CELL TECHNOLOGIES IN BIOLOGY AND MEDICINE

Peculiarities of Using Stem Cells for Regeneration of the Bone and Cartilage Tissue

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Scientific literature about the use of MSC contains clinical and experimental data on the efficiency of cell technologies for restoration of the osteoarticular apparatus. The use of MSC immobilized in the appropriate carriers and differentiation of these cells towards the bone cells and chondrocytes are of crucial importance. However, the use of MSC, both individual and in combination with other preparations and substances has a number of drawbacks and advantages. The absence of published reports on contraindications and complications of cell therapy is worthy of note, because the analysis of unsuccessful application of MSC will help to determine the indication for this treatment, and hence, to improve the efficiency of cell technologies in the future. Wider use of MSC in clinical practice and experimental studies for acceleration of reparative processes in the bone and cartilage tissue seems to be promising.

Key Words: *mesenchymal stem cells; cell technologies; regeneration of the bone and cartilaginous tissues*

Six and half million bone fractures are annually recorded in USA; in 15% cases, healing process is associated with complications, which cannot be effectively corrected. Damage to articular cartilage is the main orthopedic problem in USA; more than 500,000 cartilage repair procedures are annually performed; their total costs are billions of dollars. At present, there are no effective methods of cartilage tissue regeneration [39].

Healing of bone fractures is a complex dynamic process involving various cells and stimulating agents. The processes of bone tissue regeneration remain not completely understood and are the object of numerous researches [48].

Defects of the bone and cartilaginous tissues are usual complications of fractures and joint diseases (*e.g.* rheumatoid arthritis and osteoarthritis) producing a great social and economical influence on aging population. Despite new achievements of traumatology and orthopedics, restoration of the bone and cartilaginous tissues is associated with serious problems, because extensive defects cannot heal spontaneously. SC can be applied in regenerative biology and regenerative medicine, rapidly developing field of research opening new horizons in the treatment of wounds and traumas that cannot be effectively healed by available methods [8].

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Characteristics and sources of MSC

Mesenchymal stromal (stem) cells form the stroma of BM, while hemopoietic SC participate in the regeneration of red BM; that is why transplantation of only hemopoietic cells is not followed by BM formation. However, recent studies demonstrated the possibility of transdifferentiation of hemopoietic BM cells into tissue-specific SC and vice versa [50].

Red BM contains progenitor cells, MSC capable of differentiation into the bone, cartilage, tendon, and other types of the connective tissue. This allows using these cells for promoting regeneration of the bone tissue [8].

These properties are also typical of MSC isolated from the adipose tissue [44], umbilical cord blood [53] and Wharton's jelly matrix [26], peripheral blood [47], hair follicles [67], periosteum [22], various synovial structures (their number increases at the early stages of osteoarthritis) [65], subchondral bone [43], calvarium bone [57], ligaments with tendons [10], nervous tissue [12], *anulus fibrosus* and *nucleus pulposus* of the intervertebral disk [51], and oral mucosa [63] and embryonic SC [28], including amniotic [60] and tooth germ cells [68].

Morphologically, MSC are large fibroblast-like $D7^{-}FIB^{+}$ cells They are positive by CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD147, CD166, LNGFR, HLA-DR, and STRO-1 and negative by CD1a, CD11a, CD14, CD19, CD25, CD28, CD33, CD34, CD38, CD40, CD40L, CD45, CD117, CD133, CD144, HLA-DR, B7-1 (CD80), and B7-2 (CD86). During culturing, these cells form a monolayer and are capable of chondrogenic, osteogenic, and adipogenic differentiation [13].

MSC were isolated from periodontal ligament (ectomesenchymal origin); they were characterized by fibroblast-like morphology and a typical antigen repertoire. Moreover, periodontal MSC exhibit positive reaction to type 1 collagen and bone sialoprotein [42].

MSC isolated from *musculus orbicularis oris* were positive by $CD29^{+}$, $CD90^{+}$, $CD105^{+}$, $SH3^{+}$, $SH4^{+}$ and negative by antigens of hemopoietic $CD14^{-}$, $CD34^{-}$, $CD45^{-}$, $CD117^{-}$ and endothelial $CD31^{-}$ cells and were capable of osteogenic differentiation. These cells are easily isolated (compared to isolation from BM) and they can be used for reconstruction of the alveolar bone and correction of cleft palate in children [5].

There are data on the existence of dental MSC and dental pulp MSC. These cells can differentiate into osteogenic lineage cells and into specific tooth cells [42,68]. Dental pulp MSC (obtained from healthy 6-10-year-old children) produced mineralized matrix under certain conditions *in vitro* and differentiated into osteocytes and formed compact bone *in vivo* [68].

