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Spreading and Actin Cytoskeleton Organization of Cartilage and Bone Marrow Stromal Cells Cocultured on Various Extracellular Matrix Proteins

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Abstract—Interactions between bone marrow stromal cells (BMSCs) and cartilage cells were studied in cell cocultures. Actin cytoskeleton organization and the cell spreading on various extracellular matrix proteins (laminin 2/4, collagen type I, and fibronectin) were explored. It was found that the most pronounced morphological changes (cell shape and area, actin cytoskeleton organization) were observed in cells cultivated on fibronectin. The average spreading area of BMSCs grown on fibronectin was about four times larger than the spreading area of cartilage cells. In cocultures of these cells plated in a ratio of 1 : 1, the cell spreading area on fibronectin proved to be 1.5 times less than was theoretically calculated. To clarify what influence cells have on each other, cell spreading in the conditioned medium was assayed. It was round that the BMSC spreading area in a cartilage cell conditioned medium was significantly less than in the control serum-free medium. This shows that cartilage cells are the source of factors that affect BMSC spreading.

Keywords: bone marrow stromal cells, actin cytoskeleton, spreading, cartilage cell

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INTRODUCTION

The recovery of damaged cartilage tissue with cultured cells is a promising area of development in current regenerative medicine (Khubutiya et al., 2008; Galle et al., 2010; Pörtner and Meenen, 2010; Richardson et al., 2010; Chang et al., 2013). Cell products composed of autological chondrocytes obtained from biopsy material of undamaged cartilage have been developed for this purpose (Tobita et al., 2002; Brittberg et al., 2003; Hui et al., 2009). However, the biopsy procedure produces further damage to cartilage tissue, which is slowly repaired. Thus, research has been focused on the use of stem progenitor cells capable of chondrogenic differentiation (Ganey et al., 2009; Galle et al., 2010; Richardson et al., 2010; Chang et al., 2013) in cartilage cell replacement therapy. Mesenchymal stem cells from periosteum, bone marrow, periosteum, muscle, and derma multipotent cells have been tested. It was found that these cells differ in both differentiation potency and clinical results after their implantation into a damaged area (Yoshimura et al., 2007; Koga et al., 2007). It seems likely that the difference is due to the influence of the microenvironment, which is composed of extracellular matrix (ECM) proteins, as well as the recipient cartilage cells (Han et al., 2014).

The interaction of stem cells with the microenvironment may affect their recovery properties. Thus, further research is required to find optimal conditions for recovery of cartilage tissue with stem cells.

One approach to determining the influence that cells have on each other is to cultivate them together on different substrates. Here, we compared BMSCs and cartilage cells in serum-free medium on various proteins of the extracellular matrix: laminin 2/4, collagen type I, and fibronectin. These proteins are present in various ratios in the extracellular matrix and connective tissues (Hall, 1983; Afanasijev and Omelchenko, 2001).

It was has been shown that cell morphology, the pattern of actin cytoskeleton organization in particular, depends on the substrate and alters after a change of the surface receptors (Are et al., 1999, 2001; Petukhova et al., 2004). The study of its spatial organization provides valuable information on the difference between cultured cells and the effect of the extracellular matrix.

The purpose of this study was to investigate the pattern of the cytoskeleton in BMSCs and cartilage progenitor cells interacting with ECM proteins and the influence of these cells on each other. It was shown

Abbreviations: ECM—extracellular matrix, BMSC—bone marrow stromal cell.
† Deceased.

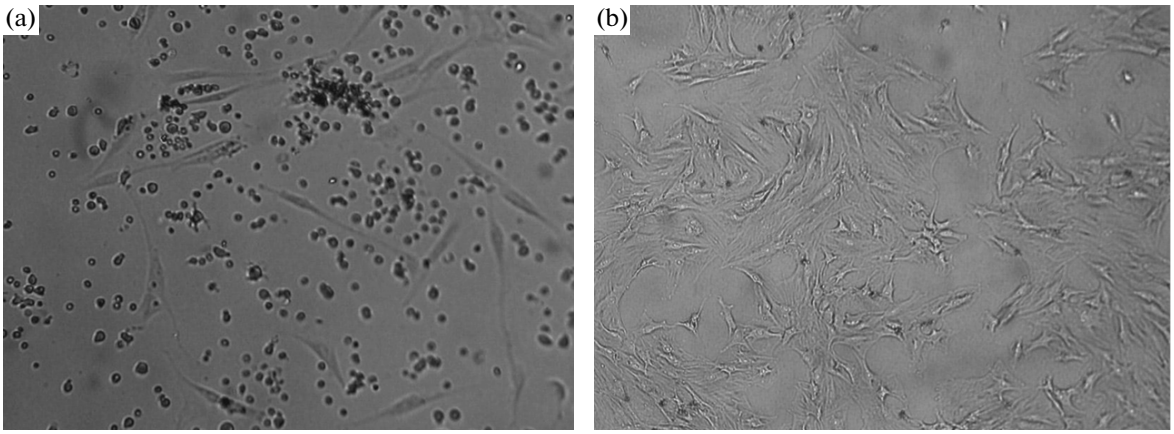

Fig. 1. Bone marrow stromal cells (BMSCs) cultured in vitro. (a) Primary culture; (b) first passage. Phase contrast. Ob. 20×.

that the cell functional changes associated with cytoskeleton reorganization during the spreading preceding their proliferation and differentiation are more informative during a period of time of a few minutes to 1 h after cell seeding onto the substrate. BMSCs and cartilage cells were cultivated separately and together on extracellular matrix proteins (laminin, collagen type I, and fibronectin).

