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EVALUATION OF THE EFFECT OF MESENCHYMAL STROMAL CELLS FROM DIFFERENT SOURCES ON HUMAN CHONDROCYTE PROLIFERATION

A.D. Belova[1], Yu.B. Basok[1], A.M. Grigoriev[1], L.A. Kirsanova[1], E.G. Kuznetsova[1], I.V. Vakhrushev[2], A.V. Kovalev[3], K.N. Yarygin[2], V.I. Sevastianov[1]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Institute of Biomedical Chemistry, Moscow, Russian Federation

[3] Priorov National Medical Research Center for Traumatology and Orthopedics, Moscow, Russian Federation

Objective: to study the effect of a conditioned medium of mesenchymal stromal cells (MSCs) from different sources on human chondrocyte proliferation. **Materials and methods.** To confirm functional activity, chondrocytes were cultured in a cartilage cell-engineered construct (CEC), including 5×10^5 cells and 5 mg of tissue-specific matrix from decellularized cartilage. The conditioned medium was obtained after culturing MSCs derived from human adipose tissue (AT), MSCs derived from the pulp of primary teeth and MSCs isolated from umbilical cord-derived Wharton's jelly in a complete cell growth medium (CCGM). To evaluate the effect of MSC-derived secretome on chondrocyte proliferation, the conditioned medium, diluted 1 : 1 with CCGM, was added to wells containing chondrocytes. The effect of MSCs on human chondrocyte proliferation was studied by indirectly co-culturing cells in CCGM using Transwell inserts. 5×10^4 MSCs were applied to the bottom of the lower chamber, and 5×10^4 human chondrocytes and 5 mg of matrix were placed in the upper chamber. Chondrocyte proliferation was assessed at days 7 and 14 by DNA quantification. Interleukin-6 content was determined as a marker of secretory activity of MSCs in the conditioned medium. The morphology of the samples was studied using histological staining methods. **Results.** The ability of chondrocytes to produce cartilage-specific extracellular matrix was confirmed when forming cartilage CEC with tissue-specific matrix in a chondrogenic differentiation medium. When comparing the effect of the conditioned medium of MSCs obtained from different sources on the growth of human chondrocytes in vitro, increased proliferation was observed in all samples compared to controls. Indirect co-culture of MSCs with chondrocytes as part of CEC showed increased DNA amount in all samples at day 14, with the amount of DNA in the sample with MSC conditioned medium significantly higher than the control. **Conclusion.** Studies on the effect of MSC conditioned medium on chondrocyte proliferation in 2D culture indicate a possible regenerative potential of MSCs for cartilage tissue repair. Within the scope of this work, we did not identify significant differences in the effect of secretome derived from MSCs that were obtained from different sources on chondrocyte proliferation. However, additional in vivo studies are warranted in the future.

Keywords: cartilage tissue, mesenchymal stromal cells, conditioned medium, tissue engineering.

INTRODUCTION

Osteoarthritis (OA) is a disease resulting in "joint failure". It is based on destructive structural changes in the hyaline cartilage with subsequent degenerative processes of the underlying bone [1, 2]. OA incidence worldwide is rising every year, and this has seen an increasing level of disability globally [3].

Articular cartilage is composed of chondrocytes and extracellular matrix (ECM). The ECM is composed of collagens (mainly type II collagen), proteoglycans, and non-collagenous proteins [4]. In the early stages of knee OA, there are changes in the structure of collagen and proteoglycans, leading to articular cartilage erosion. In response to cartilage erosion, chondrocytes undergo a phase of hypertrophic activity, producing inflammatory mediators that promote further degradative changes in articular cartilage. The final stage is chondrocyte apoptosis, shifting the balance between synthesis and catabolism of collagen and proteoglycans toward catabolism. Expression of type II collagen, one of the prominent components of cartilage, decreases during the growth of chondrocytes; therefore, mature chondrocytes are unable to produce type II collagen *de novo* [3, 5–7]. The situation is aggravated by the lack of blood supply and low

Corresponding author: Alexandra Belova. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (963) 633-94-34. E-mail: sashak1994@mail.ru

metabolic rate in cartilage, leading to its limited ability to self-repair [8, 9].

Treatment modalities for OA include symptomatic therapy consisting of the use of analgesics, as well as radical surgical intervention [10, 11]. However, the use of such methods does not always result in desired outcomes [12]. Therefore, in recent years, there has been a great interest in less invasive but more promising cellular technologies for cartilage structure restoration and OA treatment.

Over the past few decades, mesenchymal stem cells (MSCs) have been in the center of attention due to their high therapeutic potential. MSC cell therapy is used to treat various diseases, including OA, which affects 13% of the Russian population over 18 years of age [13–16]. The advantages of MSCs include their high chondrogenic potential, wide availability of sources for isolation (bone marrow, adipose tissue, pulp of primary teeth, Wharton's jelly, umbilical cord), as well as the fact that MSCs do not induce graft-versus-host disease [17–22].