According to published data, MSC of the bone marrow (BM MSC) and periosteum by their osteogenic activity are superior to cells from the adipose tissue [22,44] and peripheral blood, because BM and periosteum contain greater number of $CD105^{+}$, $CD34^{+}$, and $CD14^{+}$ cells [56].

BM MSC obtained from young and old humans and experimental animals do not differ by their properties and characteristics, but the number of these cells decreases with age [7]. However, other authors reported that proliferative activity of MSC decreases with age, which can be related to microenvironment, genome abnormalities, oxidative stress, and initiation of mechanisms of growth factor suppression (prevention of tumor development) [49].

In elderly individuals, reparative potential of MSC can vary depending on the source. BM MSC from elderly patients (mean age 64.4 years) demonstrated higher differentiation potential in the chondogenous lineage in comparison with MSC isolated from a trabecular bone [13].

Estrogens dose-dependently modulate osteogenic and adipogenic differentiation of human MSC. Thus, the results of MSC transplantation to male and female patients and the efficiency of cells isolated from male and female donors can be different [58].

Some peculiarities of MSC application

Some papers set the use of MSC for regenerative medicine in opposition to their application in tissue engineering not only restoring the tissue, but also reconstructing a 3D structure of lost or damaged tissues (e.g. articular cartilage and subchondral bone) or even organs [66].

Various carriers can be used for MSC introduction into the organism; 3D carriers provide better cell interaction and communication [61].

The matrix or substrate for tissue engineering, cell growth, or their introduction into the body is a critical determinant for clinical improvement of regeneration and reparation of body tissues. For bone tissue recovery, biocompatible materials mechanically comparable with the bone are required, they can be integrated into the skeleton, vascularized, and promote osteoinduction of implanted MSC. The carriers for MSC delivery into the bone tissue should meet the following requirements [21]:

- biocompatibility;
- stimulation of bone growth;
- spacious porous structure resembling the structure of the bone tissue to be replaced;
- mechanical durability;
- biodegradability.

Calcium triphosphate, a promoter of osteogenic differentiation of MSC [54,75], hydroxyapatite [16],

and type 1 collagen are proposed for these purposes. According to published data, MSC culturing with collagen promote their osteogenic differentiation [53].

Xeno- and autologous bone and cartilage tissues, acellular, or demineralized bone matrix can be used as the carriers for MSC [20,34].

Transplantation of BM MSC suspension and demineralized bone matrix to rodents with experimental articular cartilage damage led to the formation of a complex osteochondral structure including the articular cartilage and subchondral bone, their introduction into empty medullary cavity of the long bone resulted into the formation of trabeculae and stroma supporting hemopoiesis, while their use for the correction of a flat bone defect stimulated the growth of the bone [20].

Under conditions of co-culturing of rat MSC with intervertebral disk tissues, MSC differentiated into cells of the disk (nucleus pulposus-like cells) [34].

There are data on the growth of human BM MSC on a titanium plate. Titanium constructions are used as dental implants, for replacement of lost flat bone fragments, and for fabrication of joins for arthroplasty. The formation of bone tissue islets and activation of alkaline phosphatase were attained [15].

Round titanium implants 2×7.3 mm were passively populated with MSC and implanted to sheep into osteochondral defects of the medial condyle of the femur. Untreated defects of implants without cells served as the control. Complete regeneration of the subchondral bone plate was observed in 50% animals receiving the implants with MSC. Under these conditions, only type 1 collagen was revealed, when high expression of mRNA was detected, and regeneration of hyaline-like cartilage was noted. Osseointegration and formation of the fibrocartilaginous tissue were detected in 50% animals receiving implants with MSC and in all animals reveiving MSC-free titanium implants. Histological examination demonstrated better results in case of MSC-populated implants (the area of osseointegration and implants 8.8±6.4 mm^2), than in case of implants without cells (5.5±3.9 mm^2) or spontaneous reparation of the defect (area of newly formed bone 2.8±2.5 mm^2) [15].

Hydrogels can also be used for MSC injection into the damaged area [37] or for supplying transplanted MSC with oxygen [33] and growth factors [14].

Good results were obtained in experiments with combined application of MSC and platelet-rich plasma for improving regeneration of the bone tissue and osteointegration of implants. This plasma promotes the growth of the bone tissue and serves as the scaffold for bone growth from MSC [44,68,72]. This can be explained by the fact that cytokines released by megakaryocytes stimulate MSC differentiation, while interaction of thrombopoietic structures and MSC promote endochondral ossification [59]. MSC with plasma or platelets can be injected to regenerating bone and cartilage [72]. Fibrin gels, glue, or sponges, modifications of platelet-rich plasma, are successfully used as BM MSC carriers [38].

