Grogan S.P., Rieser F., Winkelmann V., Berardi S., Mainil-Varlet P. 2003. A static, closed and scaffold-free bioreactor system that permits chondrogenesis in vitro. Osteoarthritis and Cartilage. V. 11. P. 403.
https://doi.org/10.1016/s1063-4584(03)00053-0

Kesti M., Eberhardt C., Pagliccia G., Kenkel D., Grande D., Boss A., Zenobi-Wong M. 2015. Bioprinting complex cartilaginous structures with clinically compliant biomaterials. Adv. Funct. Mater. V. 25. P. 7406.
https://doi.org/10.1002/adfm.201503423

Levato R., Webb W.R., Otto I.A., Mensinga A., Zhang Y., van Rijen M., van Weeren R., Khan I.M., Malda J. 2017. The bio in the ink: cartilage regeneration with bioprintable hydrogels and articular cartilage-derived progenitor cells. Acta Biomater. V. 1. P. 41.
https://doi.org/10.1016/j.actbio.2017.08.005

Lewis M.C., Macarthur B.D., Malda J., Pettet G., Please C.P. 2005. Heterogeneous proliferation within engineered cartilaginous tissue: the role of oxygen tension. Biotechnol. Bioeng. V. 91. P.607.
https://doi.org/10.1002/bit.20508

Liu C.Z., Xia Z.D., Han Z.W., Hulley P.A., Triffitt J.T., Czernuszka J.T. 2008. Novel 3d collagen scaffolds fabricated by indirect printing technique for tissue engineering. J. Biomed. Mater. Res. B Appl. Biomater. V. 85. P. 519.
https://doi.org/10.1002/jbm.b.30975

Malda J., Woodfield T.B., Vloodt F.V., Kooy F.K., Martens D.E., Tramper J., Blitterswijk C.A, Riesle J.U. 2004. The effect of PEGT/PBT scaffold architecture on oxygen gradients in tissue engineered cartilaginous constructs. Biomaterials. V. 25. P. 5773.
https://doi.org/10.1016/j.biomaterials.2004.01.028

Murphy S.V., Atala A. 2014. 3D bioprinting of tissues and organs. Nat. Biotechnol. V. 32. P. 773.
https://doi.org/10.1038/nbt.2958

Murphy S.V., De Coppi P., Atala A. 2020. Opportunities and challenges of translational 3d bioprinting. Nat. Biomed. Eng. V. 4. P. 370.
https://doi.org/10.1038/s41551-019-0471-7

Nguyen D., Hägg D.A, Forsman A., Ekholm J., Nimkingratana P, Brantsing C., Kalogeropoulos T., Zaunz S., Concaro S., Brittberg M., Lindahl A., Gatenholm P., Enejder A., Simonsson S. 2017. Cartilage tissue engineering by the 3d bioprinting of ips cells in a nanocellulose/alginate bioink. Sci. Rep. V. 7. P. 658.
https://doi.org/10.1038/s41598-017-00690-y

Okubo R., Asawa Y., Watanabe M., Nagata S., Nio M., Takato T., Hikita A., Hoshi K. 2019. Proliferation medium in three-dimensional culture of auricular chondrocytes promotes effective cartilage regeneration *in vivo*. Regen. Ther. V. 15. P. 306.
https://doi.org/10.1016/j.reth.2019.10.002

Osidak E.O., Karalkin P.A., Osidak M.S., Parfenov V.A., Sivogrivov D.E., Pereira A.S., Gryadunova A.A., Koudan E.V., Khesuani Y.D., Kasyanov V.A., Belousov S.I., Krasheninnikov S.V., Grigoriev T.E., Chvalun S.N., Bulanova E.A. et al. 2019. Viscoll collagen solution as a novel bioink for direct 3d bioprinting. J. Mater. Sci. Mate. Med. V. 30. P. 31.
https://doi.org/10.1007/s10856-019-6233-y