Although MSCs are present in many tissues, their total count in the body is small, while cell therapy protocols typically require hundreds of millions of MSCs per treatment course, which requires additional time to culture them *in vitro*. Studies have shown that the implantation time of MSCs is usually too short to have an effective therapeutic effect [22]. In addition, several studies indicate that MSCs have a low survival rate (<1%) one week after their administration [23]. This suggests that the main effects of MSCs are based on paracrine mechanisms mediated by the production and secretion of a wide range of cytokines, chemokines, and growth factors by MSCs [24].

In this regard, the use of cell-free preparations based on MSC-derived secretome – conditioned media – is of great interest. *In vivo* models have demonstrated that a conditioned medium of MSCs obtained from various sources is as effective as transplantation of the corresponding MSCs [22]. It is hypothesized that the MSC-derived secretome will be able to stimulate the intrinsic regenerative potential of cartilage through secretion of various molecules such as interleukin (IL)-1β, IL-6, IL-10, vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE2), transforming growth factor (TGF-β), and others [25].

Note that such a method is aimed not only at symptomatic treatment or at slowing down the development of OA, but most importantly at restoring the structure of cartilage tissue. Moreover, the advantage of using cell-free preparations is immunocompatibility, which excludes the choice of donors and recipients in therapy [26]. At the same time, MSCs have different characteristics depending on their origin, and the ideal source of MSCs for use in the treatment of knee OA has not yet been determined.

The aim of this work was to study the effect of a conditioned medium of MSCs obtained from different sources on human chondrocyte proliferation.

MATERIALS AND METHODS

Cell isolation

Cultures of MSCs isolated from umbilical cord-derived Wharton's jelly and MSCs derived from the pulp of primary teeth (PPT-derived MSCs) were obtained from the collection of cell cultures of the cell biology laboratory of the Institute of Biomedical Chemistry, Moscow. Human costal cartilage chondrocytes were obtained from the collection of cell cultures of the Priorov National Medical Research Center for Traumatology and Orthopedics, Moscow. The source of MSCs derived from human adipose tissue (hAT-derived MSCs) was subcutaneous adipose tissue from a healthy donor obtained with informed voluntary consent.

The phenotype of PPT-derived MSCs, hAT-derived MSCs, and Wharton's jelly MSCs was investigated for multipotency criteria by flow cytometry in previous studies [27].

Culture of chondrocytes on tissue-specific matrix

To confirm the ability to form a cell-engineered construct (CEC) of cartilage, human chondrocytes were cultured on a tissue-specific decellularized porcine cartilage scaffold, which is close in composition to natural ECM and was obtained according to our previously developed method [28].

The cartilage CECs consisted of 5×10^5 cells and 5 mg of matrix. The matrix was populated with cells by spinning in test tubes with the culture medium on a Multi Bio 3D programmable shaker (Biosan, Latvia). The first 5 days, CEC were cultured in a complete cell growth medium (CCGM) containing DMEM/F12 (PanEco, Russia) (1:1) supplemented with 10% fetal bovine serum (Cytiva, USA), 1% antibiotic/antimycotic solution (Thermo Fisher Scientific, USA) and 2 mM L-glutamine (PanEco, Russia). The CCGM was then replaced with chondrogenic differentiation medium containing DMEM/high Glucose, supplemented with GlutaMAX (Thermo Fisher Scientific, USA), 10% ITS+ (Corning, USA), 1% sodium pyruvate (Thermo Fisher Scientific, USA), 0.25% L-ascorbic acid 2-phosphate (Sigma-Aldrich, USA), 0.0001% dexamethasone (Merck, FRG), 0.002% TGF-β1 (Thermo Fisher Scientific, USA) and 1% antibiotic/antimycotic solution (Thermo Fisher Scientific, USA). The medium was changed every three days.

Cell viability in the CEC was assessed by fluorescent staining using the LIVE/DEAD dye (Thermo Fisher Scientific, USA) and Leica DMi8 Thunder microscope (Leica Microsystems, Germany).

CEC morphology was examined on day 21 of culture using histological staining.

Obtaining conditioned medium

hAT-derived MSCs, PPT-derived MSCs, umbilical cord-derived Wharton's jelly MSCs and human chondrocytes were cultured in 75 cm^2 vials. Third-passage cells were used for the experiment. The CCGM was replaced every three days. The conditioned medium was collected on day 10 of culture (when the monolayer confluency reached >70%) before the experiment and stored at +4 °C. The degree of cell monolayer confluency was determined visually using a Nikon Eclipse TS100 inverted light microscope (Nikon, Japan).

Study of chondrocyte proliferation in 2D culture

To evaluate the effect of the conditioned media of different types of MSCs, chondrocytes were cultured in 24-well plates. A conditioned medium diluted 1:1 with CCGM was introduced into wells containing chondrocytes (3000 cells per well). Intravital observation of cells and photography, as well as determination of the degree of confluency of the chondrocyte monolayer, were performed using the IncuCyte Zoom System (Essen BioScience, USA) for intravital observation of cells and analysis of dynamic processes in the culture medium. The conditioned medium of chondrocytes diluted 1:1 with CCGM served as a control.

