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EDITORIAL

Mesenchymal stem cells: From bench to bedside

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Abstract

Human mesenchymal stem cells (hMSCs) have tremendous promise for use in a variety of clinical applications. The ability of these cells to self-renew and differentiate into multiple tissues makes them an attractive cell source for a new generation of cell-based regenerative therapies. Encouraging results from clinical trials have also generated growing enthusiasm regarding MSC therapy and related treatment, but gaps remain in understanding MSC tissue repair mechanisms and in clinical strategies for efficient cell delivery and consistent therapeutic outcomes. For these reasons, discoveries from basic research and their implementation in clinical trials are essential to advance MSC therapy from the laboratory bench to the patient's bedside.

Key words: Mesenchymal stem cells; Cell therapy; Cell expansion and processing

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MESENCHYMAL STEM CELLS

Friedenstein and coworkers were the first to investigate the characteristics of the colony forming fibroblastic cells, which were isolated from the bone marrow by their selective adherence to tissue culture plastics[1]. Several other groups extended the pioneering work of Friedenstein *et al*[1] showing that these plastic adherent human cells derived from bone marrow were able to differentiate into a number of mesenchymal cell types including osteoblasts, chondrocytes and adipocytes[2-4]. These cells were called "mesenchymal stem cells (MSCs)" in reference to their high self-renewing properties and ability to form cartilage and bone, and were suggested to be responsible for the normal turnover and maintenance of adult mesenchymal tissues[5]. Although the initial application of MSC was to form feeder layers for hematopoietic stem cells, hence the alternate name "marrow stromal cells", MSC's therapeutic potential to cure a plethora of debilitating diseases was soon discovered and has generated significant excitement in the field of regenerative medicine[6]. Over the last two decades, the field of MSC has progressed rapidly from the preclinical to the early clinical trial arena for a wide range of diseases.

MSCs IN CELL THERAPY

MSCs hold tremendous promise for a variety of clinical applications. Ongoing clinical trials using human mesenchymal stem cell (hMSC) include ischemic stroke, multiple sclerosis, acute leukemia, graft-versus-host disease, critical limb ischemia, articular cartilage and bone defects among others (for the clinical trials presently tested, please see: www.clinicaltrial.gov). At the time of writing this article, there were about 90 clinical trials involving hMSC at various stages world-wide. The progress in clinical trials with MSCs in various diseases has been reviewed extensively[7-12].

Although the concept of cell-based therapy is not new and bone marrow transplants have been the standard of care for years, MSC-based cell therapies represent a new generation of regenerative therapies that extend into other organ systems and meet pressing clinical needs for a broad range of diseases. MSCs are among the most widely used stem cell types in cell therapy owing to several favorable biological characteristics, including their convenient isolation from adult donors, ease of expansion in culture while maintaining genetic stability[13], lack of significant immunogenicity and feasibility for allogenic transplantation[14,15], and the homing capacity that facilitates intra-arterial/intravenous administration under minimally invasive conditions[16]. MSCs or MSC-like cells are now being isolated from blood[17], adipose tissue[18], trabecular bone[19], umbilical cord blood[20], and placenta[21] among other tissues. MSCs also have the remarkable property that they home to sites of tissue injury and institute repair, either by differentiating into tissue-specific cell phenotypes[22-25] or by creating a milieu that increases the capacity of the endogenous cells to repair tissue and modulates the immune response[26-28]. While the early studies have focused on cell differentiation, the recent results that demonstrate MSC's ability to repair tissues without significant engraftment or differentiation have led to new concepts for hMSC therapeutic effects. Critical features of this new paradigm are MSC's ability to not only secrete a rich mixture of soluble factors but also the ability to specifically respond to the immediate needs of the injured tissues. One specific example of the responsiveness of MSCs to microenvironment was the report that hMSCs injected into the hippocampus of mice following transient global ischemia decrease neuronal death by modulating inflammatory and immune responses. The transcriptomes of the hMSC changed with upregulation of 170 human genes that were largely involved in anti-inflammatory or anti-immune genes[29]. As another example, MSCs were activated by interferon-γ together with proinflammatory cytokines to express nitric oxide (NO) and several chemokines, suggesting that MSC-mediated immuno-suppression occurs through the concerted action of chemokines and NO[30]. MSC's responsiveness to the microenvironment of injured tissues suggests that the MSCs can be injected locally to enhance tissue repair, which could be one of the most useful cell therapy strategies.

