subcutaneous adipose tissue, which is why they can be considered as a source of adipose mesenchymal stem cells, and in addition, they are characterized by dense vascularization.

The stromal-vascular fraction of Hoff's infrapatellar fat pad is a promising source of adipose mesenchymal stem cells for regenerative medicine, in particular for cartilage regeneration.

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

References

1. Bacakova, L., Zarubova, J., Travnickova, M., Musilkova, J., Pajorova, J., Slepicka, P., Kasalkova, N. S., Svorcik, V., Kolska, Z., Motarjemi, H., & Molitor, M. (2018). Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells – a review. *Biotechnology Advances, 36* (4), 1111–1126. https://doi.org/10.1016/j.biotechadv.2018.03.011
2. Huang, S.-J., Fu, R.-H., Shyu, W.-C., Liu, S.-P., Jong, G.-P., Chiu, Y.-W., Wu, H.-S., Tsou, Y.-A., Cheng, C.-W., & Lin, S.-Z. (2013). Adipose-Derived Stem Cells: Isolation, Characterization, and Differentiation Potential. *Cell Transplantation, 22*(4), 701–709. https://doi.org/10.3727/096368912x655127
3. Yoshimura, K., Shigeura, T., Matsumoto, D., Sato, T., Takaki, Y., Aiba-Kojima, E., Sato, K., Inoue, K., Nagase, T., Koshima, I., & Gonda, K. (2006). Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *Journal of Cellular Physiology, 208* (1), 64–76. https://doi.org/10.1002/jcp.20636
4. Cutrona, G., Tasso, P., Dono, M., Roncella, S., Ulivi, M., Carpaneto, E. M., Fontana, V., Comis, M., Morabito, F., Spinelli, M., Frascella, E., Boffa, L. C., Basso, G., Pistoia, V., & Ferrarini, M. (2002). CD10 is a marker for cycling cells with propensity to apoptosis in childhood ALL. *British Journal of Cancer, 86* (11), 1776–1785. https://doi.org/10.1038/sj.bjc.6600329
5. Tapp, H., Hanley, E. N., Patt, J. C., & Gruber, H. E. (2009). Adipose-Derived Stem Cells: Characterization and Current Application in Orthopaedic Tissue Repair. *Experimental Biology and Medicine, 234* (1), 1–9. https://doi.org/10.3181/0805-mr-170
6. Kern, S., Eichler, H., Stoeve, J., Klüter, H., & Bieback, K. (2006). Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue. *Stem Cells, 24* (5), 1294–1301. https://doi.org/10.1634/stemcells.2005-0342
7. Munoz-Criado, I., Meseguer-Ripolles, J., Mellado-Lopez, M., Alastrue-Agudo, A., Griffeth, R. J., Forteza-Vila, J., Cugat, R., García, M., & Moreno-Manzano, V. (2017). Human Suprapatellar Fat Pad-Derived Mesenchymal Stem Cells Induce Chondrogenesis and Cartilage Repair in a Model of Severe Osteoarthritis. *Stem Cells International, 2017*, 1–12. https://doi.org/10.1155/2017/4758930
8. Zeng, N., Yan, Z.-P., Chen, X.-Y., & Ni, G.-X. (2020). Infrapatellar Fat Pad and Knee Osteoarthritis. *Aging and disease, 11* (5), 1317. https://doi.org/10.14336/ad.2019.1116
9. Esteve, D., Boulet, N., Belles, C., Zakaroff-Girard, A., Decaunes, P., Briot, A., Veeranagouda, Y., Didier, M., Remaury, A., Guillemot, J. C., Ledoux, S., Dani, C., Bouloumie, A., & Galitzky, J. (2019). Lobular architecture of human adipose tissue defines the niche and fate of progenitor cells. *Nature Communications, 10* (1). https://doi.org/10.1038/s41467-019-09992-3
10. Girousse, A., Gil-Ortega, M., Bourlier, V., Bergeaud, C., Sastourné-Arrey, Q., Moro, C., Barreau, C., Guissard, C., Vion, J., Arnaud, E., Pradere, J.-P., Juin, N., Casteilla, L., & Sengenès, C. (2019b). 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
11. Stephen, J. M., Sopher, R., Tullie, S., Amis, A. A., Ball, S., & Williams, A. (2018). The infrapatellar fat pad is a dynamic and mobile structure, which deforms during knee motion, and has proximal extensions which wrap around the patella. *Knee Surgery, Sports Traumatology, Arthroscopy, 26* (11), 3515–3524. https://doi.org/10.1007/s00167-018-4943-1.
12. 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
13. 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
14. Schoettl, T., Fischer, I. P., & Ussar, S. (2018). Heterogeneity of adipose tissue in development and metabolic function. *The Journal of Experimental Biology, 221* (Suppl 1), jeb162958. https://doi.org/10.1242/jeb.162958
15. Silva, K. R., & Baptista, L. S. (2019). Adipose-derived stromal/stem cells from different adipose depots in obesity development. *World Journal of Stem Cells, 11* (3), 147–166. https://doi.org/10.4252/wjsc.v11.i3.147
16. Sun, Y., Chen, S., Zhang, X., & Pei, M. (2019). Significance of Cellular Cross-Talk in Stromal Vascular Fraction of Adipose Tissue in Neovascularization. *Arteriosclerosis, Thrombosis, and Vascular Biology, 39* (6), 1034-1044. https://doi.org/10.1161/atvbaha.119.312425
17. Lin, G., Garcia, M., Ning, H., Banie, L., Guo, Y.-L., Lue, T. F., & Lin, C.-S. (2008). Defining Stem and Progenitor Cells within Adipose Tissue. *Stem Cells and Development, 17* (6), 1053–1063. https://doi.org/10.1089/scd.2008.0117
18. Harvanova, D., Matejova, J., Slovinska, L., Lacko, M., Gulova, S., Fecskeova, L. K., Janockova, J., Spakova, T., & Rosocha, J. (2022). The Role of Synovial Membrane in the Development of a Potential In Vitro Model of Osteoarthritis. *International Journal of Molecular Sciences, 23* (5), 2475.

https://doi.org/10.3390/ijms23052475
19. Caplan, A. I., & Dennis, J. E. (2006). Mesenchymal stem cells as trophic mediators. *Journal of Cellular Biochemistry, 98* (5), 1076–1084. https://doi.org/10.1002/jcb.20886

The article has been sent to the editors 09.08.2023

MORPHOLOGICAL COMPARISON OF THE STROMAL-VASCULAR FRACTION OF THE SUBCUTANEOUS FAT CELL AND INFRAPATELLAR FAT PAD

S. O. Maslennikov, Yu. M. Avramenko, M. L. Golovakha

Zaporizhzhya State Medical and Pharmaceutical University. Ukraine

✉ Serhiy Maslenikov, MD, PhD: travmatology1@i.ua
✉ Yulia Avramenko, MD, PhD: juliya_avramenko@ukr.net
✉ Maxim Golovakha, MD, Prof. in Traumatology and Orthopaedics: golovahaml@gmail.com