A promising approach is the use of matrices with substances modulating the direction and rate of MSC differentiation. It was demonstrated that osteogenic and chondrogenic differentiation of MSC can be modulated by dexamethasone, ascorbic acid, β-glycerophosphate [34,65], FGF [29], transforming growth factor [14,34], platelet-derived growth factor [40], basic FGF [18], vascular endothelial growth factor (VEGF) promoting the formation of blood vessels and thereby additionally accelerating tissue regeneration [73], TNF [41], and fibronectin [54].

MSC and osteoprogenitor cells not only participate in bone formation, but also express various factors modulating this process, *e.g.* chemotactic factors for endothelial cells promoting induction of angiogenesis at the site of MSC transplantation [38].

Combined use of MSC and endothelial cell precursors is a very promising approach for tissue engineering and bone formation. It ensures rapid neovascularization of growing tissues; endothelial cell precursors thus stimulate osteogenic differentiation of MSC. On week 12 after combined subcutaneous transplantation of these cells from dogs in a collagen capsule to nude mice we observed a significant increase in the number of capillaries and volume of the bone tissue (its thickness increased by 1.6 times) in comparison with transplantation of MSC alone [54].

It is now possible to introduce certain genes into MSC and thereby stimulate their directed maturation via production of required factors by these cells. Co-expression of VEGF and BMP (bone morphogenetic protein) genes in BM MSC, *e.g.* using an adenovirus-associated vector. This accelerates the formation of the vascular network and promotes osteogenesis in the ischemic extremity [73].

Angiogenesis plays a central role in regeneration of the bone tissue due to not only nutrient supply, but also direct effects of precursor cells. After ectopic transplantation of ceramic containers with GFP-labeled mouse MSC, two waves of cell migration to the container from the host organism were observed. The first migration wave was noted on day 7 after implantation and was presented primarily by $CD31^+$-cells (endotheliocyte precursors) and the second wave started on day 11 and consisted predominantly of $CD146^+$-cells (pericytes). Both cell populations did not arise from implanted elements (minor admixtures of non-stem and non-mesenchymal cells among MSC can never excluded). Migration of pericyte-like cells always de-

pended on preceding migration of endothelial cells. It should be noted that pericyte precursors retain some properties of MSC, such as high proliferative activity *in vitro* and osteogenic potential [61].

Fate of transplanted MSC

For detection of MSC after their transplantation, GFP gene is usually introduced into cell DNA. This gene persists in cells for a sufficiently long time and allows detection of structures formed by labeled MSC even after their long-term considerable differentiation [61].

GFP-labeled BM MSC from transgenic rats with fibrin glue were introduced into osteochondral defects of wild-type rats. After 24 weeks all defects were filled with hyaline chartilage and subchondral bone. GFP-positive cells were found in reconstructed tissues, but their number was considerably reduced [46].

Transplantation of MSC from GFP-transgenic Sprague–Dawley rats into knee joint of normal rats with injuries to the cruciate ligaments, medial meniscus, and articular cartilage was performed. After 4 weeks, mobilization of transplanted MSC was proven by the presence of GFP. More intensive synthesis of extracellular matrix around MSC also attests to tissue regeneration [4].

The fate of transplanted MSC during reconstruction of the articular cartilage was studied using fluorescent dye PKH26. In experiments on rabbits, labeled MSC on polyglycolic acid matrix were transplanted into a full-thickness defect of the condyle of the femur. After 2 weeks, the formation of immature cartilage containing type 2 collagen was observed. After 8 weeks, the cartilage became thinner and partially lost type 2 collagen in the basal region, where positive reaction to type 1 collagen appeared. Most chondrocytes in the regenerating cartilage were PKH26-positive, osteoblasts in the regenerating bone had mixed origin (from the donor and recipient). The thickness of the cartilage decreased up to week 8 and then remained stable to week 42 after surgery [62].

Rabbit MSC were incubated with supermagmetic iron particles and 5-bromo-2-deoxyuridine for subsequent detection by transmission electron microscopy after staining with Prussian blue. These MSC were transplanted into knee joint cavity of rabbits with modeled condyle lesion in the form of a gel with chitosan and glycerophosphate. The presence of iron nanoparticles in cell cytoplasm was detected by transmission electron microscopy. Staining with safranin O demonstrated accumulation of proteoglycans and type 2 collagens outside the cells, which confirmed chondrogenesis. Magnetic resonance imaging showed persistence of transplanted MSC for at least 12 weeks. It was concluded that these methods can be used for tracing the fate of transplanted MSC [30].

Immunomodulating properties of MSC

Nondifferentiated MSC do not express surface immunogenicity markers and do not induce immune response after xeno- and allogeneic transplantation; proliferation of allogeneic T cells and inflammatory and immune processes are also suppressed [45,76].

Xenogeneic transplantation of human BM MSC for regeneration of a 3-mm femoral bone defect in sheep promoted healing of the damaged tissues. No local or systemic reactions to long-term presence of xenogeneic MSC were detected. The use of autologous MSC led to even more rapid formation of the bone, which was seen from accelerated collagen mineralization [45].