MATERIALS AND METHODS

Chinchilla newborn rabbits and young (not more 6 months) nonbred rats were used in the experiments. The rats received water and natural food ad libitum. To isolate cells, the animals were sacrificed by cervical dislocation and sterilized with 30% alcohol for 10–15 min.

BMSC isolation and cultivation. Rabbit flat bones were taken from a plate and placed in Ca^{2+} and Mg^{2+}-free PBS supplemented with penicillin and streptomycin (100 μg/mL each). Bones were purified from soft tissues and washed with PBS. They were then dissected with a scalpel, and bone marrow was carefully washed with PBS using a syringe with a 23-gauge needle.

Rat bone marrow was isolated form tibia and femur bones. Bones were washed with PBS containing antibiotics, and epiphyses were removed. Bone marrow was washed with a syringe with a 23-gauge needle.

Bone marrow nuclear cells were isolated using density gradient centrifugation. It was placed in a suspension with 2 mL PBS, and the suspension was layered on a 3-mL histopaque (Sigma, United States) with a density of 1.077 g/mL and centrifuged (800 *g*, 20 min) at room temperature. Cells from the Histopaque interphase (mostly nuclear cells) were transferred into another tube and centrifuged in PBS in ten volumes at 600 *g* for 15 min at room temperature to remove Histopague. Washed cells were suspended in αMEM medium (Sigma, United States) supplemented with 20% fetal calf serum (HyClone, United States) and penicillin/streptomycin mixture (100 μg/mL each). The cells were counted under an inverted microscope using blood formed element register.

The cells were seeded at 0.5×10^6 cells/cm^2 in Petri dishes and incubated at 37°C and 5% CO_2. Two cellular types, weakly attached round ones and attenuated elongated ones, were observed in the primary BMSC culture (Fig. 1a). Round nonadhesive cells (bone marrow hematopoietic cells) were unable to divide and were practically eliminated by the first passage. Only elongated fibroblast-like cells (bone marrow nuclear cells) continued to proliferate (Fig. 1b).

The cells were passaged using 0.25% trypsin with 0.02% EDTA in physiological buffer (Gibco, United States). Cells at the second to seventh passages were used in experiments.

Cartilage cell isolation and cultivation. Cartilage cells of newborn rabbits or adult rats were established from migrating cells of cartilage fragments obtained from the iliac crest or intervertebral discs. The tissue was washed with PBS and minced with a 0.5- to 2.0-mm scalpel. The fragments were placed in Petri dishes, covered with a slide fragment, and incubated in the culture medium (αMEM, 20% fetal calf serum, penicillin/streptomycin mixture, 100 μg/mL each).

Single elongated cells with irregular shape and weakly adhesive round cells were observed in the primary culture. Figure 2a shows cartilage cells that migrated from cartilage in the primary culture. Cartilage-derived cells were cultivated in the growth medium. Cultures that reached an 80% monolayer were passaged. This density is the most effective for the subculturing procedure. Figure 2b shows the cartilage cell culture at the third passage. Round weakly adhered cells were eliminated; only attenuated fibroblast-like cells survived. The cells were subcultured with trypsin/EDTA. Cells at the second to seventh passages were used in experiments.

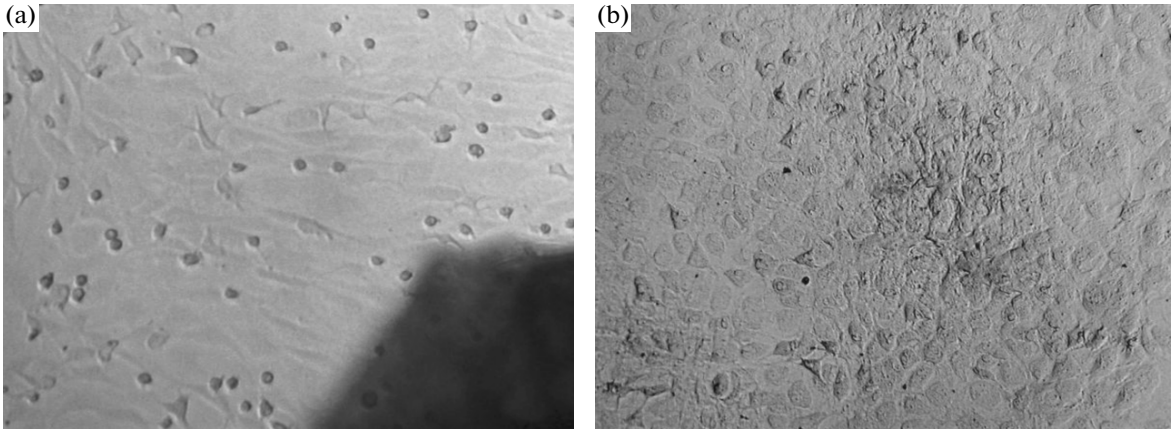

Fig. 2. Rat articular cartilage cells in culture. (a) Cells migrating from the cartilage, primary culture; (b) cells at the third passage. Phase contrast. Ob. 20×.

