Book Chapter

Exercise as an Adjuvant to Cartilage Regeneration Therapy

John Kelly Smith

Departments of Academic Affairs and Biomedical Sciences, James H. Quillen College of Medicine, East Tennessee State University, USA

***Corresponding Author:** John Kelly Smith, Departments of Academic Affairs and Biomedical Sciences, James H. Quillen College of Medicine, East Tennessee State University, P.O. Box 70300, Johnson City, TN 37614, USA

Published **September 14, 2022**

This Book Chapter is a republication of an article published by John Kelly Smith at International Journal of Molecular Sciences in December 2020. (Smith, J.K. Exercise as an Adjuvant to Cartilage Regeneration Therapy. Int. J. Mol. Sci. 2020, 21, 9471. https://doi.org/10.3390/ijms21249471)

How to cite this book chapter: John Kelly Smith. Exercise as an Adjuvant to Cartilage Regeneration Therapy. In: Saeed Tarighi, editor. Prime Archives in Molecular Sciences: 3rd Edition. Hyderabad, India: Vide Leaf. 2022.

Foreword

In the global burden of disease 2010 study, osteoarthritis accounted for 17,135 years of life lived with disability (YLD), an increase of 64% when compared to YLD of 1990. Overall, musculoskeletal disorders (which included inflammatory causes of arthritis) accounted for 6.8% of total YLDs [1] with osteoarthritis ranked as the 11th leading cause of disability worldwide [2]. And the global prevalence of osteoarthritis is expected to increase as the average age and weight of the World's population increases.

Physical exercise has long been recognized as an essential factor in the maintenance of skeletal health, particularly during adolescence when ~ 50% of bone mass accretion occurs [3]. The 2019 American College of Rheumatology/Arthritis Foundation guidelines for the management of osteoarthritis of the hip and knee emphasized the importance of regularly performed physical exercise [4]. Both traditional (resistance, aerobic and flexibility) and non-traditional (Tai Chi, yoga, aquatic) exercises have been shown to be effective in the management of knee and hip osteoarthritis [5]. In their systematic review of 44 clinical trials involving patients with knee osteoarthritis, Fransen and associates found that land-based therapeutic exercises reduced pain and improved physical function and the quality of life for at least 2-6 months after cessation of formal treatment [6]. In this regard, The World Health Organization recommends that adult men and women should accumulate at least 150 min of moderate intensity physical exercise per week and young people aged 5–17 years should accumulate at least 60 min of physical exercise of moderate to vigorous intensity daily [7].

There is increasing interest in treating articular cartilage and subchondral bone defects and osteoarthritis with autologous chondrocyte implants (ACI), matrix autologous chondrocyte implants (MACI) and bone marrow-derived mesenchymal cell implants or injections [8-14]. ACI and MACI procedures have been shown to produce durable long-term outcomes in the treatment of partial and full-thickness articular cartilage defects in knee joints [15-19]. In addition, MSCs mobilized to joints

from the peripheral blood or placed on implantation matrices have the potential to repair cartilage by differentiating into chondrocytes [20].

In this chapter I review the pathophysiology of osteoarthritis and detail how physical exercise improves the health of articular cartilage and chondrocytes in osteoarthritis and enhances the potential of mesenchymal stem cells and chondrocytes for successful implantation therapy. I discuss how exercise protects the skeletal system by upregulating the production of anti-osteoclastogenic cytokines and downregulating the production of osteoclastogenic cytokines by chondrocytes and peripheral blood mononuclear cells. Also provided is a summary of scaffolds currently approved or undergoing clinical trials for MACI and exercise protocols that can be used to rehabilitate osteoarthritis patients and recipients of chondrocyte and mesenchymal stem cell implants. I also provide an aerobic exercise protocol that can be used to condition tissue donors.

Definition of Exercise

In this chapter I have used the following definition of exercise: A physical activity that involves repetitive voluntary contractions of limb, back or abdominal muscles that are of sufficient force to maintain or improve physical conditioning.

Pathophysiology of Osteoarthritis

Osteoarthritis (OA) is a complex polygenetic disease involving structural and functional alterations of the entire joint, including the articular cartilage, menisci, subchondral bone, capsule, synovium, ligaments and periarticular muscles. The knee is the most common joint to be involved followed by the hand and hip. OA is especially prevalent in the elderly, particularly elderly women, in overweight individuals, and in persons with traumatic joint injuries including those that are work related (e.g., lifting of heavy loads, constant knee bending). The contribution of genetics in OA is estimated to be between 40-80% with late onset OA being associated with many common DNA variants [21].