While the original focus of hMSC's therapeutic potential was their ability to engraft and their plasticity, recent findings suggest that MSC's primary function is to inhibit immune responses and to establish a favorable microenvironment for tissue repair through immune modulation, down-regulation of inflammatory responses and paracrine effects[31]. Thus, the defining properties for hMSC should include not only their multi-lineage potential but also their robustness to respond to biological cues and to modulate the microenvironment. It is also likely that the therapeutic benefits of hMSC are a combined result of multiple contributing factors, generating both short-term tissue responses and long-term tissue repair and regeneration. For this reason, basic science studies are important to elucidate the controlling factors and to gain mechanistic insights underpinning MSC therapies.

CLINICAL APPLICATIONS OF MSCs

The beneficial outcomes from an increasing number of clinical trials using hMSCs without any major side effects has been a major driving force behind interest in MSCs' clinical application. As scientists learn more about MSC biology and tissue repair mechanisms, the encouraging clinical results, most notably in cardiac repair and bone disorders, have generated a growing enthusiasm.

Cardiac repair

A compelling clinical need exists in cardiovascular therapies to protect, restore and regenerate cardiomyocytes that are lost due to myocardial infarctions and heart failure. Bone marrow-derived cells, including both hematopoietic and MSCs, have shown remarkable clinical efficacy in terms of functional improvements including ejection fraction, ventricular volumes, infarct size and myocardial perfusion[32-34]. The functional improvement that occurred within 72 h was far earlier than would be expected for cell regeneration, leading to intense debate about repair mechanisms after cell transplantation[35]. The prevailing concept of stem cell efficacy has now shifted toward the cytokine-paracrine effects, which have been shown to modulate angiogenesis, inflammation, cytoprotection, metabolism and apoptosis. Despite the exciting possibilities that stem cell therapy have major beneficial effects on myocyte regeneration, inconsistent outcomes and, in some cases, poor engraftment and modest improvement have been reported in human trials[36-38]. These results highlight the need to understand the MSC tissue repair mechanisms and exact biology of stem cells in order to address the limitations such as the optimal cell type, mode of cell processing and delivery. The focus of improving and standardizing cell processing and delivery methods should be on enhancing cell engraftment while maintaining their therapeutic potency.

Bone disorders

MSCs have considerable potential for treatment of musculoskeletal disorders owing to their expansion capacity, immunosuppressive properties and ability to differentiate into bone and cartilage. Autologous bone marrow-derived MSCs have been used in fracture nonunion, osteogenesis imperfecta, and bone metabolic diseases, and demonstrated bone formation and limb function recovery in patients[39-42]. In addition, MSCs are also combined with scaffolds that are inductive or instructive to direct MSCs down specific lineage pathways and augment the therapeutic effect. Considerable *in vitro* and animal studies suggest MSCs have the potential for rapid bone regeneration and are the cell of choice in bone repair. However, in contrast with most studies in cardiovascular therapies, the numbers of patients studied in stem cell therapy for bone diseases and repair are relatively low and more long-term and sufficiently controlled clinical trials are needed to assess the therapeutic outcome. As MSCs are the progenitors responsible for the normal turnover of adult mesenchymal tissues and have high responsiveness to tissue injury, "intelligent" materials that are able to recruit endogenous MSCs *in vivo* and direct them down specific pathways will be a useful therapeutic avenue.

PROMISE AND OBSTACLES OF MSC THERAPY

The last few years have witnessed a growing enthusiasm for the clinical application of MSC-based therapy. Despite the significant potential, challenges in MSC's clinical applications include low survival of transplanted cells, limited targeting capabilities, and low grafting efficiency and potency, which often requires use of a high number of cells to achieve therapeutic benefits. To date, clinical studies using stem cells have not been conclusive and are, in many cases, less impressive than what has been observed in preclinical models. A major obstacle limiting MSC clinical application is the lack of defining markers due to the inherent heterogeneity of MSC populations and variation associated with cell processing and expansion. The lack of standardization and variation in cell characterization and processing may help explain the discrepancies observed in some of the clinical studies[43]. Standardization is also critical for meaningful interpretation and comparison of experimental outcomes and understanding the mechanisms underlying the potential benefits of stem cells.