For cartilage formation, allogeneic MSC were transplanted on a porous ceramic container made of β-tricalcium phosphate into the joint cavity without immunosuppression. After 8 weeks, no histological (lymphocyte infiltration) and biochemical (antibodies to allogeneic MSC) signs of immunological conflict were noted. No differences in cartilage formation from allogeneic and autologous MSC were reveale. Implantation of the container without MSC was not followed by cartilage tissue formation [76].

Coral hydroxyapatite with poly(lactic-co-glycolic acid) and absorbed human MSC were subcutaneously implanted to immunodeficient CB17 mice. Ten weeks after implantation, the formation of bone-like tissue containing human cells was noted. In the control (scaffold without MSC), only fibrous tissue was formed [16].

According to another report, the use of autologous BM MSC for reparation of the bone tissue is accompanied by enhanced resorption of the damaged bone and activation of inflammatory and some immune reactions [1]. There are data that allogeneic undifferentiated MSC are low effective stimulators of T cell proliferation [74].

MSC from human umbilical cord blood immobilized in tricalcium phosphate–collagen scaffold were transplanted to rats into a 4 mm femoral bone defect. Four weeks after xenotransplantation, proliferation of MSC within the matrix was observed, but later these MSC were eliminated by the host organism. At the same time, considerably acceleration of bone formation was observed in comparison with the control [27].

Human MSC can be detected within 1-4 weeks after their transplantation to rats with critical bone defects of the skull. At that term, MSC formed aggregates and looked like osteoblasts, and later infiltration by macrophages, $CD3^+$ and $CD8^+$ cells appeared.

Administration of immunosuppressive drugs prolongs lifetime of MSC and improves bone regeneration, but it did not completely suppress the immune response and hampered completion of reparative processes [11].

MSC application for reparation of the bone tissue

In experiments on Wistar-Kyoto rats, demineralized bone matrix without cells or with BM MSC was placed into a lateral skull bone defect. Transplantation of MSC stimulated angiogenesis and osteogenesis in the zone of lesion leading to complete restoration of the bone. Moreover, the use of MSC suppressed the inflammatory response [2].

Regeneration of the bone tissue after xenotransplantation of suspension of human MSC or chondroblasts was studied on rats with traumas of both femoral bones. No signs of immunological reactions or other pathological changes were revealed, but acceleration of bone regeneration on day 10 through 30 in comparison with the control group was noted. MSC implantation more markedly stimulated reparative osteogenesis than implantation of chondroblasts, which was associated with accelerated maturation of bone lamellae. Moreover, injection of MSC suspension stimulated tissue regeneration in the controlateral extremity. The formed bone tissue was completely integrated into the surrounding bone and underwent remodeling [3].

Eight weeks after MSC implantation for the treatment of radial bone fracture in mice, the elasticity of the new bone tissue corresponded to that of the intact bone. Regenerating bone is characterized by lower volume, but higher mineral density. Ten and 35 weeks after MSC implantation, the axial strength of elements reconstructed with the use of MSC was by 1.5-2 times higher than that of contralateral undamaged bones [32].

Different scaffolds (cube 2 mm on edge) with human MSC were subcutaneously implanted to mice for 5 weeks. The results of histomorphometric analysis attest to the absence of bone tissue formation in the scaffolds made of coralline apatite and bovine bones. Bone formation was observed in hydroxyapatite and calcium triphosphate scaffold. Only mineral resorption of the scaffold material was noted at this stage [21].

Transplantation of a hydroxyapatite–collagen gel composite for replacement of the middle third of the femur in rabbits was followed by the formation of new bone tissue with gradual biodegradation of the artificial material. After transplantation of the construct with MSC, the bone formed earlier and its density was higher (0.99±0.11 without cells and 1.29±0.14 with MSC); simultaneous development of the fibrovascular network and osteoids was noted. Moreover, six months after transplantation of the composite with MSC, BM structures were found [9].

After transplantation of fetal human MSC on a macroporous scaffold made of poly(ε-caprolactone) tricalcium phosphate for the treatment of a 7-mm femoral bone defect, the rate of bone formation increased by more than 2-fold (43.3±10.5 mm^3 *vs*. 21.0±7.4 mm^3 in cell-free scaffold, $p<0.05$) and density of the bone tissue also increased (3.9±1.7 *vs*. 0.4±0.3 mN×m/degree, $p<0.05$). Despite the fact that human MSC persisted for less than 4 weeks, well-developed vascular network in the damaged area was observed after their disappearance (35.2±11.1 mm^3 vs. 6.5±3.6 mm^3, $p<0.05$). Complete union of bone fragments was observed only after MSC transplantation [75].