Osteoarthritis is an ongoing process involving mechanical, inflammatory, and metabolic derangements of the articular cartilage, subchondral bone and synovium. This is reflected in the finding of elevated blood levels of C-reactive protein (CRP) and picrogram amounts of the proinflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β early in the disease and proportionate to the patients' symptoms and, in the case of TNF-α, in keeping with radiographic findings of OA [22].

Articular cartilage is comprised primarily of tissue fluid, which accounts for 65-85% of its mass, and Type II collagen and proteoglycans, which account for 15-22% and 4-7% of its mass, respectively. Proteoglycans include the small leucine rich decorin, lumican, biglycan, fibromodulin, lumican and epiphycan, and the heparin sulfate proteoglycan perlecan. Other collagens (types V, VI, IX, X, XI, XII, IV), cell adhesins, growth factors, and cytokines are also present. The predominant cell in articular cartilage is the chondrocyte, which is responsible for maintaining articular homeostasis by replacing degraded matrix with newly synthesized components [23]. Also present in articular cartilage, synovium and synovial fluid are mesenchymal stem cells, which may serve as a valuable source of stem cell transplantation in the treatment of OA [24].

Osteoarthritis is characterized by early alterations in the organization and molecular composition of the articular cartilage extracellular matrix. This change is met with a compensatory proliferative response of chondrocytes and an increase in chondrocyte matrix synthesis. With time, hypertrophied chondrocytes become senescent, a phenotype associated with the secretion of proinflammatory cytokines and matrix degrading proteases and a reduction in the secretion of antiinflammatory cytokines [25-27]; this, in turn, stimulates proliferative and proinflammatory responses in adjacent synovium and periarticular bone causing synovial hypertrophy and contributing to the development of osteophytes. Subchondral bone turnover and angiogenesis is increased with consequent vascular invasion of the cartilage [21]. Senescent chondrocytes eventually undergo apoptosis terminating articular cartilage synthesis [25]. Other

metabolic derangements involving transforming growth factor (TGF)-β, fibroblast growth factor (FGF)-2, FGF-18, growth differentiation factor (GDF)-5, and hypoxia-induced factor (HIF)-2a may also contribute to the pathogenesis of osteoarthritis [23].

Studies on menisci, which contain multiple subpopulations of cells responsible for tissue repair and maintenance ("fibrochondrocytes") have shown that their production of IL-1 is elevated in OA (109-288 pg/mL). IL-1 is a potent proinflammatory and osteoclastogenic cytokine whose effects on menisci and articular cartilage are catabolic [28].

Studies in mice indicate that nuclear factor of activated T cells 1 (NFAT1), a member of NFAT transcription factors, plays a critical role in maintaining the anabolic functions of adult articular chondrocytes by regulating their expression of matrix degrading proteinases and proinflammatory cytokines. Deletion of NFAT1 in adult mice results in a loss of type-II collagen and aggrecan and an over-expression of matrix degrading proteases and proinflammatory cytokines; this is followed by chondrocyte proliferation and hypertrophy, destruction of articular surfaces, osteophyte formation, and exposure of subchondral bone – findings characteristic of OA [29]. Rodova and associates determined that NFAT1 expression in articular cartilage is regulated epigenetically by histone methylation [30]. The anabolic activity of chondrocytes is maintained by their secretion of growth factors TGF-β, insulin-like growth factor (IGF)-1, FGF-2, FGF-18, GDF-5, and bone morphogenic proteins (BMPs) (Figure 1).