Media conditioning. BMSCs or cartilage cells (80–90% confluence) were cultivated in serum-free αMEM medium for 24 h. The conditioned medium was collected for further experiments.

Cell preparation for cytoskeleton research. Localization of fibrillar actin. Cells at the logarithmic growth phase were removed from the plate surface with trypsin/EDTA. The cell suspension had serum-containing growth medium added to inactivate trypsin, was centrifuged at 200 *g* for 3 min, was washed with serum-free-medium twice, and was diluted to 10^4 cells/mL, which is optimal for studying the cytoskeleton in single cells.

Coverslip coating with extracellular matrix proteins. Extracellular matrix proteins were placed on siliconized coverslips. Before silicon coating, coverslips were degreased and cleaned with concentrated nitric acid for 30 min. They were then repeatedly washed with distilled water to reach neutral pH: they were incubated in distilled water for 30 min, pH was measured, and they were washed again until stable pH was reached.

After reaching neutral pH, the glasses were placed with tweezers in a special container and treated with rectified alcohol for 20 min at room temperature. Coverslips were dried and placed in silicon (Repel-Silane ES, Sigma, United States) for 30 min. The coverslips were then put in the stand, washed with a distilled water jet, rinsed with alcohol, and air-dried. Dried glasses were carefully transferred from the container into a clean Petri dish. Treated glasses may be stored for a long period of time (several months).

Coating with fibronectin and laminin 2/4. Fibronectin (Sigma, United States) was diluted to 10 μg/mL. Laminin 2/4 isolated from human placenta in accordance with (Palm and Furcht, 1983) in a modified way and kindly provided by Dr. I.V. Voronkina (Institute of Cytology, Russia) was also diluted to 10 μg/mL. One hundred milliliters of solution was placed into the center of the prepared coverslips and kept overnight at 4°C.

Coating with fibrillar collagen. Collagen solution was kindly provided by Dr. L.V. Kukhareva (Institute of Cytology, Russia). It was diluted to 100 μg/mL with low-acidity water (glacial acetic acid/$H_2)_2$—1 : 1000). One hundred milliliters of solution was placed in the center of the siliconized coverslips. The coverslips were incubated for 30 min at room temperature, washed with 100 μL PBS three times, and another sample of protein was positioned. The procedure was repeated three times.

Coating, incubation, and cell fixation. Glasses coated with extracellular matrix proteins were washed three times with 100 μL PBS and treated with 100 μL 0.2% bovine albumin (Sigma, United States) in PBS for 1 h at 37°C to prevent unspecific binding. Residual solution was poured off, and glasses were washed three times with 100 μL PBS.

One hundred milliliters of cell suspension containing 10^4 cells/mL in serum-free culture or conditioned medium (Are et al., 2001) were placed on washed glasses and incubated for 1 h at 37°C and 5% CO_2 for cell attachment and spreading. Residual medium was removed, and cells were fixed with 50 μL 4% neutral formaldehyde (Sigma, United States) for 10–20 min at room temperature. The fixator was removed, and cells were washed three times with 100 μL PBS.

Rhodamine–phalloidin staining. Actin cytoskeleton was visualized by staining with rhodamine–phalloidin. The cells fixed on the substrate and washed with PBS^- were permeabilized with 0.1% Triton X-100 for 30 min at 37°C and treated with rhodamine–phalloidin (Invitrogen, United States) diluted 1 : 20 with PBS^- for 10 min in the dark. Samples were washed with PBS^- and embedded into Mounting medium (Pharmacia Biotech, Sweden).

Confocal microscopy. Preparations were assayed under a Leica TCS SL confocal microscope (Ger-

many). Rhodamine fluorescence was excited with a 543-nm argon laser. Images were recorded using the microscope software.

Staining quantitative assay. The BMSC and cartilage cell spreading area stained with rhodamine–phalloidin was measured using an Axiophot microscope with a color CCD camera and VideoTest-Size software. This software allows an object area on to be measured on the photos. About 30 cells were measured on each preparation.

The results were statistically treated with Student's t-test and χ^2 criterion. The data were considered significant at $p < 0.01$.

The reagents were Triton X-100, formaldehyde, BSA, Histopaque, fibronectin, and αMEM (Sigma, United States); fetal calf serum (HyClone, United States; trypsin (Sigma, United States); rhodamine–phalloidin (Invitrogen, United States); and Mounting medium (Pharmacia Biotech, Sweden).

RESULTS AND DISCUSSION

Different forms of actin cytoskeleton organization in BMSC cells produced by various ECM proteins. Three basic extracellular matrix proteins generated different distributions of actin filaments in BMSCs.

