charide (LPS) challenge. Immunol. Lett. V. 163. P. 187. https://doi.org/10.1016/j.imlet.2014.10.019

Bunggulawa E.J., Wang W., Yin T., Wang N., Durkan C., Wang Y., Wang G. 2018. Recent advancements in the use of exosomes as drug delivery systems. J. Nanobiotech. V. 16. P. 81. https://doi.org/10.1186/s12951-018-0403-9

Caby M.P., Lankar D., Vincendeau-Scherrer C., Raposo G., Bonnerot C. 2005. Exosomal-like vesicles are present in human blood plasma. Int. Immunol. V. 17. P. 879. https://doi.org/10.1093/intimm/dxh267

Colombo M., Raposo G., Théry C. 2014. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell. Dev. Biol. V. 30. P. 255. https://doi.org/10.1146/annurev-cellbio-101512-122326

Conde-Vancells J., Rodriguez-Suarez E., Gonzalez E., Berisa A., Embade N., Gil D., Matthiesen R., Valle M., Elortza F., Wagner C., Lu S.C., Mato J.M., Falcon-Perez J.M. 2010. Candidate biomarkers in exosome-like vesicles purified from rat and mouse urine samples. Proteomics Clin. Appl. V. 4. P. 416. https://doi.org/10.1002/prca.200900103

Keerthikumar S., Chisanga D., Ariyaratne D., Al Saffar H., Anand S., Zhao K., Samuel M., Pathan M., Jois M., Chilamkurti N., Gangoda L., Mathivanan S. 2016. ExoCarta: A web-based compendium of exosomal cargo. J. Mol. Biol. V. 428. P. 688. https://doi.org/10.1016/j.jmb.2015.09.019

Keller S., Rupp C., Stoeck A., Runz S., Fogel M., Lugert S., Hager H.D., Abdel-Bakky M.S., Gutwein P., Altevogt P. 2007. CD24 is a marker of exosomes secreted into urine and amniotic fluid. Kidney Int. V. 72. P.1095. https://doi.org/10.1038/sj.ki.5002486

Lehrich B.M., Liang Y., Khosrav P., Federoff H.J., Fiandac M.S. 2018. Fetal bovine serum-derived extracellular vesicles persist within vesicle-depleted culture media. Int. J. Mol. Sci. V. 19. P. 3538. https://doi.org/10.3390/ijms19113538

Marcos-Vadillo E. García-Sánchez A. 2016. Cell culture techniques: corticosteroid treatment in A549 human lung epithelial cell. Methods Mol. Biol. V. 1434. P. 169. https://doi.org/10.1007/978-1-4939-3652-6_12

Michael A., Bajracharya S.D., Yuen P.S.T., Zhou H., Star R.A., Illei G.G., Alevizos I. 2010. Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis. V. 16. P. 34. https://doi.org/10.1111/j.1601-0825.2009.01604.x

Palmirotta R., Lovero D., Cafforio P., Felici C., Mannavola F., Pellè E., Quaresmini D., Tucci M., Silvestris F. 2018. Liquid biopsy of cancer: A multimodal diagnostic tool in clinical oncology. Ther. Adv. Med. Oncol. V. 10. P. 758835918794630. https://doi.org/10.1177/1758835918794630

Prado N., Marazuela E.G., Segura E., Fernández-García H., Villalba M., Théry C., Rodríguez R., Batanero E. 2008. Exosomes from bronchoalveolar fluid of tolerized mice prevent allergic reaction. J. Immunol. V. 181. P. 1519. https://doi.org/10.4049/jimmunol.181.2.1519

Purushothaman A., Bandari S.K., Liu J., Mobley J. A., Brown E.E., Sanderson R.D. 2016. Fibronectin on the surface of myeloma cell-derived exosomes mediates exosome-cell interactions. J. Biol. Chem. V. 291. P. 1652. https://doi.org/10.1074/jbc.M115.686295