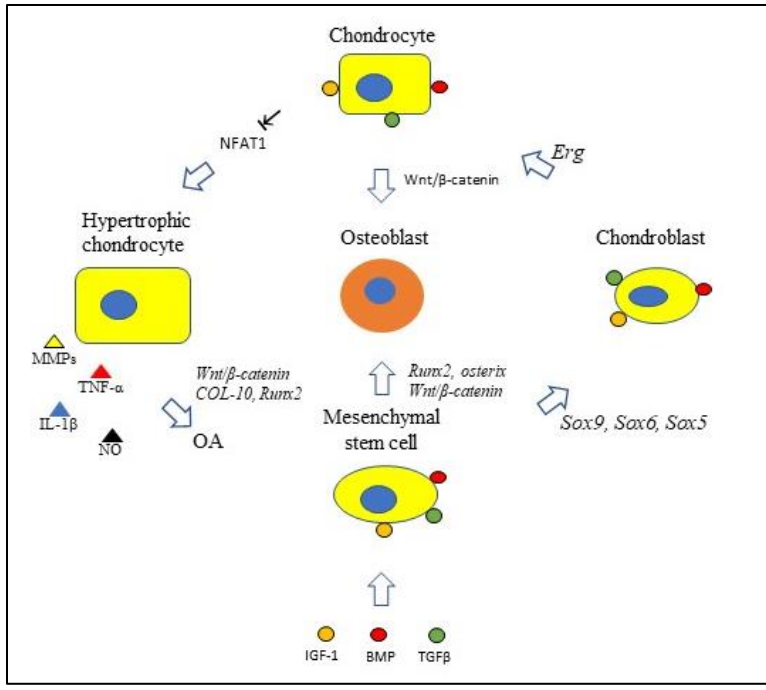

Figure 1: With the aid of growth factors IGF-1, TGF-β, and BMPs, bone marrow mesenchymal stem cells (BM-MSC) expressing *Sox9* and/or *Sox6 or Sox5* differentiate into chondroblasts. Ets-related gene (*Erg*) transcriptional activation prompts chondroblasts to differentiation into mature chondrocytes expressing the transcription factor NFAT1 which plays a critical role in maintaining chondrocyte homeostasis. In adult mice, deletion of chondrocyte NFAT1 results in a loss of type-II collagen and aggrecan and an over-expression of cartilage degrading proteases, proinflammatory cytokines, and nitric oxide (NO); this is accompanied by chondrocyte proliferation and hypertrophy, destruction of articular surfaces, osteophyte formation, and exposure of subchondral bone – findings characteristic of OA.

Wnt/β-catenin signaling in chondrocytes can prompt their differentiation into osteoblasts, and activation of *runx2* and *osterix* in BM-MSCs prompts their differentiation into osteoblasts. Other growth factors involved in chondrocyte homeostasis are FGF-2, FGF-18, and GDF-5 (not shown).

Exercise and Osteoarthritis

Although regularly performed moderate intensity exercise is recognized as the mainstay treatment of OA [4,5] there are a limited number of studies sampling constituents of the OA joint before and after supervised exercise training of men and women. One of these was published by Roos and Dahlberg and involved 45 subjects who had undergone medial meniscus resection 3-5 years prior to the study and were at risk of developing OA. Subjects underwent supervised exercise training 3 times weekly for 4 months or were assigned to a noninterventional group. All subjects had the content of their knee cartilage glycosaminoglycan content assessed by delayed gadolinium-enhanced magnetic resonance imaging. Exercise increased cartilage levels of glycosaminoglycan in proportion to the level of physical activity [31].

In a similar study, Munukka and associates assessed the effects of 12 months of leisure time physical activity on the glycosaminoglycan content of femoral cartilages in 76 post-menopausal women with knee OA using delayed gadolinium-enhanced magnetic resonance imaging. They also found that exercise increased the amount of cartilage glycosaminoglycan [32].

Iijima and associates studied the effects of 2-4 weeks of treadmill walking in 24 male Wistar rats with induced damage to their knee joints using micro-computed tomography, histology and immunohistochemistry analysis. They found that exercise prevented the progression of post-traumatic bone and cartilage lesions and increased BMP-2 and BMP-6 expression in the joint superficial zone chondrocytes [33].

Assis and associates studied the effects of aerobic exercise training on an experimental model of knee osteoarthritis in 50 male Wistar rats. Twenty of the rats were trained on treadmills 3 days/week at 16 meters/minute for 50 minutes/day for 8 weeks. The exercising and control rats were sacrificed, and their knee joints assessed by histologic, morphometric and immunohistochemical analysis. Compared to the controls,

exercising animals had a better pattern of cartilage organization and less cartilage degeneration. Exercising animals also had lower chondrocyte nuclear or nucleolar expression of IL-1β, caspase-3 and matrix metalloproteinase (MMP)-13, confirming the ability of aerobic exercise to downregulate proinflammatory and proteolytic pathways in this model of OA [34].

Exercise and Mesenchymal Stem Cells

The author is using the International Society for Cell and Gene Therapy committee's recommendation that the acronym "MSC" be used for both mesenchymal stem cells and mesenchymal stromal cells and that the MSC acronym be preceded by "BM" for bone marrow origin and "AD" for adipose tissue origin [35].

Exercise Studies in Rodents

Liu et al. reported the effects of 8 weeks of treadmill exercise (60 minutes per day at 19.3 meters/minute, 5 degree incline) on the proliferative, differential and apoptotic abilities of cultured femoral BM-MSC. They found that exercise enhanced their osteogenic potential and decreased their adipogenic potential and posited that "BMSC derived from exercised rats on early passage may be a good cell source for bone tissue engineering" [36]. Emmons et. al. report that 15 and 60 minutes of treadmill exercise done by C57 BI/6 mice increased the proliferative capacity of their bone marrow hematopoietic stem cells (BM-HSC) and multipotential HSC progenitors by 40-61%. They attribute these findings to a change in the BM-HSC secretome which included an upregulation of granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF) [37]. Bourzac et. al. reviewed literature reports on the effects of physical exercise on MSC proliferation, differentiation and homing and found that the effects of exercise varied depending on the exercise protocol and the tissue from which MSC were obtained; they concluded that "the combination of physical exercise and MSC engraftment improves neural, cartilage, and muscular tissue recovery, but it is not clear whether the effects of MSCs and exercise are additive or synergistic" [38]. Ocarino et. al. studied the effects of exercise on BM-MSC in osteopenic