Co-culture of chondrocytes and mesenchymal stromal cells

The effect of MSCs on human chondrocyte proliferation was studied by indirect cell co-culture in CCGM using a Transwell plate with polycarbonate membrane inserts for 24-well plates with a 3 μm pore size (Corning, USA). 5×10^4 MSCs were plated on the bottom of the lower chamber. In the upper chamber, 5×10^4 human chondrocytes and 5 mg of tissue-specific decellularized porcine cartilage scaffold were placed. Transwell plates containing chondrocytes in both chambers served as control. Chondrocyte proliferation on days 7 and 14 was assessed by DNA quantification using fluorescent dye Quant-iT PicoGreen (Thermo Fisher Scientific, USA).

DNA quantification

DNA was isolated using the DNeasy Blood&Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions. For DNA measurement, Quant-iT Picogreen kit (Thermo Fisher Scientific, USA) was used according to the manufacturer's instructions and a Spark 10M plate reader (Tecan Trading, Switzerland) at 520 nm wavelength.

Quantification of IL-6 level in the conditioned medium

As a marker of secretory activity of MSCs in conditioned medium, we determined the level of cytokine interleukin-6 (IL-6) by solid-phase enzyme-linked immunosorbent assay (Vector-Best, Russia). The method is based on a three-step analysis using mono- and polyclonal antibodies to IL-6. The procedure for the analysis is recommended by the manufacturer in the kit instructions. A plate reader was used to quantify IL-6 level at 450 nm wavelength.

Histological examination

The samples were fixed in a 10% formalin solution, washed in running water and dehydrated in alcohols of increasing concentration, kept in an ethanol + chloroform mixture, then in chloroform and embedded in paraffin. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin, Alcian blue, and Masson's trichrome. Analysis and photography of the preparations were carried out using an inverted Nikon Eclipse Ti microscope (Nikon, Japan).

RESULTS AND DISCUSSION

The immunophenotypic profile of marker expression in PPT-derived MSCs, hAT-derived MSCs and Wharton's jelly MSCs was investigated by us in previous work, and met the International Society for Cell & Gene Therapy (ISCT) criteria for multipotency of MSCs [29]. All primary cultures were characterized by high expression of CD29, CD44, CD49b, CD73 and CD90, while no expression of CD34, CD45 or HLA-DR was observed [27].

Staining of CEC with fluorescent dye LIVE/DEAD revealed a significant mass of viable chondrocytes on the surface of the matrix from decellularized cartilage (Fig. 1).

When chondrocytes were cultured in the differentiation medium as part of cartilage CEC on day 21 of cultivation, we observed the formation of large clusters of cartilage microparticles united by cells – conglomerates (Fig. 2). Cells were visualized on the surface of all cartilage microparticles, and the entire cell population was characterized by polymorphism. Thus, fibroblast-like cells could be detected in the periphery, whereas round-shaped cells were distributed in the central zone. In addition, cell growth was accompanied by significant ECM production.

The specimens were fixed in 10% formalin solution, washed in running water and dehydrated in alcohols of

ascending concentration, incubated in a mixture of ethanol and chloroform, then in chloroform and embedded in paraffin. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin, alcian blue, and Masson's trichrome. The preparations were analyzed and photographed using a Nikon Eclipse Ti inverted microscope (Nikon, Japan).

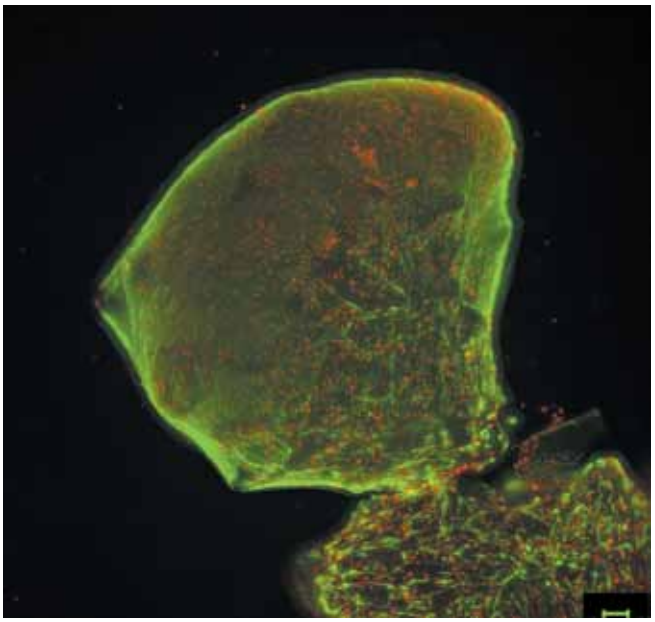

Fig. 1. Examination of human chondrocyte viability in cartilage CEC at day 21 of culturing. LIVE/DEAD staining. Scale bar: 100 μm

Due to their regenerative properties, MSC-derived secretome is considered as a promising treatment for articular cartilage diseases [30]. However, the composition and effect of a conditioned medium of MSCs will differ depending on the source of cells, and the methods and conditions of their cultivation. Therefore, we compared the effects of a conditioned medium, derived from MSCs that were obtained from different sources, on the growth of human chondrocyte culture *in vitro*.