A hallmark of stem cells is their ability to expand in culture without phenotypic alternations. In the bone marrow obtained from human donors, hMSC's are rare and in the range of approximately 1 in 10^5 nucleated cells. Because of the low occurrence of MSC in bone marrow, only culture-expanded MSCs are likely to meet the demand in clinical application. However, DNA replication is not a perfect process and *in vitro* cell processing and expansion could induce potential changes to the cell and increase risks in their therapeutic applications. In addition to the safety concerns, the impact of culture expansion and cell processing on hMSC therapeutic potency is largely unknown and requires further investigation. Recent studies have shown that sequential passaging of MSC using standard culture methods has been associated with a decrease in expression of adhesion molecules, the loss of chemokine receptors, enlargement of cell size and lack of chemotactic response to chemokines, thus compromising their therapeutic potency[44-46].

Several recent studies have illustrated the increasingly recognized importance of cell processing of MSC for specific clinical indications. Le Blanc's group has recently shown that cryopreservation reduces the yield of *ex vivo* expanded MSC obtained from freshly harvested bone marrow mononuclear cells (MNC). In addition, MSC from fresh MNC were more potent in suppressing the lymphocyte responses in a mixed lymphocyte culture compared with MSC prepared from cryopreserved MNC[47]. In still another study, MSC pre-conditioned under hypoxic condition (0.5% O_2 for 24 h) increased expression of pro-survival and pro-angiogenic factors and enhanced the capacity of MSC to repair infarcted myocardium, owing to reduced cell death and apoptosis of transplanted cells, increased angiogenesis, and paracrine effects[48]. While these studies confirmed the seemingly obvious notion that MSC properties and functional capacity vary depending on the processing protocols, they also represent the beginning of an important research arena that addresses a bottleneck in MSC therapy.

PROSPECTIVE

Stem cells produce all multi-cellular tissues in the body in tightly controlled microenvironments. As a result, they are particularly sensitive to their immediate environmental cues. A case in point is the importance of a seemingly pedestrian factor of oxygenation for stem cell fate. Low oxygen tension, traditionally termed "hypoxia", is known to profoundly influence cellular events, cytokine physiology, and regenerative potential, and may in fact represent an "*in situ*" normoxia[49,50]. Although oxygen tension has been recognized as a developmentally important stimulus *in vivo*, it has not been adequately accounted for in *in vitro* cultures[51]. As the concept of MSC therapy shifted from the early proliferation-differentiation-engraftment assumption to the paracrine hypothesis, MSC therapeutic properties are now defined not only by their proliferative and multi-lineage potentials but also their ability to respond to and influence their immediate surrounding environments. To this end, basic and preclinical research will continue to play an important role in uncovering the dynamic interplay between stem cells and their microenvironments. Implementing these discoveries in clinical trials will be critical to advance MSC therapy from bench to a clinical reality.

REFERENCES

1. **Friedenstein AJ**, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation* 1968; **6**: 230-247
2. **Ashton BA**, Allen TD, Howlett CR, Eaglesom CC, Hattori A, Owen M. Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. *Clin Orthop Relat Res* 1980; 294-307
3. **Bab I**, Ashton BA, Gazit D, Marx G, Williamson MC, Owen ME. Kinetics and differentiation of marrow stromal cells in diffusion chambers in vivo. *J Cell Sci* 1986; **84**: 139-151
4. **Castro-Malaspina H**, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D, McKenzie S, Broxmeyer HE, Moore MA. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood* 1980; **56**: 289-301
5. **Caplan AI**. Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. *Tissue Eng* 2005; **11**: 1198-1211
6. **Gerson SL**. Mesenchymal stem cells: no longer second class marrow citizens. *Nat Med* 1999; **5**: 262-264
7. **Giordano A**, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. *J Cell Physiol* 2007; **211**: 27-35
8. **García-Castro J**, Trigueros C, Madrenas J, Pérez-Simón JA,