Radisic M., Malda J., Epping E., Geng W., Langer R., Vunjak-Novakovic G. 2005. Oxygen gradients correlate with cell density and cell viability in engineered cardiac tissue. Biotechnol.Bioeng. V. 93. P. 332.
https://doi.org/10.1002/bit.20722

Ren X., Wang F., Chen C., Gong X., Yin L., Yang L. 2016. Engineering zonal cartilage through bioprinting collagen type ii hydrogel constructs with biomimetic chondrocyte density gradient. BMC Musculoskelet Disord. V. 20. P. 301.
https://doi.org/10.1186/s12891-016-1130-8

Rowland C.R., Lennon D.P., Caplan A.I., Guilak F. 2013. The effects of crosslinking of scaffolds engineered from cartilage ECM on the chondrogenic differentiation of MSCs. Biomaterials. V. 34. P. 5802.
https://doi.org/10.1016/j.biomaterials.2013.04.027

Schuurman W., Levett P.A., Pot M.W., Weeren P., Dhert W.J.A., Hutmacher D.W., Melchels F.P.W., Klein T.J., Malda J. 2013. Gelatin-methacrylamide hydrogels as potential biomaterials for fabrication of tissue-engineered cartilage constructs. Macromol. Biosci. V. 13. P. 551.
https://doi.org/10.1002/mabi.201200471

Skardal A., Devarasetty M., Kang H.W., Mead I., Bishop C., Shupe T., Lee S.J., Jackson J., Yoo J., Soker S., Atala A. 2015. A Hydrogel bioink toolkit for mimicking native tissue biochemical and mechanical properties in bioprinted tissue constructs. Acta Biomater. V. 25. P. 24.
https://doi.org/10.1016/j.actbio.2015.07.030

Zhang Y., Zhou D., Chen J., Zhang X., Li X., Zhao W., Xu T. 2019. Biomaterials based on marine resources for 3d bioprinting applications. Mar. Drugs. V. 17. P. 555.
https://doi.org/10.3390/md17100555

The Use of Collagen in a High Concentration for the Engeineering of Cartilage Tissue with 3D-Bioprinting

E. V. Isaeva[a,] *, E. E. Beketov[a], V. V. Yuzhakov[a], N. V. Arguchinskaya[a], A. A. Kisel[a], E. P. Malakhov[a], T. S. Lagoda[a], N. D. Yakovleva[a], P. V. Shegai[b], S. A. Ivanov[a], and A. D. Kaprin[b]

[a]*Tsyb MRRC — Branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation, Obninsk, 249036 Russia*

[b]*National Medical Research Radiological Center of the Ministry of Health of the Russian Federation, Obninsk, 249036 Russia*

**e-mail: e1esa@yandex.ru*

3D-bioprinting is a promising technology for tissue engineering structure formation in the case of damaged tissue and organ replacement. Collagen is one of the most appropriate hydrogels for the purpose, due to its exceptional biocompatibility. However, the use of collagen with conventionally low concentration makes bioprinting difficult and does not provide high accuracy. The purpose of this study was evaluation of suitability of collagen with high concentration in case of chondrocyte-laden scaffold fabrication via 3D-bioprinting for cartilage formation *in vitro* and *in vivo*. Immediately after the beginning of the scaffold incubation, cell migration to the surface and out of the scaffold was observed. The results of the study showed that the 4% porosity inherent for collagen is not enough for cells to survive in case of long-term incubation *in vitro*. The cells remaining in the collagen mostly died within 4 weeks. As for *in vivo* study, 2 weeks after implantation of the scaffold, a weak granulomatous inflammation was observed. After 6 weeks, connective tissue was formed in the area of implantation, where macrophages and the groups of small cells with rounded nuclei were found. In accordance with morphological criteria, these cells could be considered as young chondrocytes. However, their amount was not sufficient to start the formation of cartilage tissue.

Keywords: tissue engineering, 3D-bioprinting, scaffold, bio-ink, collagen, chondrocytes, histology, immunohistochemistry