Images obtained using a lifetime cell imaging system demonstrate an increase in the number of chondrocytes on the culture plate over time. Cell count in all samples did not differ significantly at day 24 of culturing. At both day 7 and 14 of observation, a decrease in proliferation could be observed in the control sample (CCGM diluted 1:1 with conditioned medium from chondrocytes) compared to the samples of the experimental groups.

Chondrocyte monolayer confluency on day 7, when cultured in the presence of a conditioned medium of PPT-

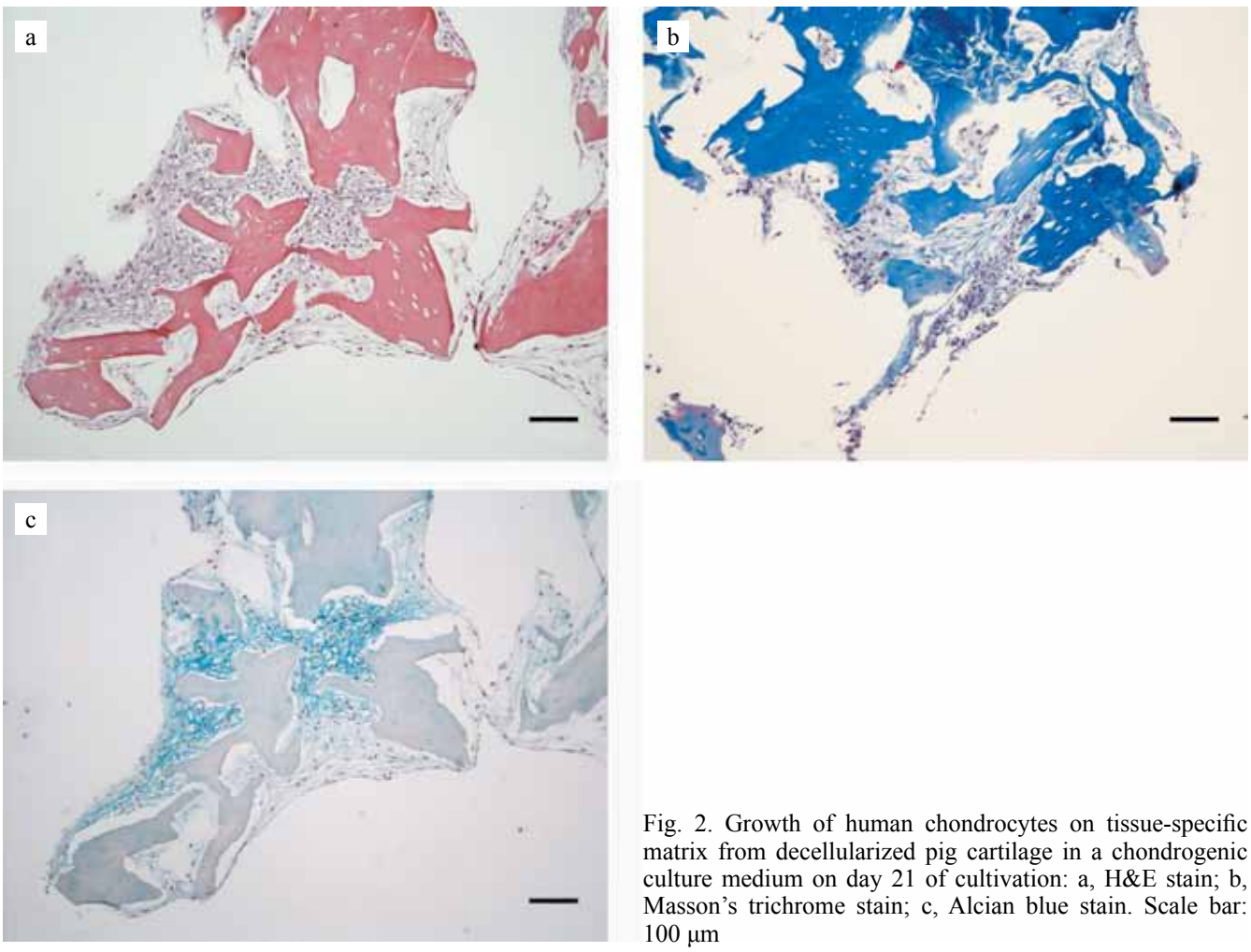

Fig. 2. Growth of human chondrocytes on tissue-specific matrix from decellularized pig cartilage in a chondrogenic culture medium on day 21 of cultivation: a, H&E stain; b, Masson's trichrome stain; c, Alcian blue stain. Scale bar: 100 μm

derived MSCs and hAT-derived MSCs was 1.2 times higher than that of the control sample, and 1.4 times when cultured in the presence of a conditioned medium of umbilical cord-derived Wharton's jelly MSCs. On day 14, monolayer confluency in the experimental samples was 1.2 times higher than that of the control (Fig. 4).