female Wistar rats with and without nitric oxidase inhibition. BM-MSC were isolated from their femurs and cultured in osteogenic medium for 7, 14 and 21 days, phenotyped and analyzed for alkaline phosphatase, collagen synthesis and formation of mineralized nodules. They found that exercise increased BM-MSC osteogenesis and that inhibition of nitric oxide diminished their osteogenic response. They concluded that "nitric oxide mediates the beneficial effects of physical activity upon MSCs osteogenic differentiation" [39]. Hell and associates measured the effects of treadmill exercise on the osteogenic potential of BM-MSC in young and adult female Wistar rats by measuring cell viability, percentage of cells per field, mineralized nodular number and gene expression for telomerase reverse transcriptase (TERT), alkaline phosphatase (AP), caspase 3, osteocalcin, collagen I and sialoprotein. They found that exercise increased the differentiation of BM-MSCs in both study groups, but the effect was greater in young animals than in adults [40]. Using mice, Wallace and associates measured the effects of 5 days of treadmill exercise (30 minutes/day) on BM-MSC and found that exercise increased their osteogenic potential [41]. Yamaguchi measure the effects of exercise on the ability of BM-MSC obtained from male Wistar rats to repair experimentally induced femoral groove osteochondral defects in female Wistar rats. Two weeks after BM-MSC were injected into the defective joints, rats were either sedentary or subjected to 2, 4, or 8 weeks of treadmill exercises performed 5 days/week at 12 meters/minute for 30 minutes; the animals were then sacrificed, and their joints subjected to immuno-histochemical staining. Compared to the sedentary group, they found that exercise enhanced cartilage repair and concluded that their study "highlights the importance of exercise following cell transplantation therapy" [42].

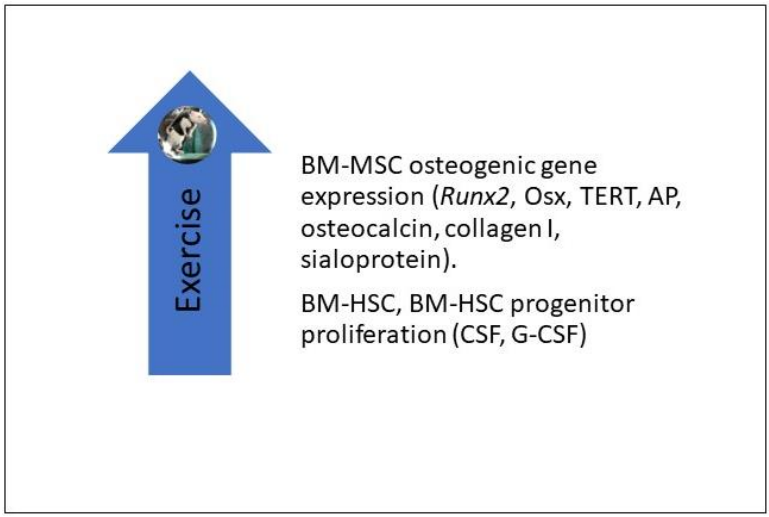

Figure 2: Studies in rodents have shown that treadmill exercise upregulates the osteogenic potential of BM-MSC including their expression of osteogenic genes. Exercise also increases the proliferative capacity of BM-HSC and BM-HSC progenitors by upregulating G-CSF and CSF levels in their secretome.

AP, alkaline phosphatase; BM-HSC, bone marrow-derived hematopoietic stem cells; BM-MSC, bone marrow-derived mesenchymal stem cells; C/EBPα, CCAAT enhancer-binding protein-α; CSF, colony stimulating factor; G-CSF, granulocyte colony stimulating factor; Osx, osterix; PPARγ-2, peroxisome proliferator-activated receptor-γ-2; *Runx2*, runt-related transcription factor 2; TERT, telomerase reverse transcriptase.

Mechanical Strain Study in Rodents

Using cultures of BM-MSC flushed from the femurs and tibia of Sprague-Dawley rats, Runguang et. al. found that mechanical strain exerted on the cultures by a FLEXcell-500 device promoted increased BM-MSC expression of osteogenic markers *Runx2*, osterix (Osx) and type I-collagen, and decreased their expression of adipogenic markers peroxisome proliferator-activated receptor-γ (PPARγ-2) and CCAAT enhancer-binding protein α (C/EBPα). *Runx2* is the main regulatory gene controlling skeletal development and morphogenesis in vertebrates, Osx is a transcription factor for osteoblasts, PPARγ-

2 is a transcription factor that regulates differentiation of MSCs into adipocytes, and C/EBPα induces spleen focus-forming virus proviral integrin 1 (PU.1) and interacts with activator protein-1 (AP-1) and nuclear factor kappa-B (NF_KB) to regulate myeloid development. The authors concluded that mechanical strain promotes BM-MSC differentiation into osteoblasts while impeding their differentiation into adipocytes [43].