After transplantation of autologous BM MSC on a ceramic container for regeneration of full-thickness defect of the femoral diaphysis in sheep, the following stages of the reparative processes were observed [6]:

1) bone formation on the external surface of the container;
2) bone formation in the inner cylindrical canal;
3) formation of cracks and fissures in the implant;
4) bone formation in pores of the bioceramic implant.

Autologous MSC isolated from BM and suspended in fibrin gel were applied onto the surface of surgical implants and used in experiments on sheep. Radiological method showed larger area of bone growth after transplantation of MSC after 2, 3, and 6 months. However, no significant histological differences from the control were revealed (19.833±8.729 and 8.667±8.667% of the area of implant contact with the bone, respectively) [31].

The density of the bone tissue under conditions of distraction osteosynthesis under experimental conditions was higher after transplantation of MSC: the bone tissue formed earlier and more intensively, whereas in the control only large cartilage tissue islets were found [29].

Constructs with MSC were implanted at the lesion sites (necrosis of the femur head) of three patients; external fixation was used. Patients have been followed for more than three years and promising results were obtained [6].

The use of MSC for joint regeneration

Routine histological procedure, special staining, ultrastructural studies, and morphometry showed that culturing of human MSC in chondrogenic medium leads to the formation of cartilage structures similar (by chondrocyte phenotype, appearance of collagen fibrils, and matrix distribution) to native cartilage. The results of histomorphometric analysis suggest that the

density of formed structures is higher than that of fetal cartilage, but lower than that of adult cartilage tissue. The proportion between the cells and matrix and cellular density were closer to those of adult human cartilage tissue [24].

After transplantation of luciferase-labeled MSC for regeneration of the knee joint meniscus after its removal in rats, direct differentiation of these cells into meniscus cells without MSC mobilization from other organs and tissues was observed [25].

Evaluation of possible effects of BM MSC and a scaffold for these cells prepared from normal meniscus tissues (2×10^5 MSC per meniscus) on regeneration of the meniscus in rabbits showed the presence of GFP-labeled MSC on the surface of the meniscus after culturing for 1 week and in deep zones after 2 weeks. After 4 weeks, production of extracellular matrix was detected histologically and by the analysis of the expression of aggrecan and type 10 collagen mRNA. The density of cultured tissues significantly increased after 2 weeks and approached that of normal meniscus [69].

Allogeneic BM MSC were implanted on different scaffolds into defects (4 mm diameter, 5 mm depth) of the medial femoral condyle in rabbits. When caprolactone was used as the scaffold, the formation of mature trabecular bone was observed 3 months after surgery. The results of computer tomography attest to progressive mineralization from the recipient tissues into the internal region of the implant; satisfactory reparation of the cartilage was noted at the same term. Moreover, viability of transplanted MSC over 5 weeks was demonstrated using a fluorescent label [55].

After transplantation of synthetic extracellular matrix in composition with hyaluronic acid and gelatin (hydrogel for injection) as the carrier for autologous BM MSC, complete healing of the damaged articular cartilage of the knee joint (femur) in rabbits was attained after 12 weeks. After transplantation of MSC without the matrix, hyaline-like cartilage was formed only at the periphery of the defect, while the central zone was occupied by the fibrous tissue [37].

In 27 rabbits, artificial femoral cartilage defect was filled by BM MSC alone or in a complex with collagen membrane, or left untreated. Regeneration of the cartilage and subchondral bone, restoration of articular cartilage, and its integration with the bone were observed only in the group with membrane transplantation 12 weeks after surgery. On other groups the damaged structures were replaced by the fibrous tissue [36].

MSC in the treatment of spinal diseases

Degeneration of the intervertebral disk is the most frequent cause of low back pains. These processes become more and more incident and not always can be effectively corrected. Symptomatic treatment remains most effective. The possibility of using MSC for the treatment of various spinal diseases is now actively studied [35,51,52].

Autologous BM MSC were transplanted into lumbar intervertebral disks after induction of their degeneration in rabbits. The height of the disk and its density 24 weeks after transplantation attained 91 and 81% of the control values (magnetic resonance imaging data). In untreated animals, the corresponding parameters were 67 and 60% of normal, respectively. Macroscopic and histological examinations revealed relatively preserved nucleus with circular annulus structure in MSC-transplanted discs compared to indistinct structure seen in untreated controls. Restoration of proteoglycan accumulation in MSC-transplanted discs confirmed from immunohistochemistry and gene expression analysis [52].

Numerous transplanted MSC were detected (by the presence of GFP) in nucleus pulposus after 2 weeks. These MSC express phenotypic markers typical of cartilage tissue cells (type 2 collagen, keratan sulfate, chondroitin sulfate, aggregan) and nucleus pulposus cells (hypoxia-inducible factor 1α, glutamine-1 transporter, and matrix metalloproteinase-2) [35].