Table presents data on the influence of the paracrine effect of MSCs of different origins on the quantitative

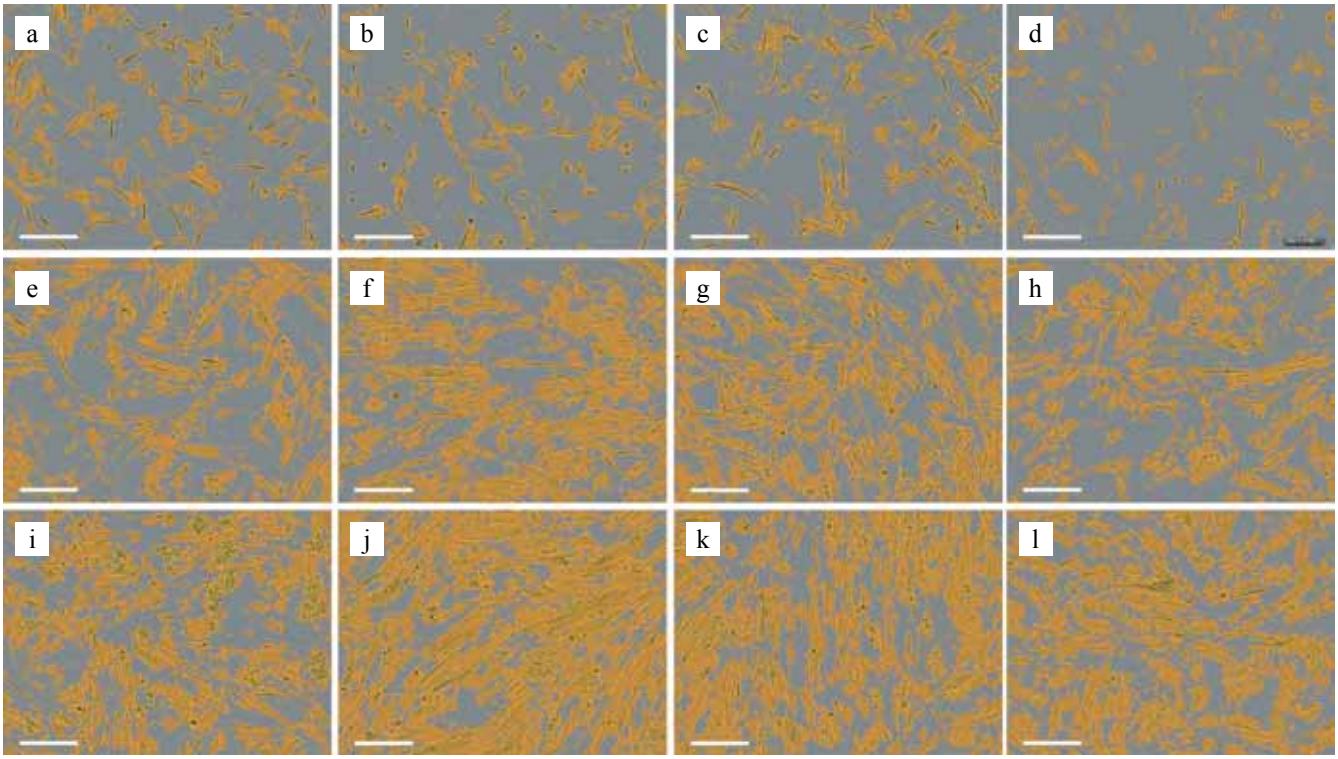

Fig. 3. Effect of conditioned medium on human chondrocyte proliferation in a 2D culture. (a, b, c, d), 24 hours; (e, f, g, h), day 7; (i, j, k, l), day 14; (a, e, i), conditioned medium of dental pulp MSCs; (b, f, j), conditioned medium of AT-MSCs; (c, g, k), conditioned medium of umbilical cord-derived Wharton's jelly MSCs; (d, h, l), conditioned medium of chondrocytes (control). Scale bar: 300 μm