Rodriguez R, Menendez P. Mesenchymal stem cells and their use as cell replacement therapy and disease modelling tool. *J Cell Mol Med* 2008; **12**: 2552-2565
9 **Undale AH**, Westendorf JJ, Yaszemski MJ, Khosla S. Mesenchymal stem cells for bone repair and metabolic bone diseases. *Mayo Clin Proc* 2009; **84**: 893-902
10 **Gersh BJ**, Simari RD, Behfar A, Terzic CM, Terzic A. Cardiac cell repair therapy: a clinical perspective. *Mayo Clin Proc* 2009; **84**: 876-892
11 **Battiwalla M**, Hematti P. Mesenchymal stem cells in hematopoietic stem cell transplantation. *Cytotherapy* 2009; **11**: 503-515
12 **Locatelli F**, Bersano A, Ballabio E, Lanfranconi S, Papadimitriou D, Strazzer S, Bresolin N, Comi GP, Corti S. Stem cell therapy in stroke. *Cell Mol Life Sci* 2009; **66**: 757-772
13 **Bernardo ME**, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* 2007; **67**: 9142-9149
14 **Aggarwal S**, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005; **105**: 1815-1822
15 **Le Blanc K**, Tammik L, Sundberg B, Haynesworth SE, Ringdén O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol* 2003; **57**: 11-20
16 **Zhang M**, Mal N, Kiedrowski M, Chacko M, Askari AT, Popovic ZB, Koc ON, Penn MS. SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. *FASEB J* 2007; **21**: 3197-3207
17 **Zvaifler NJ**, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, Maini RN. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res* 2000; **2**: 477-488
18 **Zuk PA**, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001; **7**: 211-228
19 **Sottile V**, Halleux C, Bassilana F, Keller H, Seuwen K. Stem cell characteristics of human trabecular bone-derived cells. *Bone* 2002; **30**: 699-704
20 **Erices A**, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol* 2000; **109**: 235-242
21 **Igura K**, Zhang X, Takahashi K, Mitsuru A, Yamaguchi S, Takashi TA. Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. *Cytotherapy* 2004; **6**: 543-553
22 **Pereira RF**, O'Hara MD, Laptev AV, Halford KW, Pollard MD, Class R, Simon D, Livezey K, Prockop DJ. Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1998; **95**: 1142-1147
23 **Quevedo HC**, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D, Pattany PM, Zambrano JP, Hu Q, McNiece I, Heldman AW, Hare JM. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proc Natl Acad Sci USA* 2009; **106**: 14022-14027
24 **Conrad C**, Niess H, Huss R, Huber S, von Luettichau I, Nelson PJ, Ott HC, Jauch KW, Bruns CJ. Multipotent mesenchymal stem cells acquire a lymphendothelial phenotype and enhance lymphatic regeneration in vivo. *Circulation* 2009; **119**: 281-289
25 **Tadokoro M**, Kanai R, Taketani T, Uchio Y, Yamaguchi S, Ohgushi H. New bone formation by allogeneic mesenchymal stem cell transplantation in a patient with perinatal hypophosphatasia. *J Pediatr* 2009; **154**: 924-930
26 **Chen J**, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke* 2001; **32**: 2682-2688
27 **Hofstetter CP**, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ, Olson L. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci USA* 2002; **99**: 2199-2204
28 **Ankeny DP**, McTigue DM, Jakeman LB. Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. *Exp Neurol* 2004; **190**: 17-31
29 **Ohtaki H**, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, Prockop DJ. Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. *Proc Natl Acad Sci USA* 2008; **105**: 14638-14643
30 **Ren G**, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008; **2**: 141-150
31 **Prockop DJ**. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. *Mol Ther* 2009; **17**: 939-946
32 **Mazhari R**, Hare JM. Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche. *Nat Clin Pract Cardiovasc Med* 2007; **4** Suppl 1: S21-S26
33 **Schuleri KH**, Amado LC, Boyle AJ, Centola M, Saliaris AP, Gutman MR, Hatzistergos KE, Oskouei BN, Zimmet JM, Young RG, Heldman AW, Lardo AC, Hare JM. Early improvement in cardiac tissue perfusion due to mesenchymal stem cells. *Am J Physiol Heart Circ Physiol* 2008; **294**: H2002-H2011
34 **Mazo M**, Planat-Bénard V, Abizanda G, Pelacho B, Léobon B, Gavira JJ, Peñuelas I, Cemborain A, Pénicaud L, Laharrague P, Joffre C, Boisson M, Ecay M, Collantes M, Barba J, Casteilla L, Prósper F. Transplantation of adipose derived stromal cells is associated with functional improvement in a rat model of chronic myocardial infarction. *Eur J Heart Fail* 2008; **10**: 454-462
35 **Gnecchi M**, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res* 2008; **103**: 1204-1019
36 **Tendera M**, Wojakowski W, Ruzyłło W, Chojnowska L, Kepka C, Tracz W, Musiałek P, Piwowarska W, Nessler J, Buszman P, Grajek S, Breborowicz P, Majka M, Ratajczak MZ. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial. *Eur Heart J* 2009; **30**: 1313-1321
37 **Lunde K**, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grøgaard HK, Bjørnerheim R, Brekke M, Müller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med* 2006; **355**: 1199-1209
38 **Schächinger V**, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Süselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med* 2006; **355**: 1210-1221
39 **Horwitz EM**, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 1999; **5**: 309-313
40 **Horwitz EM**, Gordon PL, Koo WK, Marx JC, Neel MD, McNall