Most BMSCs spread on collagen type I have an irregular shape with a rough edge due to numeral long pseudopodia (Fig. 3a). Some cells have a "treelike" structure. Actin is mostly concentrated on the cell edge and protrusions in filament bundles of irregular shape. No organized actin structures was observed in the cytoplasm central part. Usually, actin is seen in numerous aggregates interacting with each other and forming smooth networklike structure. Radial filament bundles filling protrusions, which probably stimulate novel protrusions produced during the spreading process, are observed in cells with a smaller number of pseudopodia.

Most stromal cells that are spread out on immobilized laminin 2/4, conversely, have an oval, slightly polarized shape with an even edge and wide lamella along the whole leading edge (Fig. 3b). Actin is concentrated mostly in numerous parallel stress fibers passing through the entire cell. Moreover, densely packed short actin structures are arranged along the whole membrane of the leading edge. Single actin aggregates are observed on the cytoplasm periphery between the ends of stress fibers and the membrane.

BMSCs spread well on fibronectin. They have an oval shape with thin wide lamella along the cytoplasm periphery (Fig. 3c). Ruffles terminating in conic projections are seen along the whole cell perimeter. Just under the membrane, radial filament bundles are located with adjacent long circular microfilament bundles followed by brightly stained bundles of actin structures that make up the background of thin short protrusions. A poorly defined rough network of actin filaments is visualized under these structures. Actin was practically not revealed in the cytoplasm central region.

The results showed that bone marrow stromal cells are able to spread on three major proteins of the extracellular proteins: fibronectin, collagen type I and laminin 2/4. The population is composed of cells with a distinct organization of actin cytoskeleton. Only single BMSCs have a cytoskeleton that is typical for fibroblasts. A similar population heterogeneity has been shown for cultured rat fibroblasts (Are et al., 1999).

Organization of actin cytoskeleton in cartilage cells cultivated on various extracellular matrix proteins. Cartilage cells spread on collagen type I have a predominately elongated shape with a leading front edge (Fig. 4a). Parallel stress fibers pass along the long cellular axis. The front cellular region contains a powerful polygonal network of actin filaments with well-defined "foci" at their points of intersection. Neither free actin aggregates nor actin concentration under the cell membrane common for bone marrow stromal cells were revealed.

Cartilage cells spread on laminin have a similar phenotype with wide lamella having large protrusions on the leading edge and a long "tail" on the posterior cell region (Fig. 4b). Unlike BMSCs, cartilage cell cytoplasm has no stress fibers. However, similarly to BMSCs, a powerful microfilament network arranged into the entire structure is observed along the whole cell edge. Radial actin filament bundles reaching the membrane move out from this structure. Some of the fibrillar actin is arranged as a thin layer under the membrane. Fibrillar actin is not visible in the central region of cytoplasm, as in the case of BMSCs.

Cartilage cells practically are not spread on fibronectin (Fig. 4c). They are small and round-shaped with numerous very short actin-rich protrusions along the whole plasma membrane. In these cells, actin is concentrated only under the membrane and cellular protrusions. It is also revealed in diffuse aggregates in the central part of the cytoplasm.

The comparison of MBSC and cartilage cell interaction with various proteins of the extracellular matrix showed that a difference in cell shape and actin cytoskeleton organization was the most evident for cell cultures plated on fibronectin. However, the difference is not apparent if the cells have been cultivated for longer than 90 min. The may be due to the slower spreading capacity of cartilage cells on fibronectin compared to BMSCs. Fibronectin is located in deep layers of hyaline cartilage and colocalized with collagen type IX (Parsons et al., 2011). When the cell migration deep into the cartilage ceased, fibronectin probably became involved in generation of the cartilage dense matrix with incorporated isogenic chondrocyte groups located in so-called "lacunae."