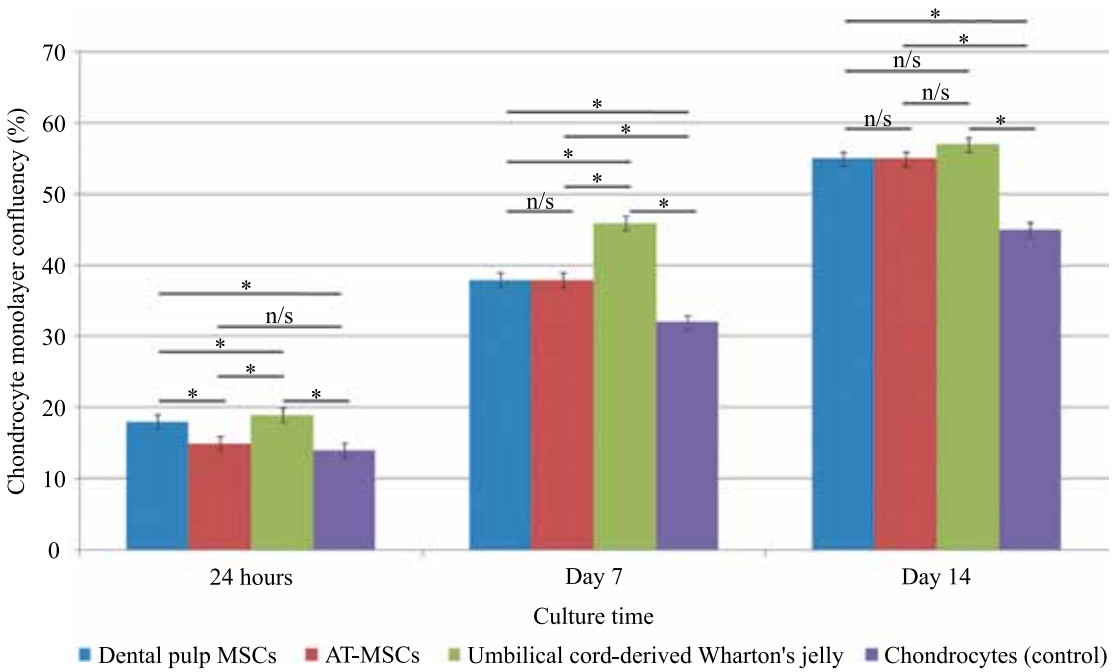

Fig. 4. Chondrocyte monolayer confluency in the presence of conditioned media from MSCs obtained from different sources (n/s, no statistically significant differences; *, there are differences at $p < 0.05$)

Table

Chondrocyte proliferation on decellularized porcine cartilage at days 7 and 14 of indirect co-culture in Transwell with mesenchymal stromal cells from different sources

Culture time	DNA (μg/CEC)			
	PPT-derived MSCs	hAT-derived MSCs	Umbilical cord-derived Wharton's jelly MSCs	Chondrocytes (control)
Day 7	0.57 ± 0.25	0.36 ± 0.14	0.80 ± 0.11	0.51 ± 0.25
Day 14	1.20 ± 0.09	1.34 ± 0.15	1.24 ± 0.27	0.90 ± 0.26

DNA content in CECs consisting of chondrocytes and tissue-specific matrix from decellularized porcine cartilage when they are indirectly co-cultured in Transwell cells. On day 7, the least amount of DNA was found in samples with conditioned medium of hAT-derived MSCs, while on day 14, all samples showed cell growth and, consequently, increased amount of DNA. DNA amount in the sample with conditioned medium of hAT-derived MSCs was significantly higher than that of the control ($p < 0.05$).

On day 14 of culture, the differences between the samples were insignificant, which may indicate a relatively similar effects of the conditioned medium of different MSCs on chondrocyte proliferation in this experiment.

Many research works have revealed the fundamental role of MSC-derived secretome as an active ingredient that can modulate cellular responses and signaling pathways, thereby promoting tissue repair [31]. One of the components of MSC-derived secretome is IL-6, a multifunctional cytokine that is an important factor in various physiological processes, including immune regulation, hematopoiesis and inflammation, and also modulates cell proliferation, differentiation and apoptosis [32].

To identify differences in cytokine secretion between PPT-derived MSCs, hAT-derived MSCs and Wharton's jelly MSCs, the IL-6 levels in the conditioned medium on days 1, 3 and 6 of culture were compared. So, on days 3 and 6 of culture, IL-6 levels in all samples were almost twofold higher (day 3: PPT-derived MSCs – 3.89 ± 0.31 ng/mL; hAT-derived MSCs – 26.99 ± 1.22 ng/mL, Wharton's jelly MSCs >70 ng/mL; day 6: 3.72 ± 0.44 ng/mL, 22.08 ± 3.71 ng/mL, and >70 ng/mL, respectively) than on day 1 of culture (1.85 ± 0.07 ng/mL, 16.94 ± 0.68 ng/mL, and >70 ng/mL, respectively). This confirms active cell proliferation and secretion of active factors over time.

Probably, the difference in the effect of conditioned medium of MSCs isolated from different sources on human chondrocyte proliferation, as well as the difference in IL-6 secretion, is mediated by the initial microenvironment (niche) of MSCs. It is worth noting that MSCs are found in many tissues of the body, but only MSCs from bone marrow and adipose tissue have been widely studied for the treatment of OA [12]. However, the number of MSCs in bone marrow is small, it also decreases with the age of the donor, and the cell collection procedure is quite traumatic. In this regard, research has begun to explore alternative sources of obtaining MSCs, including dental pulp and umbilical cord-derived Wharton's jelly. Umbilical cord-derived Wharton's jelly MSCs have been shown to have a positive effect on the regeneration of damaged hyaline cartilage in pigs [33]. Nowzari et al. showed the regenerative potential of human dental pulp MSCs and their secretome on a collagenase-induced OA model in rats [34].

CONCLUSION

Thus, studies on the effect of a conditioned medium of hAT-derived MSCs, PPT-derived MSCs and umbilical cord-derived Wharton's jelly MSCs on human chondrocyte proliferation in a 2D culture indicate that MSCs have a possible regenerative potential for cartilage tissue repair. Within the framework of this work, we did not identify any significant differences in the effect of the secretome of MSCs obtained from different sources on chondrocytes in indirect co-culture. However, further *in vivo* studies are warranted in the future.

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The authors declare no conflict of interest.