RY, Muul L, Hofmann T. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc Natl Acad Sci USA* 2002; **99**: 8932-8937
41 **Whyte MP**, Kurtzberg J, McAlister WH, Mumm S, Podgornik MN, Coburn SP, Ryan LM, Miller CR, Gottesman GS, Smith AK, Douville J, Waters-Pick B, Armstrong RD, Martin PL. Marrow cell transplantation for infantile hypophosphatasia. *J Bone Miner Res* 2003; **18**: 624-636
42 **Tseng SS**, Lee MA, Reddi AH. Nonunions and the potential of stem cells in fracture-healing. *J Bone Joint Surg Am* 2008; **90** Suppl 1: 92-98
43 **Cuomo AV**, Virk M, Petrigliano F, Morgan EF, Lieberman JR. Mesenchymal stem cell concentration and bone repair: potential pitfalls from bench to bedside. *J Bone Joint Surg Am* 2009; **91**: 1073-1083
44 **Toma C**, Wagner WR, Bowry S, Schwartz A, Villanueva F. Fate of culture-expanded mesenchymal stem cells in the microvasculature: in vivo observations of cell kinetics. *Circ Res* 2009; **104**: 398-402
45 **Wall ME**, Bernacki SH, Loboa EG. Effects of serial passaging on the adipogenic and osteogenic differentiation potential of adipose-derived human mesenchymal stem cells. *Tissue Eng* 2007; **13**: 1291-1298
46 **Kretlow JD**, Jin YQ, Liu W, Zhang WJ, Hong TH, Zhou G, Baggett LS, Mikos AG, Cao Y. Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells. *BMC Cell Biol* 2008; **9**: 60
47 **Samuelsson H**, Ringdén O, Lönnies H, Le Blanc K. Optimizing in vitro conditions for immunomodulation and expansion of mesenchymal stromal cells. *Cytotherapy* 2009; **11**: 129-136
48 **Hu X**, Yu SP, Fraser JL, Lu Z, Ogle ME, Wang JA, Wei L. Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. *J Thorac Cardiovasc Surg* 2008; **135**: 799-808
49 **Ivanovic Z**. Hypoxia or in situ normoxia: The stem cell paradigm. *J Cell Physiol* 2009; **219**: 271-275
50 **Grayson WL**, Zhao F, Izadpanah R, Bunnell B, Ma T. Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3D constructs. *J Cell Physiol* 2006; **207**: 331-339
51 **Ma T**, Grayson WL, Fröhlich M, Vunjak-Novakovic G. Hypoxia and stem cell-based engineering of mesenchymal tissues. *Biotechnol Prog* 2009; **25**: 32-42

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