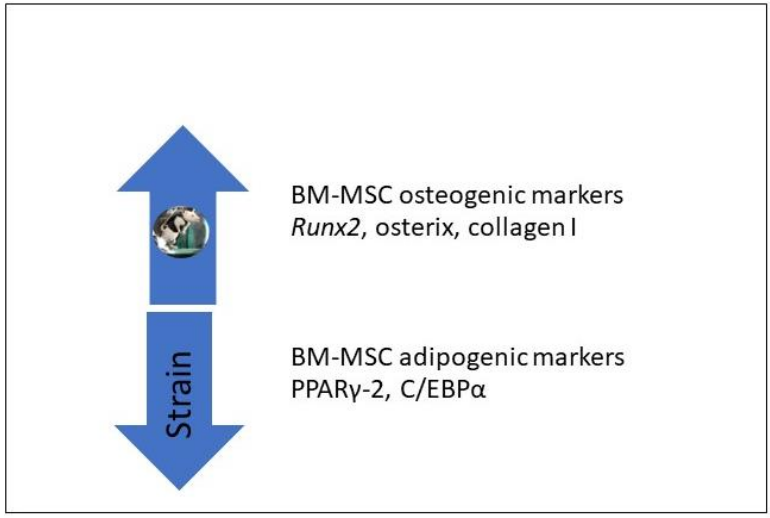

Figure 3: Mechanical strain exerted on BM-MSC cultures by a FLEXcell-500 device increased their expression of osteogenic markers *Runx2*, osterix (Osx) and type I-collagen and decreased their expression of adipogenic markers peroxisome proliferator-activated receptor-γ (PPARγ-2) and CCAAT enhancer-binding protein α (C/EBPα).

Exercise Studies in Humans

There are a limited number of studies on the effect of exercise on human BM-MSC. Schmidt and associates studied the effects of short term high intensity exercise on the ability of post-exercise sera to influence the proliferation, migration and apoptosis activity of cultured BM-MSC. They found that post-exercise sera enhanced the migratory capacity of BM-MSC, a finding they attributed to the generation of IL-6 by contracting skeletal muscles. They posited that "there is a direct relationship between exercise, IL-6 release and stem cell recruitment" [44].

Carbonare et. el. studied the effects of running one-half a marathon on the differentiation potential of mesenchymal circulating progenitor cells (M-CPCs) and on the effects of sera on a human bone marrow-derived mesenchymal stem cell line (hBM-MSC) in 22 athletes. They found that exercise upregulated the expression of osteogenic genes *Runx2, muscle segment homobox gene 1 (MSx1), secreted phosphoprotein 1 (SPP-1)* and chondrogenic genes *SRY-Box 9 (SOX9), collagen type II alpha-1 gene (COL2A1),* and apoptosis-related genes *autophagy-related gene 3 (ATG3) and Unc-like kinase gene (Ulk1)* in M-CPCs. Also upregulated were BMP2 and BMP6. The authors concluded that exercise upregulated the differentiation and apoptosis of BM-MSC [45]. In a study involving 20 amateur runners, Valenti et. al. assessed the effects of running one-half a marathon on the expression of micro-RNAs (miRNAs) in human BM-MSC incubated with pre- and post-exercise sera. They found that exercise upregulated the expression of miRNAs promoting osteoblast differentiation, including miR-21-5p, miR-129-5p, miR-378-5p, and miR-188-p, while downregulating the expression of a miRNA that promotes adipocyte differentiation (miRNA-188-5p). They also found that exercise upregulated the expression of the osteogenic gene *Runx2* [46]. Niemiro et. al. studied the kinetics of progenitor cell mobilization during 60 minute treadmill exercises (70% Vo_{2peak}) performed by seven men. They found that exercise increased circulating levels of cysteine x cysteine (CXC) chemokine ligand (CXCL)-12 and SCF in hematopoietic stem cells but not in BM-MSC. They concluded that exercise may serve as a valuable adjunct in the context of HSC transplants [47]. Zhang et. al. found that dynamic compression of the type that occurs with exercise increased the expression of chondrogenic genes in cultures of human BM-MSC [48]. Sumanasinghe and associates seeded human BM-MSC in 3D type I collagen matrices and subjected them to 0%, !0%, or 12% uniaxial cyclic tensile strain at 1 Hz for 4 hours/day for 7 or 14 days. They found that BMP2 mRNA expression and BMP2 production was upregulated in the strain samples as compared to controls indication that mechanical strain of the type associated with exercise can induce osteogenic differentiation of human BM-MSC [49].