REFERENCES

1. *Karateev AE, Lila AM*. Osteoarthritis: current clinical concept and some promising therapeutic approaches. *Rheumatology Science and Practice*. 2018; 56 (1): 70–81. [In Russ, English abstract]. doi: 10.14412/1995-4484-2018-70-81.
2. *Hunter DJ, Bierma-Zeinstra S*. Osteoarthritis. *The Lancet*. 2019; 393 (10182): 1745–1759. doi: 10.1016/s0140-6736(19)30417-9. PMID: 31034380.
3. *Giorgino R, Albano D, Fusco S, Peretti GM, Mangiavini L, Messina C*. Knee osteoarthritis: epidemiology, pathogenesis, and mesenchymal stem cells: what else is new? An Update. *Int J Mol Sci*. 2023; 24 (7): 6405. doi: 10.3390/ijms24076405. PMID: 37047377.

4. *Hsueh MF, Önnerfjord P, Kraus VB*. Biomarkers and proteomic analysis of osteoarthritis. *Matrix Biol*. 2014; 39: 56–66. doi: 10.1016/j.matbio.2014.08.012. PMID: 25179675.
5. *Chow YY, Chin KY*. The role of inflammation in the pathogenesis of osteoarthritis. *Mediators Inflamm*. 2020; 2020: 8293921. doi: 10.1155/2020/8293921. PMID: 32189997.
6. *Aaron RK, Racine J, Dyke JP.* Contribution of circulatory disturbances in subchondral bone to the pathophysiology of osteoarthritis. *Curr Rheumatol Rep*. 2017; 19 (8): 49. doi: 10.1007/s11926-017-0660-x. PMID: 28718064.
7. *Sanchez C, Bay-Jensen AC, Pap T, Dvir-Ginzberg M, Quasnichka H, Barrett-Jolley R et al.* Chondrocyte secretome: a source of novel insights and exploratory biomarkers of osteoarthritis. *Osteoarthritis Cartilage*. 2017; 25 (8): 1199–1209. doi: 10.1016/j.joca.2017.02.797. PMID: 28232143.
8. *Sevastianov VI, Dukhina GA, Grigoriev AM, Perova NV, Kirsanova LA, Skaletskiy NN et al*. The functional effectiveness of a cell-engineered construct for the regeneration of articular cartilage. *Russian Journal of Transplantology and Artificial Organs*. 2015; 17 (1): 86–96. [In Russ, English abstract]. doi: 10.15825/1995-1191-2015-1-86-96.
9. *Shariatzadeh M, Song J, Wilson S*. The efficacy of different sources of mesenchymal stem cells for the treatment of knee osteoarthritis. *Cell Tissue Res*. 2019; 378 (3): 399–410. doi: 10.1007/s00441-019-03069-9. PMID: 31309317.
10. *Roos EM, Arden NK*. Strategies for the prevention of knee osteoarthritis. *Nature Reviews Rheumatology*. 2015; 12 (2): 92–101. doi: 10.1038/nrrheum.2015.135. PMID: 26439406.
11. *Urjas'ev OM, Zaigrova NK*. Osteoartrit: patogenez, diagnostika, lechenie. *Zemskij vrach.* 2016; 1–2 (29–30): 27–35.
12. *Loo SJQ, Wong NK*. Advantages and challenges of stem cell therapy for osteoarthritis (review). *Biomed Rep*. 2021; 15 (2): 67. doi: 10.3892/br.2021.1443. PMID: 34155451.
13. *Murphy JM, Fink DJ, Hunziker EB, Barry FP*. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum*. 2003; 48 (12): 3464–3474. doi: 10.1002/art.11365. PMID: 14673997.
14. *Garay-Mendoza D, Villarreal-Martínez L, Garza-Bedolla A, Pérez-Garza DM, Acosta-Olivo C, Vilchez-Cavazos F et al*. The effect of intra-articular injection of autologous bone marrow stem cells on pain and knee function in patients with osteoarthritis. *Int J Rheum Dis*. 2018; 21 (1): 140–147. doi: 10.1111/1756-185X.13139. PMID: 28752679.
15. *Desancé M, Contentin R, Bertoni L, Gomez-Leduc T, Branly T, Jacquet S et al*. chondrogenic differentiation of defined equine mesenchymal stem cells derived from umbilical cord blood for use in cartilage repair therapy. *Int J Mol Sci*. 2018; 19 (2): 537. doi: 10.3390/ijms19020537. PMID: 29439436.
16. *Galushko EA, Bolshakova TYu, Vinogradova IB, Ivanova ON, Lesnyak OM, Menshikova LV et al*. Structure of rheumatic diseases among adult population of Russia according to data of an epidemiological study (preliminary results). *Rheumatology Science and Practice*. 2009; 47 (1): 11–17. [In Russ]. doi: 10.14412/1995-4484-2009-136.
17. *De Bari C, Roelofs AJ*. Stem cell-based therapeutic strategies for cartilage defects and osteoarthritis. *Curr Opin Pharmacol*. 2018; 40: 74–80. doi: 10.1016/j.coph.2018.03.009. PMID: 29625333.
18. *Basok YuB, Grigoryev AM, Kirsanova LA, Kirillova AD, Subbot AM, Tsvetkova AV et al*. Comparative study of chondrogenesis of human adipose-derived mesenchymal stem cells when cultured in collagen-containing media under *in vitro* conditions. *Russian Journal of Transplantology and Artificial Organs*. 2021; 23 (3): 90–100. doi: 10.15825/1995-1191-2021-3-90-100.
19. *Gronthos S, Mankani M, Brahim J, Robey PG, Shi S*. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci USA*. 2000; 97 (25): 13625–13630. doi: 10.1073/pnas.240309797. PMID: 11087820.
20. *Zhou C, Yang B, Tian Y, Jiao H, Zheng W, Wang J et al*. Immunomodulatory effect of human umbilical cord Wharton's jelly-derived mesenchymal stem cells on lymphocytes. *Cell Immunol*. 2011; 272 (1): 33–38. doi: 10.1016/j.cellimm.2011.09.010. PMID: 22004796.
21. *Marmotti A, Mattia S, Castoldi F, Barbero A, Mangiavini L, Bonasia DE et al*. Allogeneic umbilical cord-derived mesenchymal stem cells as a potential source for cartilage and bone regeneration: an *in vitro* study. *Stem Cells Int*. 2017; 2017: 1732094. doi: 10.1155/2017/1732094. PMID: 29358953.
22. *Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R*. Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. *Int J Mol Sci*. 2017; 18 (9): 1852. doi: 10.3390/ijms18091852. PMID: 28841158.
23. *Parekkadan B, Milwid JM*. Mesenchymal stem cells as therapeutics. *Annu Rev Biomed Eng*. 2010; 12: 87–117. doi: 10.1146/annurev-bioeng-070909-105309. PMID: 20415588.
24. *Maguire G*. Stem cell therapy without the cells. *Commun Integr Biol*. 2013; 6 (6): e26631. doi: 10.4161/cib.26631. PMID: 24567776.
25. *Mancuso P, Raman S, Glynn A, Barry F, Murphy JM*. Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome. *Front Bioeng Biotechnol*. 2019; 7: 9. doi: 10.3389/fbioe.2019.00009. PMID: 30761298.
26. *Gunawardena TNA, Rahman MT, Abdullah BJJ, Kasim NHA*. Conditioned media derived from mesenchymal stem cell cultures: The next generation for regenerative medicine. *J Tissue Eng Regen Med*. 2019; 13 (4): 569–586. doi: 10.1002/term.2806. PMID: 30644175.
27. *Tsvetkova AV, Vakhrushev IV, Basok YuB, Grigor'ev AM, Kirsanova LA, Lupatov AYu et al.* Chondrogeneic po-