Axial distraction in combination with implantation of BM MSC improved hydratation and nutrition of the intervertebral disk in rabbits with its degeneration. A synergistic effect of both therapies in reversing degenerative disc disease was observed, which was confirmed by measuring disk height, morphological examination, and average dead cell count [23].

Adult rabbits (n=30) underwent posterolateral intertransverse fusion at the L4-L5 level and received transplantation of MSC on different matrices. The animals were divided into four groups based on the implant material: autologous bone (group 1), porous hydroxyapatite and type 1 collagen (group 2), autologous BM MSC in a low concentration (10^6) on hydroxyapatite (group 3), and autologous BM MSC in a high concentration (10^8) on hydroxyapatite (group 4). Osteogenic differentiation was induced with dexamethasone. After transplantation of autologous BM MSC in a high concentration on hydroxyapatite, the development of mature bone tissue was observed, pores of the scaffold were filled with bone matrix. When implants with low MSC concentration was used, fibrous tissue predominated [17].

BM MSC arrested degeneration of the intervertebral disk in mice through chondrocytic differentiation and stimulation of endogenous cells; BM MSC induced an increase of endogenous notochordal cells in the nucleus pulposus and expression of chondrocytic markers [70].

Protective properties of human BM MSC against apoptosis induced by oxidative stress were demonstrated *in vitro* on nucleus pulposus cells of the intervertebral disk. The effect was shown to be mediated by indirect cell interaction [64].

Similar results were obtained in clinical settings. BM MSC were absorbed on porous tricalcium phosphate granules (short-term incubation under negative pressure) and transplanted into the area of spinal damage. After 34.5 months, good spinal fusion results were obtained in 95.1% cases (41 patients). Only four patients had minor exudation or moderate swelling not requiring additional treatment [19]. Injection of autologous BM MSC almost completely attested degeneration of the intervertebral disk in two elderly patients (67 and 70 years) with lumbar protrusion, unstable lumbago, leg pain, and numbness [71].

Conclusion

Scientific literature about the use of MSC contains clinical and experimental data on the efficiency of cell technologies for restoration of the osteoarticular apparatus. The use of MSC immobilized in the appropriate carriers and differentiation of these cells towards the bone cells and chondrocytes are of crucial importance. However, the use of MSC, both individual and in combination with other preparations and substances, has a number of drawbacks and advantages. The absence of published reports on contraindications and complications of cell therapy is worthy of note, because the analysis of unsuccessful application of MSC will help to determine the indication for this treatment, and hence, to improve the efficiency of cell technologies in the future. Wider use of MSC in clinical practice and experimental studies for acceleration of reparative processes in the bone and cartilage tissue seems to be promising.