In summary, experiments in humans, while limited in number, have shown that exercise upregulates BM-MSC and BM-HSC recruitment, enhances BM-MSC osteogenic, chondrogenic and apoptotic gene expression, and upregulates BM-MSC expression of osteogenic miRNAs and the secretion of growth factors (Figure 3, Table 1).

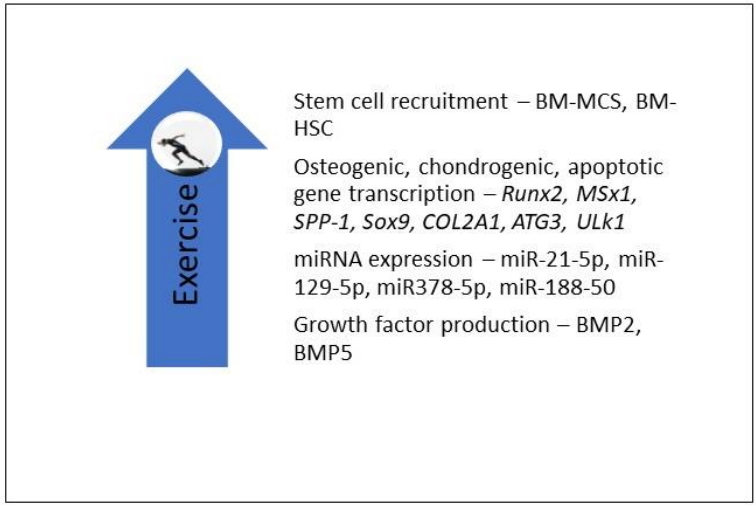

Figure 4: Studies in humans indicate that exercise increases the recruitment of BM-MSC and BM-HSC and upregulates the expression of osteogenic, chondrogenic, apoptotic genes, osteogenic micro-RNAs, and osteogenic growth factors in BM-MSC. *ATG3, autophagy-related gene 3; COL2A1, collagen type II alpha-1 gene; MSx1, muscle segment homobox gene 1*; *Runx2, runt-related transcription factor 2*; SPP-1, secreted phosphoprotein 1; *SOX-9*, SRY-Box 9; *Ulk1, Ul kinase gene 1;* miR, micro-RNA; BMP, bone morphogenic protein.

Exercise and Osteoclastogenic and Antiosteoclastogenic Cytokines

In a before and after trial involving 43 healthy adults Smith and associates measured the effect of six months of combined aerobic, resistance, and flexibility exercises on the production of osteoclastogenic cytokines (IL-1α, TNF-α), anti-osteoclastogenic cytokines (TGF-β, IL-4, IL-10), and cytokines with variable effects on osteoclastogenesis (interferon (IFN)-γ, IL-6) by cultured mitogen-stimulated peripheral blood

mononuclear cells (PBMC). Also measured were serum markers of bone formation (osteocalcin) and bone resorption (C-terminal telopeptides of Type I collagen). Exercises done on an average of 2.5 hours a week attenuated the production of osteoclastogenic cytokines and enhanced the production of antiosteoclastogenic cytokines (Figure 2). These changes were accompanied by a 16% reduction in collagen degradation products and a 9.8% increase in osteocalcin levels. They concluded that "Long-term moderate intensity exercise exerts a favorable effect on bone resorption by changing the balance between blood mononuclear cells producing osteoclastogenic cytokines and those producing antiosteoclastogenic cytokines" [50].

Other studies have shown that moderate intensity exercise performed on a regular basis decreases blood levels of osteoclastogenic cytokines and increases blood levels of antiosteoclastogenic cytokines. Santos et. al. reported that exercise training of 22 elderly men for 60 minutes/day, 3 days per week for 24 weeks reduced blood levels of IL-6 and TNF-α and increased blood levels of IL-10 [51]. In a similar study involving 6 months of aerobic or resistance exercise in 80 elderly men, El-Kader et. al. reported that aerobic exercise was superior to resistance exercise in reducing blood levels of IL-6 and TNF-α and increasing blood levels of IL-10 [52,53]. Similar results were reported by Yuan and associates, including exercise-related reductions in osteoclastogenic cytokines IL-1, IL-6, and TNF-α and exercise-related increases in antiosteoclastogenic cytokines IL-2, IL-10, IL-12, IL-13, IL-18, and IFN-γ [54]. In a study involving the effect of acute resistance knee exercise (25 sets of 10 repetitions at 60% of one repetition maximum) in 12 women with knee OA, Helmark et. al. found that exercise increased intraarticular and perisynovial levels of the antiinflammatory cytokine IL-10 as compared to levels found in 13 non-exercising controls [55].