tential of MSC from different sources in spheroid culture. *Bull Exp Biol Med*. 2021; 170 (4): 528–536. doi: 10.1007/s10517-021-05101-x. PMID: 33725253.

28. *Sevastianov VI, Basok YuB, Grigoriev AM, Nemets EA, Kirillova AD, Kirsanova LA et al.* Decellularization of cartilage microparticles: Effects of temperature, supercritical carbon dioxide and ultrasound on biochemical, mechanical, and biological properties. *J Biomed Mater Res A*. 2023; 111 (4): 543–555. doi: 10.1002/jbm.a.37474. PMID: 36478378.
29. *Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; 8 (4): 315–317. doi: 10.1080/14653240600855905. PMID: 16923606.
30. *Rosochowicz MA, Lach MS, Richter M, Suchorska WM, Trzeciak T*. Conditioned medium – is it an undervalued lab waste with the potential for osteoarthritis management? *Stem Cell Rev Rep*. 2023; 19 (5): 1185–1213. doi: 10.1007/s12015-023-10517-1. PMID: 36790694.
31. *Gangadaran P, Oh EJ, Rajendran RL, Oh JM, Kim HM, Kwak S et al*. Three-dimensional culture conditioned bone marrow MSC secretome accelerates wound healing in a burn injury mouse model. *Biochem Biophys Res Commun*. 2023; 673: 87–95. doi: 10.1016/j.bbrc.2023.05.088. PMID: 37364390.
32. *Fan Y, Ye J, Shen F, Zhu Y, Yeghiazarians Y, Zhu W et al*. Interleukin-6 stimulates circulating blood-derived endothelial progenitor cell angiogenesis *in vitro*. *J Cereb Blood Flow Metab*. 2008; 28 (1): 90–98. doi: 10.1038/sj.jcbfm.9600509. PMID: 17519976.
33. *Wu KC, Chang YH, Liu HW, Ding DC*. Transplanting human umbilical cord mesenchymal stem cells and hyaluronate hydrogel repairs cartilage of osteoarthritis in the minipig model. *Tzu Chi Med J*. 2019; 31 (1): 11–19. doi: 10.4103/tcmj.tcmj_87_18. PMID: 30692826.
34. *Nowzari F, Zare M, Tanideh N, Meimandi-Parizi A, Kavousi S, Saneian SM et al.* Comparing the healing properties of intra-articular injection of human dental pulp stem cells and cell-free-secretome on induced knee osteoarthritis in male rats. *Tissue Cell*. 2023; 82: 102055. doi: 10.1016/j.tice.2023.102055. PMID: 36948080.

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