REFERENCES

1. D. O. Zubov, *Fiziol. Zh.,* **54**, No. 4, 30-36 (2007).
2. P. V. Kruglyakov, I. B. Sokolova, N. N. Zin'kova, *et al., Thitologiya,* **47**, No. 6, 466-477 (2005).
3. T. Kh. Fatkhudinov, D. V. Gol'dshtein, A. A. Pulin, *et al., Byull. Eksp. Biol. Med*., **140**, No. 7, 109-113 (2005).
4. M. Agung, M. Ochi, S. Yanada, *et al., Knee Surg. Sports Traumatol. Arthrosc.,* **14**, No. 12, 1307-1314 (2006).
5. D. F. Bueno , I. Kerkis, A. M. Costa, *et al., Tissue Eng. Part A.,* **15**, No. 2, 427-435 (2009).
6. R. Cancedda, M. Mastrogiacomo, G. Bianchi, *et al., Novartis Found Symp.,* **249**, 133-143 (2003).
7. S. Cei, B. Kandler, A. Fugl, *et al., J. Periodontol*., **77**, No. 4, 699-706 (2006).
8. D. Chanda, S. Kumar, and S. Ponnazhagan, *J. Cell. Biochem*., **111**, No. 2, 249-257 (2010).
9. S. H. Chang , K. Y. Tung, Y. J. Wang, *et al., Plast Reconstr Surg*., **125**, No. 5, 1393-1402 (2010).
10. M. T. Cheng, H. W. Yang, T. H. Chen, and O. K. Lee, *Cell Prolif.,* **42**, No. 4, 448-460 (2009).
11. C. K. Chuang, K. J. Lin, C. Y. Lin, *et al., Tissue Eng. Part A.,* **16**, No. 2, 479-488 (2010).
12. I. H. Chung, T. Yamaza, H. Zhao*, et al., Stem Cells*, **27**, No. 4, 866-877 (2009).
13. P. Coipeau, P. Rosset, A. Langonne, *et al., Cytotherapy*, **11**, No. 5, 584-594 (2009).
14. M. Endres, N. Wenda, H. Woehlecke, *et al., Acta Biomater*., **6**, No. 2, 436-444 (2010).
15. K. H. Frosch, A. Drengk, P. Krause, *et al., Biomaterials*, **27**, No. 12, 2542-2549 (2006).
16. K. Fu, Q. Xu, J. Czernuszka, *et al., J. Biomed. Mater. Res. A.*, **92**, No. 4, 1256-1264 (2010).
17. T. S. Fu, W. J. Chen, L. H. Chen, *et al., J. Orthop. Res*., **27**, No. 3, 380-384 (2009).
18. M. Fukui, S. Akita, and K. Akino, *Wound Repair Regen.,* **13**, No. 3, 332-340 (2005).
19. Y. Gan, K. Dai, P. Zhang, *et al., Biomaterials*, **29**, No. 29, 3973-3982 (2008).
20. O. Gurevitch, B. G. Kurkalli, T. Prigozhina, *et al., Stem Cells*, **21**, No. 5, 588-597 (2003).
21. C. T. Harris and L. F. Cooper, *J. Biomed. Mater. Res. A.*, **68**, No. 4, 747-755 (2004).
22. O. Hayashi, Y. Katsube, M. Hirose, *et al., Calcif. Tissue Int*., **82**, No. 3, 238-247 (2008).
23. H. T. Hee, H. D. Ismail, C. T. Lim, *et al.,* J. Bone Joint Surg. Br., **92**, No. 5, 726-736 (2010).
24. A. T. Hillel, J. M. Taube, T. C. Cornish, *et al., Cells Tissues Organs.*, **191**, No. 1, 12-20 (2010).
25. M. Horie, I. Sekiya, T. Muneta, *et al., Stem Cells*, **27**, No. 4, 878-887 (2009).
26. J. Y. Hsieh, Y. S. Fu, S. J. Chang, *et al., Stem Cells Dev*., **19**, No. 12, 1895-1910 (2010).
27. M. Jager, O. Degistirici, A. Knipper, *et al., J. Bone Miner Res*., **22**, No. 8, 1224-1233 (2007).
28. Y. H. Ji, J. L. Ji, F. Y. Sun, *et al., Mol. Cell. Proteomics*, **9**, No. 3, 550-564 (2010).
29. X. Jiang, S. Zou, B. Ye, *et al., Bone*, **46**, No. 4, 1156-1161 (2010).
30. X. H. Jin, L. Yang, X. J. Duan, *et al., Zhonghua Yi Xue Za Zhi.* **87**, No. 45, 3213-3218 (2007).
31. P. Kalia, G.W. Blunn, J. Miller, *et al., Tissue Eng*., **12**, No. 6, 1617-1626 (2006).
32. I. Kallai, G.H. van Lenthe, D. Ruffoni, *et al., J. Biomech*., **43**, No. 12, 2315-2320 (2010).
33. N. Kimelman-Bleich, G. Pelled, D. Sheyn, *et al., Biomaterials*, **30**, No. 27, 4639-4648 (2009).
34. C. L. Korecki, J. M. Taboas, R. S. Tuan, and J. C. Iatridis, *Stem Cell. Res. Ther.,* **1**, No. 2, 18 (2010).
35. C. L. Le Maitre, P. Baird, A. J. Freemont, and J. A. Hoyland, Arthritis. Res. Ther., **11**, No. 1, R20 (2009).
36. J. Lin, R. Wang, and L. Cheng, Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, **20**, No. 12, 1229-1234 (2006).
37. Y. Liu, X. Z. Shu, and G. D. Prestwich, *Tissue Eng*., **12**, No. 12, 3405-3416 (2006).
38. E. Lucarelli, *Eur. Cell Mater.,* **20**, 13-23 (2010).
39. J. M. Mason, A.S. Breitbart, M. Barcia, *et al., Clin. Orthop. Relat. Res*., **379**, Suppl., S171-S178 (2000).