In summary, exercise upregulates PBMC production and serum levels of antiosteoclastogenic cytokines and downregulates PBMC production and serum levels of osteoclastogenic

cytokines. In one study, exercise increased intraarticular and perisynovial levels of IL-10 in patients with knee OA (Figure 4).

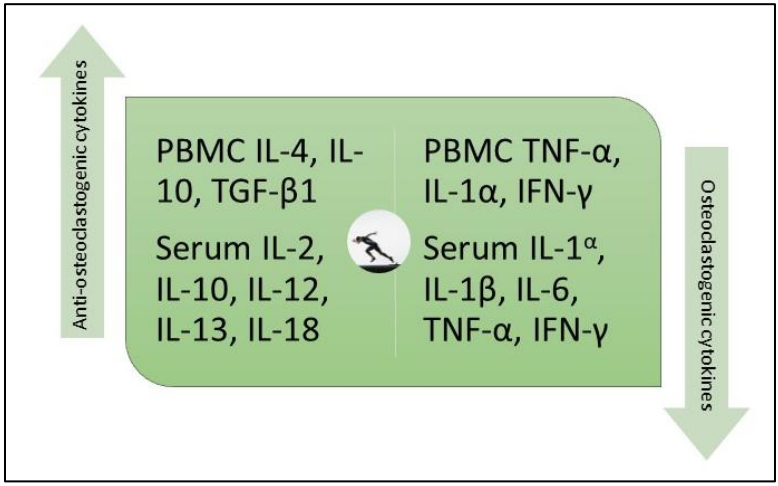

Figure 5: Exercise increases PBMC secretion and serum levels of antiosteoclastogenic cytokines while reducing PBMC secretion and serum levels of osteoclastogenic cytokines. PBMC, peripheral blood mononuclear cells; IL, interleukin; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ. IFN-γ is a pleotropic cytokines with both antiosteoclastogenic and osteoclastogenic activities which are context dependent.

Scaffolds

A variety of scaffolds are in development for use in MACI therapy, including hydrogels containing type-I collagen, hyaluronic acid, albumin, fibrin, agarose, or alginate, and composite scaffolds composed of type-I and type III collagen, hyaluronic acid and poly-glycolic acid, polyacetic acid, or polydioxanone. Currently, clinical trial entry for osteochondral repair is only approved for a nanocomposite three-layered collagen-hydroxyapatite scaffold, a poly (lactic-co-glycolic acid)-calcium-sulfate bilayer scaffold, and an aragonite-based scaffold [152][56]. There is emerging interest in scaffolds that can deliver gene carriers that upregulate the production of growth factors in articular cartilage and subchondral bone [57].

Rehabilitation Protocols
For Osteoarthritis

There is no consensus on what constitutes an optimal rehabilitation exercise program for patients with OA with the exception that regularly performed physical exercise should become a lifetime commitment and should be done in sufficient volume to relieve pain and improve function. In designing a rehabilitation program, consideration should be given to exercises that simulate the type of muscle activity that patients use in their daily routines. The American College of Sports Medicine (ACSM) guidelines suggest that aerobic training should include a minimum of 150 minutes of moderate intensity or 75 minutes of vigorous intensity aerobic exercise per week in bouts of at least 10 minutes. For resistance training, two sessions per week, with two sets of 8 to 12 repetitions at a load of 60% to 70% of one repetition maximum with a rest period of $\geq$ 48 hours between resistance training sessions is indicated. Resistance training can produce favorable responses independent of the type of equipment (dynamometers, weights, bands) utilized, the type of exercise (e.g., isokinetic, isotonic), and the muscle action (i.e., isometric, eccentric concentric) performed [58].

For Post-Transplant Rehabilitation

In their comprehensive review of postoperative procedures used to rehabilitate matrix-induced autologous chondrocyte implants of the tibial-femoral joint Edward and associates concluded that allowing patients to bear full weight at 6 weeks postoperatively allowed a quicker return to normal activity. Their review included 6-, 8-, 10- and 12-week rehabilitation programs all of which began with two weeks of 20% weight bearing coupled with continuous passive motion (CPM) of 0-40 degrees flexion knee exercises starting 12-24 hours post-surgery [ ]. Upon full recovery from transplant surgery, applying the ACSM exercise guidelines for rehabilitation of osteoarthritis would seem appropriate.