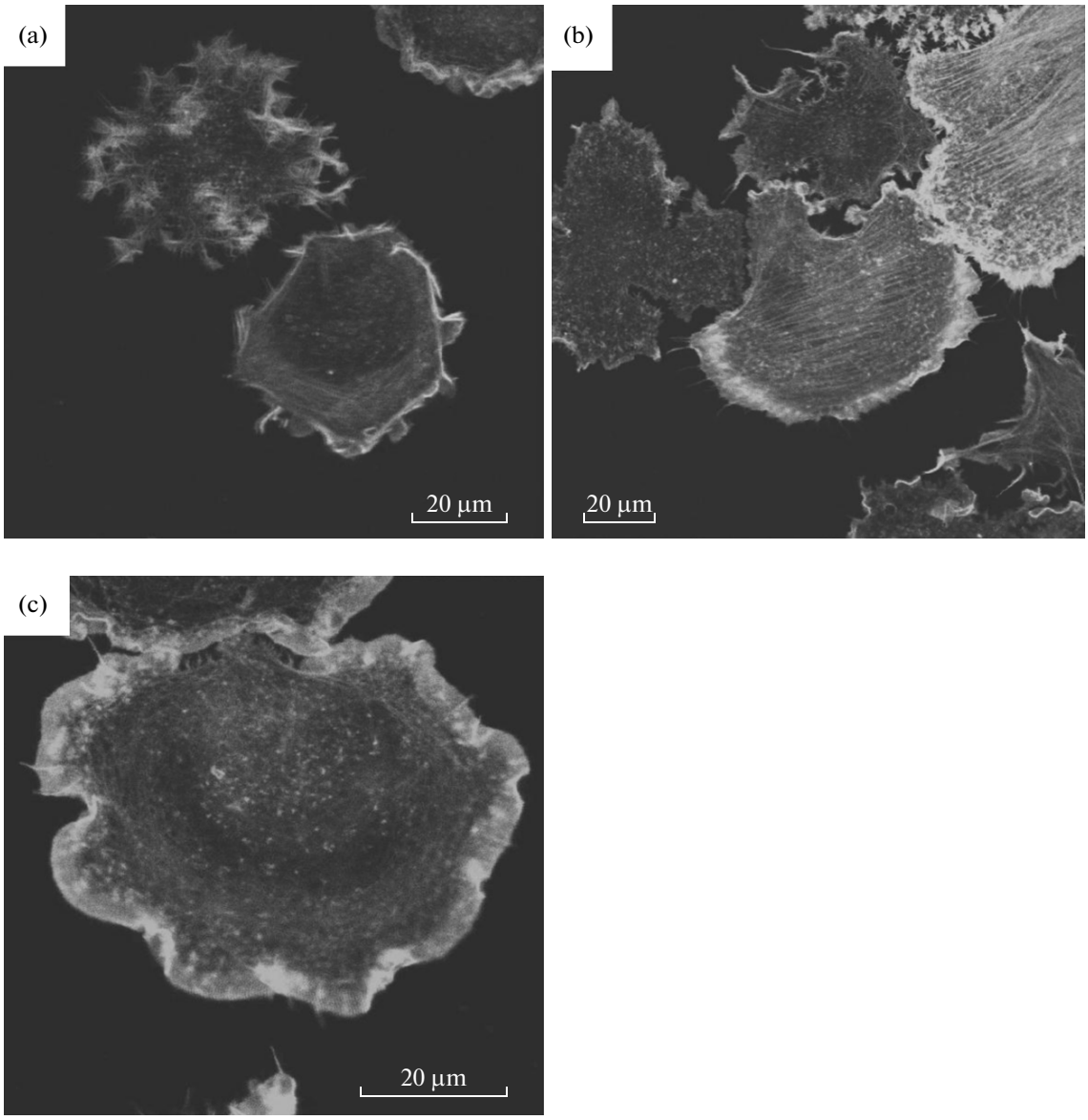

Fig. 3. Actin cytoskeleton of rat bone marrow stromal cells cultivated on the extracellular matrix proteins. (a) Collagen type I, (b) laminin 2/4, and (c) fibronectin. Rhodamine–phalloidin staining. Confocal microscopy. Scale bar is 20 μm.

BMSCs and cartilage cells spreading on collagen also displayed a difference in the cell shape and organization of actin structures. During the spreading process, BMSCs generate outgrowths, whereas cartilage cells have a smooth edge and a network with "foci" (bright spots) in sites of thin actin filament intersection. This network presumably may be used as a phenotypic marker of chondrogenic cells. However, further experiments are required to confirm this notion.

The results obtained showed that different cell types, undifferentiated BMSCs and cartilage progenitor cells, are distinguished by their interaction with extracellular matrix proteins. The difference was most obvious with fibronectin and, to a lesser extent, collagen type I.

Thus, we have shown that the pattern of interaction of cartilage and bone marrow stromal cells with major proteins of the extracellular matrix (collagen type I, fibronectin, and laminin 2/4) is diverse. It manifests in the cell shape and spreading, as well as in the cytoskeleton organization.

Spreading area of cartilage and bone marrow cells cocultured on fibronectin in serum-free culture or conditioned medium. We found that the difference in the cell shape and organization of actin cytoskeleton was most obvious in cultures maintained on fibronectin for 1 h (Figs. 3, 4). Cell spreading was used as a criterion for quantitative assay of the cell interaction with the substrate.