40. R. C. McCarty, S. Gronthos, A. C. Zannettino, *et al., J. Cell Physiol*., **219**, No. 2, 324-333 (2009).
41. P. M. Mountziaris, S. N. Tzouanas, and A. G. Mikos, *Biomaterials*, **31**, No. 7, 1666-1675 (2010).
42. K. M. Mrozik, P. S. Zilm, C. Bagley, *et al., Stem Cells Dev*., **19**, No. 10, 1485-1499 (2010).
43. K. Neumann, T. Dehne, M. Endres, *et al*., *J. Orthop. Res*., **26**, No. 11, 1449-1456 (2008).
44. P. Niemeyer, K. Fechner, S. Milz, *et al., Biomaterials*, **31**, No. 13, 3572-3579 (2010).
45. P. Niemeyer, K. Szalay, R. Luginbuhl, *et al., Acta Biomater*., **6**, No. 3, 900-908 (2010).
46. Y. Oshima, N. Watanabe, K. Matsuda, *et al., J. Histochem. Cytochem.*, **53**, No. 2, 207-216 (2005).
47. A. Peterbauer-Scherb, M. van Griensven, A. Meinl, *et al., J. Tissue Eng. Regen. Med.,* **4**, No. 6, 485-490 (2010).
48. A. M. Phillips, *Injury*, **36**, Suppl. 3, S5-S7 (2005).
49. N. Quarto, B. Behr, and M. T. Longaker, *Tissue Eng. Part A.,* **16**, No. 10, 3185-3197 (2010).
50. M. Z. Ratajczak, M. Kucia, R. Reca, *et al., Leukemia*, **18**, No. 1, 29-40 (2004).
51. S. M. Richardson, R. V. Walker, S. Parker, *et al., Stem Cells*, **24**, No. 3, 707-716 (2006).
52. D. Sakai, J. Mochida, T. Iwashina, *et al., Biomaterials*, **27**, No. 3, 335-345 (2006).
53. R. K. Schneider, A. Puellen, R. Kramann, *et al., Biomaterials*, **31**, No. 3, 467-480 (2010).
54. C. Seebach, D. Henrich, C. Kähling, *et al., Tissue Eng. Part A.,* **16**, No. 6, 1961-1970 (2010).
55. X. X. Shao, D. W. Hutmacher, S. T. Ho, *et al., Biomaterials*, **27**, No. 7, 1071-1080 (2006).
56. D. Smiler, M. Soltan, and M. Albitar, *Implant Dent.,* **17**, No. 3, 236-247 (2008).
57. P. Steenhuis, K. M. Carr, G. J. Pettway, and M. A. Ignelzi Jr., *Cells Tissues Organs.*, **190**, No. 3, 150-157 (2009).
58. P. Strube, M. Mehta, A. Baerenwaldt, *et al., Bone*, **45**, No. 6, 1065-1072 (2009).
59. K. Sumiyoshi, S. Kubota, R. A. Furuta, *et al., J. Cell. Commun. Signal.*, **4**, No. 1, 5-14 (2010).
60. H. Sun, K. Feng, J. Hu, *et al., Biomaterials*, **31**, No. 6, 1133-1139 (2010).
61. R. Tasso, F. Fais, D. Reverberi, *et al., Biomaterials*, **31**, No. 8, 2121-2129 (2010).
62. M. Tatebe, R. Nakamura, H. Kagami, *et al., Cytotherapy*, **7**, No. 6, 520-530 (2005).
63. G. B. Tomar, R. K. Srivastava, N. Gupta, *et al., Biochem. Biophys. Res. Commun*., **393**, No. 3, 377-383 (2010).
64. F. Wang, X. Wu, Y. Wang, *et al., Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi,* **24**, No. 4, 391-398 (2010).
65. Y. Wang, X. Shi, L. Ren, *et al., J. Biomater. Sci. Polym. Ed.*, **21**, Nos. 8-9, 1227-1238 (2010).
66. C. Weinand, R. Gupta, E. Weinberg, *et al., Tissue Eng. Part A.,* **15**, No. 9, 2605-2615 (2009).
67. G. Wu, Z. H. Deng, X. J. Fan, *et al., Stem Cells Dev*., **18**, No. 4, 583-589 (2009).
68. Y. Yamada, S. Nakamura, K. Ito, *et al., Tissue Eng. Part A.,* **16**, No. 6, 1891-1900 (2010).
69 T. Yamasaki, M. Deie, R. Shinomiya, *et al., Artif. Organs,* **32**, No. 7, 519-524 (2008).
70. F. Yang, V. Y. Leung, K. D. Luk, *et al., Mol. Ther.*, **17**, No. 11, 1959-1966 (2009).
71. T. Yoshikawa, Y. Ueda, K. Miyazaki, *et al., Spine*, **35**, No. 11, E475-E480 (2010).
72. R. Yoshimi, Y. Yamada, K. Ito, *et al., J. Craniofac. Surg*., **20**, No. 5, 1523-1530 (2009).
73. C. Zhang, K. Z. Wang, H. Qiang, *et al., Acta Pharmacol. Sin.,* **31**, No. 7, 821-830 (2010).
74. X. Zhang, T. Tang, Q. Shi, *et al., Immunobiology,* **214**, No. 3, 179-186 (2009).
75. Z. Y. Zhang, S. H. Teoh, M. S. Chong, *et al., Biomaterials*, **31**, No. 4, 608-620 (2010).
76. Z. Y. Zhao, L. Yang, P. Xu, *et al., Zhonghua Wai Ke Za Zhi*., **43**, No. 20, 1340-1343 (2005).