Pre-Implantation Exercises

For healthy donors of bone marrow-derived MSC, the author recommends prescribing an aerobic exercise regimen using the following guidelines: 1. Calculate the patient's age-based maximum heart rate (MHR) using the following formula: MHR = 220 – age. 2. Start the exercise program at 40-50% of MHR with weekly increments until the patient reaches 75-85% of MHR. The exercises should be done ≥ 30 minutes/day 5 days/week. This exercise formula may also be useful in setting aerobic exercise goals in OA patients and in patients with implants following their period of rehabilitation.

Mesenchymal stem cells are fibroblast-like cells that arise from embryonic mesenchyme and serve as the source of bone, cartilage, tendon, and adipose tissue. In addition to bone marrow and peripheral blood, MSC are found in the vascular niches of adipose tissue, skeletal and cardiac muscle, lung, cartilage, and tendons. These progenitor cells synthesize and secrete a number of growth factors, extracellular matrix proteins and cytokines that support the growth and survival of hematopoietic stem cells. They are identified by their expression of cell surface markers CD73, CD90, and CD105 and the absence of hematopoietic markers CD14 and CD45. Other defining criteria for MSC include evidence of clonal expansion and/or the capacity to differentiate into multiple cell types including tendon, cartilage, bone and adipose tissue. MSC represent a small fraction of the mononuclear cell population in bone marrow (0.001-0.01%) [52].

At present, implant techniques used to treat knee OA include third generation scaffold implantations and chondrocyte and MSC injectable techniques. Importantly, methods using biomaterials that incorporate chondroprogenitors, chondrocytes and MSC have led to significant progress in regenerating OA joints [Richardson, 2016]. Scaffold implantation has produced a hyaline-like cartilage repair, durable pain and function improvement, and no donor site morbidity. BM-MSC injections have resulted in pain and functional improvement but have limited evidence of efficacy [11].

There is an emerging interest in developing scaffolds using viral and nonviral vectors that deliver gene or gene products that stimulate the production of growth factors by articular chondrocytes and subchondral bone. These growth factors include TGF-β, insulin-like growth factor (IGF)-1, BMPs (particularly BMP-2 and BMP-7), and basic fibroblast growth factor (FGF-2). Also planned is the use of transcription factors to promote osteochondral healing, including Sox5, Sox6, Sox9, *Runx2* and Osx as well as the use of mRNAs [Madry 2020]. It is reassuring that physical exercise has been shown to achieve similar goals, including the upregulation of the expression of osteochondral genes and transcription factors (*Runx2*, Osx, MSx1, SPP-1, Sox9, COL2A1, ATG3 and Ulk1), mRNAs encoding ALP, BMP2, BMP4, OCL, and Collagen I, and the production of osteochondral end products including TERT, ALP, caspase3, osteocalcin, BMP2 and BMP6 (see Figures 2 and 3).

In light of the beneficial effects of exercise on articular cartilage chondrocytes and on BM-MSC expression of osteochondral genes, transcription factors, and products, it is anticipated that exercise will improve the results of ACI and MACI in patients with osteochondral defects. ACI involves an arthroscopic biopsy of a small piece of articular cartilage taken from an area not subjected to major pressure, culturing the chondrocytes and injecting them into the diseased joint of the donor/recipient [53]; in MACI the chondrocytes are incorporated into a scaffold matrix and placed in the osteochondral defect during an open procedure. Preclinical trials have shown that both ACI and MACI are successful in producing hyaline-like cartilage regrowth [54-56] with reasonable long-term durability [57]. However, ACI is associated with up to 40% dedifferentiation of chondrocytes during culturing and/or after the transplant [58]. Whether exercise will improve this aspect of ACT is yet to be determined.

Whether other joint preservation techniques are benefitted by exercise is also to be determined. Osteoplasty involves drilling or punching of holes through the subchondral plate at the site of the chondral defect; this incites an inflammatory response which includes the mobilization of BM-MSC to the articular surface

[59]. Since exercise has been shown to increase circulating levels of BM-MSC, exercise done before and after osteoplasty has the potential to improve the results of this regenerative technique.

The evidence provided in this review supports a policy of recommending physical exercise for autologous chondrocyte donors/recipients and BM-MSC donors and recipients. In the author's view, patients with OA and recipients of osteochondral implants should make a lifetime commitment to perform physical exercise in keeping with ACSM guidelines.

Recommending exercise for patients undergoing regenerative procedures has the added benefit of reducing their risks for ischemic cardiovascular disease, hypertension, diabetes mellitus, the metabolic syndrome, and certain forms of cancer [60].

Conclusion

Physical exercise done both by bone marrow-derived mesenchymal stem cell donors and recipients and by autologous chondrocyte donor-recipients may improve the outcome of osteochondral regeneration therapies and improve skeletal health by downregulating the production of osteoclastogenic cytokines and upregulating the production of antiosteoclastogenic cytokines.

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