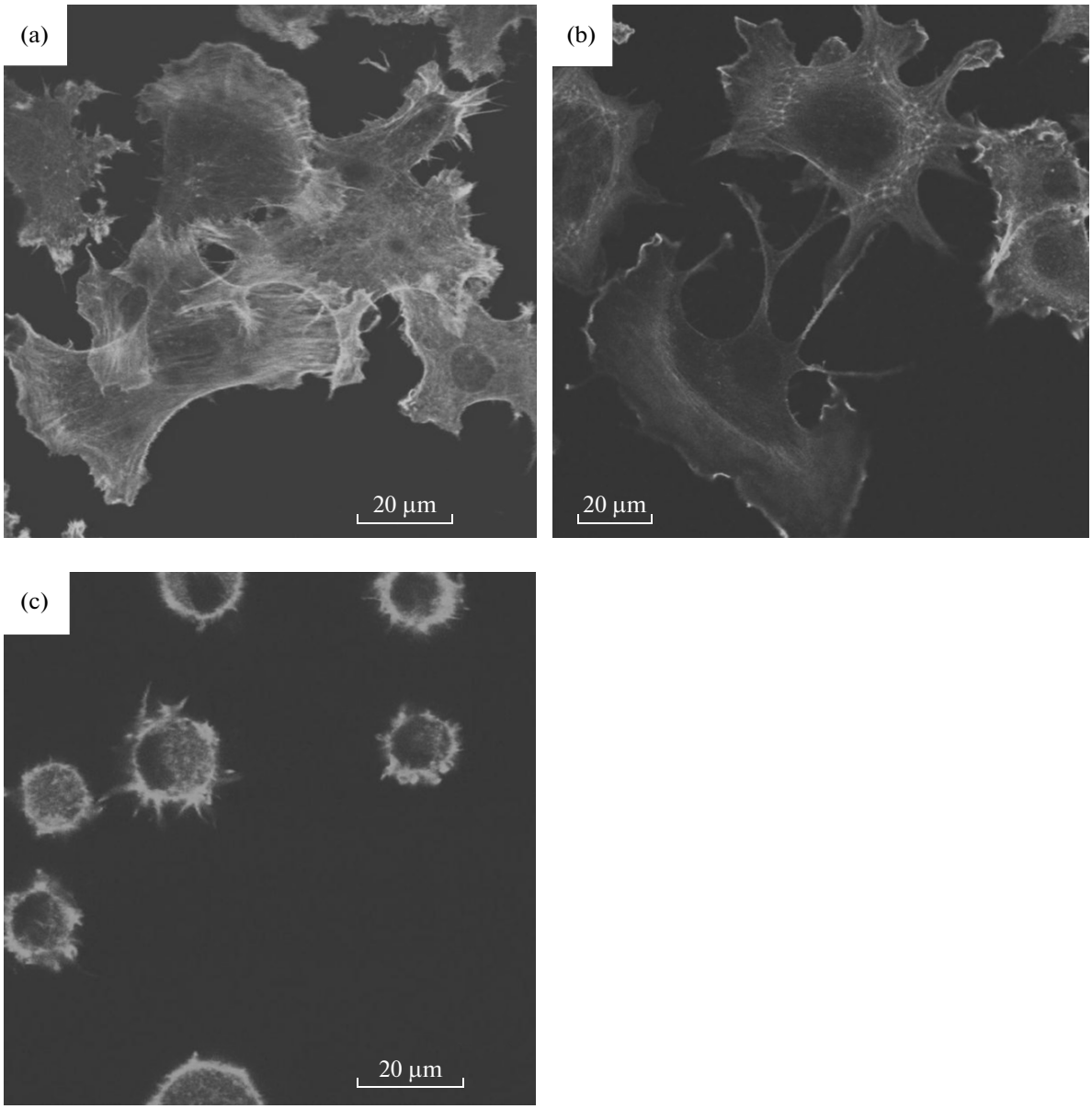

Fig. 4. Actin cytoskeleton of rat cartilage cells cultivated on the extracellular matrix proteins. (a) Collagen type I, (b) laminin 2/4, and (c) fibronectin. Rhodamine–phalloidin staining. Confocal microscopy. Scale bar is 20 μm.

It was found that the BMSC average spreading area is higher than cartilage cells (763 ± 49 and 189 ± 7.5 μm^2, respectively, $p < 0.01$) in serum-free cultures with fibronectin. In cocultures of these cells in a ratio of 1 : 1, their average spreading was 283 ± 25 μm^2 (Fig. 5c), which, according to Student's *t*-test, was significantly less than the BMSC and higher than the cartilage cell spreading area ($p < 0.01$).

The expected average area of fibronectin spreading cells is 763 ± 189 μm^2/2 = 476 μm^2. Using the χ^2 criterion, it was shown that the difference between the expected (476 μm^2) and observed (283 μm^2) spreading area of BMSCs and cartilage cells in short-term cocultures was significant ($\chi^2 = 78.3$, $p < 0.01$). Thus, the observed spreading area was significantly less the expected one (Fig. 5c). This means that BMSCs and cartilage cells cocultured on fibronectin directly interact with each other or via released factors that resulted in their reduced spreading area.

Similar results were obtained in cocultures of rabbit BMSCs and cartilage cells on fibronectin (Fig. 6). Further experiments have been performed with rabbit cells.

To exclude direct cell interactions, conditioned media were used for BMSC and cartilage cell cultivation.

It was found (Fig. 7) that the spreading area of a BMSC cartilage cell conditioned medium was 767 ±

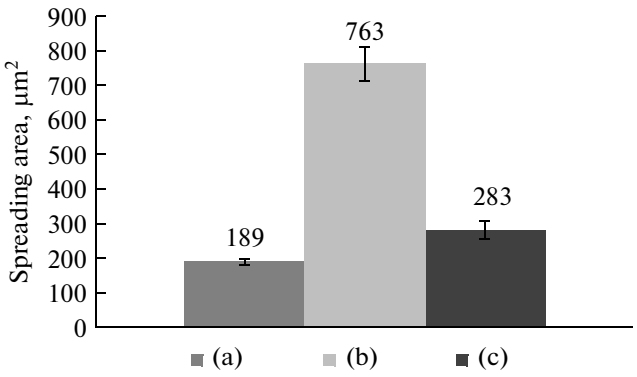

Fig. 5. Rat cell spreading area in serum-free culture medium. (a) Cartilage cells, (b) BMSC, and (c) coculture of BMSCs and cartilage cells. Vertical lines show mean value error; numbers above columns show average spreading area.