Samanta S., Rajasingh S., Drosos N., Zhou Z., Dawn B., Rajasingh J. 2018. Exosomes: New molecular targets of diseases. Acta Pharmacol. Sin. V. 39. P. 501. https://doi.org/10.1038/aps.2017.162

Shelke G.V. Lässer C., Gho Y.S. Lötvall J. 2014. Importance of exosome depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. J. Extracell. Ves. V. 3. P. 24783. https://doi.org/10.3402/jev.v3.24783

Stoorvogel W., Kleijmeer M.J., Geuze H.J., Raposo G. 2002. The biogenesis and functions of exosomes. Traffic. V. 3. P. 321. https://doi.org/10.1034/j.1600-0854.2002.30502.x

Vlassov A.V., Magdaleno S., Setterquist R., Conrad R. 2012. Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim. Biophys. Acta. V. 1 820. P. 940. https://doi.org/10.1016/j.bbagen.2012.03.017

Whiteside T.L. 2015. The potential of tumor-derived exosomes for noninvasive cancer monitoring. Expert Rev. Mol. Diagn. V. 15. P. 1293. https://doi.org/10.1586/14737159.2015.1071666

Zhou Q., Xie F., Zhou B., Li C., Kang Y., Wu B., Li L., Dai R. 2020. Fetal bovine serum-derived exosomes regulate the adipogenic differentiation of human bone marrow mesenchymal stromal cells in a cross-species manner. Differentiation. V. 115. P. 11. https://doi.org/10.1016/j.diff.2020.06.004

Comparative Analysis of Methods for Isolating Exosomes from the Culture Medium

A. N. Gorshkov[a, *], L. V. Purvinsh[a, b], A. V. Protasov[a, b], P. A. Nekrasov[a], B. A. Shaldzhyan[a], and A. V. Vasin[a, b]

[a] *Smorodintsev Research Institute of Influenza, Ministry of Health of the Russian Federation, St. Petersburg, 197376 Russia*

[b] *Peter the Great St. Petersburg Polytechnic University, St. Petersburg, 195251 Russia*

**e-mail: angorsh@yahoo.com*

Exosomes are extracellular membrane vesicles with a diameter of 40–100 nm. They are formed as invaginations of the membrane of the late endosome, with the release of the vesicles into the lumen of the multivesicular body. The fusion of the multivesicular body with the plasma membrane leads to the secretion of exosomes into the extracellular environment. Exosomes are a key vehicle for intercellular communication. They are used to transfer a wide range of biologically active molecules, including lipids, proteins, mRNA and microRNA. The transfer of these molecules to recipient cells regulates their functions normally and contributes to the pathogenesis of many diseases. The unique features of the molecular composition of exosomes make them a potentially important diagnostic and prognostic marker in medicine. The communicative function of exosomes allows them to be considered as a promising delivery system for therapeutic drugs, including the latest gene therapy tools. For the practical implementation of these pos-

sibilities, fully reproducible, standardized protocols for obtaining highly purified exosome preparations from various biological fluids and culture media, known for their complex heterogeneous composition, are required. To date, a number of methods have been proposed for the isolation and purification of exosomes. Nevertheless, there is currently no standard approach that allows one to obtain pure preparations fully suitable for the subsequent application of highly sensitive methods for the analysis of exosomal proteins and RNA. In this work, we compared the efficiency of exosome isolation from the cell culture medium by three methods: 1) ultracentrifugation, 2) concentration by tangential flow filtration followed by gel filtration, 3) precipitation using the commercial Total Exosome Isolation Reagent. Evaluation of the quality of exosome samples obtained by the above methods included transmission electron microscopy, dynamic light scattering and detection of the marker exosomal protein annexin A2 by Western blotting. According to our data, the highest purity of exosome preparations is achieved when they are concentrated by tangential flow filtration followed by gel filtration.

Keywords: exosomes, ultracentrifugation, gel filtration