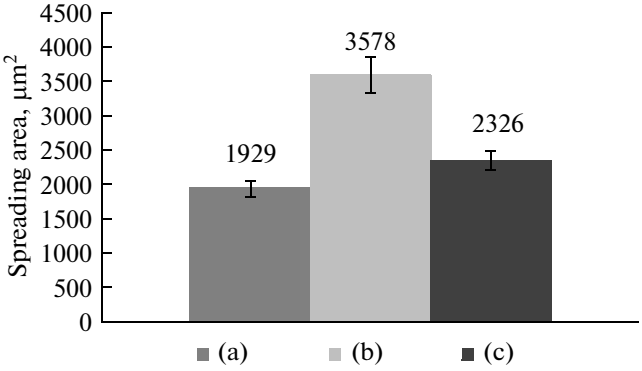

Fig. 6. Rabbit cell spreading area in serum-free culture medium. (a) Cartilage cells, (b) BMSCs, and (c) coculture of BMSCs and cartilage cells. Vertical lines show mean value error; numbers above columns show average spreading area.

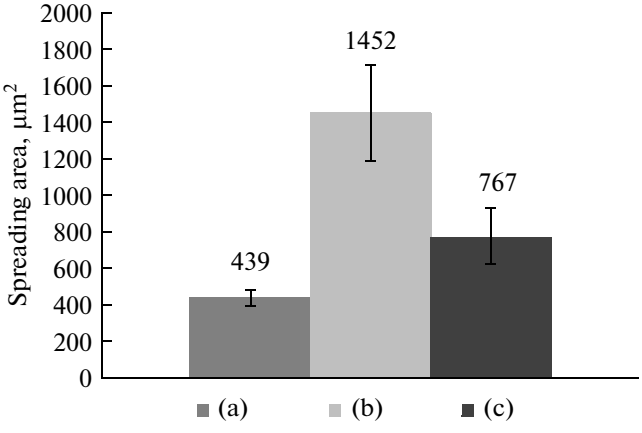

Fig. 7. Spreading area of (a) cartilage cells and (b) rabbit BMSCs in (a, b) control and (c) and conditioned serum-free culture media. Vertical lines show mean value error; numbers above columns show average spreading area.

138 μm². This was significantly less than their spreading area in the serum-free medium (1452 ± 263 μm²). The cartilage cell spreading area in BMSC conditioned and serum-free media was similar (data are not presented).

Thus, it can be thought that cartilage cells release factors affecting BMSC spreading; however, the qualitative and quantitative compositions of these factors probably differ.

Various proteins of the extracellular matrix and proteoglycans may be released by cells and affect the cell spreading (Ermakova et al., 2008). They include collagen type I, fibronectin, and proteoglycan aggrecan in the case of BMSCs (Kopesky et al., 2010) and collagens type 2 and 9 and aggrecans for cartilage cells (Kopesky et al., 2010; Parsons et al., 2011). In this paper, we have shown that cartilage cells spread less on fibronectin than on collagen type I and laminin 2/4. It is possible that the clue to BMSC and cartilage cell interaction will be found in extracellular matrix components that they have released (Imoto et al., 2002). Components such as collagen type I and hyaluronic acid are already used in the creation of the bioengineering counterpart to cartilage tissue, a technology that is steadily improving (Dai et al., 2010; Richardson et al., 2010). Studying the effect of cartilage conditioned medium on BMSCs will foster the development of a new technology for using BMSCs in regenerative medicine of cartilage tissue.

REFERENCES

Afanasjev, Yu.I. and Omelyanenko, N.P., Connective tissues, in *Rukovodstvo po gistologii* (Histology Manual), St. Petersburg: SpetsLit, 2001, vol. 1, pp. 249–283.

Are, A., Pinaev, G., Burova, E., and Lindberg, U., Attachment of A-431 cells on immobilized antibodies to the EGF receptor promotes cell spreading and reorganization of the microfilament system, *Cell Motil. Cytoskeleton*, 2001, vol. 48, pp. 24–36.

Are, A.F., Pospelova, T.V., and Pinaev, G.P., The characteristics of actin cytoskeleton structure and its rearrangements by extracellular matrix proteins in normal, immortalized and transformed rat fibroblasts, *Tsitologiia*, 1999, vol. 41, no. 8, pp. 707–715.

Brittberg, M., Peterson, L., Sjogren-Jansson, E., Tallheden, T., and Lindahl, A., Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments, *Bone Joint Surg. Am.*, 2003, vol. 85-A, pp. 109–115.

Chang, N.J., Lam, C.F., Lin, C.C., Chen, W.L., Li, C.F., Lin, Y.T., and Yeh, M.L., Transplantation of autologous endothelial progenitor cells in porous PLGA scaffolds create a microenvironment for the regeneration of hyaline cartilage in rabbits, *Osteoarthritis Cartilage*, 2013, vol. 21, pp. 1613–1622.

Dai, W., Kawazoe, N., Lin, X., Dong, J., and Chen, G., The influence of structural design of PLGA/collagen hybrid scaffolds in cartilage tissue engineering, *Biomaterials*, 2010, vol. 31, pp. 2141–2152.

Ermakova, I.I., Chertkova, T.A., Mokrushin, A.L., Romaniouk, A.V., Sakuta, G.A., and Morozov, V.I., Proteoglycans of L6J1 myoblast extracellular matrix. Characteristics and effect on myoblast adhesion, *Tsitologiia*, 2008, vol. 50, no. 8, pp. 692–699.

Galle, J., Bader, A., Hepp, P., Grill, W., Fuchs, B., Käs, J.A., Krinner, A., Marquass, B., Müller, K., Schiller, J., Schulz, R.M., von Buttlar, M., von der Burg, E., Zscharnack, M., and Löffler, M., Mesenchymal stem cells in cartilage repair: state of the art and methods to monitor cell growth, differentiation and cartilage regeneration, *Curr. Med. Chem.*, 2010, vol. 17, pp. 2274–2291.

Ganey, T., Hutton, W.C., Moseley, T., Hedrick, M., and Meisel, H.J., Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: experiments in a canine model, *Spine*, 2009, vol. 34, pp. 2297–2304.

Hall, B.K., *Cartilage*, Vol. 1: *Structure, Function, and Biochemistry*, New York: Academic Press, 1983.

Han, Y.L., Wang, S., Zhang, X., Li, Y., Huang, G., Qi, H., Pingguan-Murphy, B., Lu, T.J., Xu, F., Engineering physical microenvironment for stem cell based regenerative medicine, *Drug Discov. Today*, 2014, vols. 1359–6446, pp. 00033–00036.

Hui, J.H.P., Azura, M., and Lee, E.H., Review article: stem cell therapy in orthopaedic surgery: current status and ethical considerations, *Malaysian Orthopaedic J.*, 2009, vol. 3, pp. 4–12.

Imoto, E., Kakuta, S., Hori, M., Yagami, K., and Nagumo, M., Adhesion of a chondrocytic cell line (USAC) to fibronectin and its regulation by proteoglycan, *Oral Pathol. Med.*, 2002, vol. 31, pp. 35–44.

Khubutiya, M.Sh., Kliukvin, I.Y., Istranov, L.P., Khvatov, V.B., Shekhter, A.B., Vaza, A.Y., Kanakov, I.V., and Bocharova, V.S., Stimulation of regeneration of hyaline cartilage in experimental osteochondral injury, *Bull. Exp. Biol. Med.*, 2008, vol. 146, pp. 658–661.

Koga, H., Muneta, T., Ju, Y.-J., Nagase, T., Nimura, A., Mochizuki, T., Ichinose, S., von der Mark, K., and Sekiya, I., Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration, *Stem Cells*, 2007, vol. 25, pp. 689–696.

Kopesky, P.W., Lee, H.Y., Vanderploeg, E.J., Kisiday, J.D., Frisbie, D.D., Plaas, A.H., Ortiz, C., and Grodzinsky, A.J., Adult equine bone marrow stromal cells produce a cartilage-like ECM mechanically superior to animal-matched adult chondrocytes, *Matrix Biol.*, 2010, vol. 29, pp. 427–438.

Pörtner, R. and Meenen, N.M., Technological aspects of regenerative medicine and tissue engineering of articular cartilage, *Handchir. Mikrochir. Plast. Chir.*, 2010, vol. 42, pp. 329–336.

Palm, S.L. and Furcht, L.T., Production of laminin and fibronectin by schwannoma cells: cell-protein interactions in vitro and protein localization in peripheral nerve in vivo, *Cell Biol.*, 1983, vol. 96, pp. 1218–1266.

Parsons, P., Gilbert, S.J., Vaughan-Thomas, A., Sorrell, D.A., Notman, R., Bishop, M., Hayes, A.J., Mason, D.J., and Duance, V.C., Type IX collagen interacts with fibronectin providing an important molecular bridge in articular cartilage, *Biol. Chem.*, 2011, vol. 286, pp. 34986–34997.

Petukhova, O.A., Turoverova, L.V., Kropacheva, I.V., and Pinaev, G.P., Morphological peculiarities of epidermoid carcinoma A431 cells spread on immobilized ligands, *Tsitologiia*, 2004, vol. 46, no. 1, pp. 5–15.

Richardson, S.M., Hoyland, J.A., and Mobasheri, R., Mesenchymal stem cells in regenerative medicine: opportunities and challenges for articular cartilage and intervertebral disc tissue engineering, *Cell. Physiol.*, 2010, vol. 222, pp. 23–32.

Tobita, M., Ochi, M., Uchio, Y., Mori, R., Iwasa, J., Katsube, K., and, Motomura, T., Treatment of growth plate injury with autogenous chondrocytes, *Acta Orthop. Scand.*, 2002, vol. 73, pp. 352–358.

Yoshimura, H., Muneta, T., Nimura, A., Yokoyama, A., Koga, H., and Sekiya, I., Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle, *Cell Tissue Res.*, 2007, vol. 327, pp. 449–462.

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