CONTENT

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Mesenchymal stem cells in orthopedics - a current perspective

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Abstract

Osteoarthritis is a common condition that can affect any joint in the body. It is encountered in all age groups, but with a higher incidence in the older population. There is no treatment currently available that would prevent the development or progression of osteoarthritis and the gold standard end-stage treatment is still total joint replacement surgery, which is not without its risks. Therefore, new approaches are considered daily to treat patients that are not yet at end-stage osteoarthritis, but still experience the most common symptoms of pain and joint dysfunction. Mesenchymal stem cell research offers new opportunities for osteoarthritis treatment as their paracrine effect exhibits clinical improvement in osteoarthritis patients, providing much-needed minimally invasive treatment options.

Keywords: *osteoarthritis, regenerative medicine, MSCs, lipoaspiration*

Introduction

Osteoarthritis (OA) is a progressive degenerative condition that can affect any joint in the body, but it primarily affects the knees, hips and hand joints [1-3]. The economic burden of OA is at least $89.1 billion annually [4,5]. Years of research in OA pathophysiology resulted in our better understanding of the underlying processes, as OA is now recognized as a whole joint disease that affects articular cartilage and subchondral bone, Hoffa's fat pad, synovia, ligaments, and muscles (Figure 1) [6-8]. The dominant symptoms of OA are joint pain and reduced motion that can be treated either pharmacologically or surgically, with total joint replacement surgery as an end-stage treatment [9].

OA affects 40% of people older than 70, presenting with first symptoms at the age of 55, suggesting that patients are living with decreased mobility and pain for more than 20 years [10,11]. It is approximated that 250 million people suffer from OA worldwide, with the female sex being at a higher risk of developing OA than men [12,13]. The observed difference in sex distribution can be attributed to different female anatomy compared to the one in males, such as narrower femurs, thinner patellae, larger angles of quadriceps and differences in the size of tibial condyles; leading to different kinematics and making women more likely to develop OA [14]. Obesity, increased body mass index (BMI), previous knee injury or malalignment are strong risk factors for knee OA, whereas hip deformities play a great role in

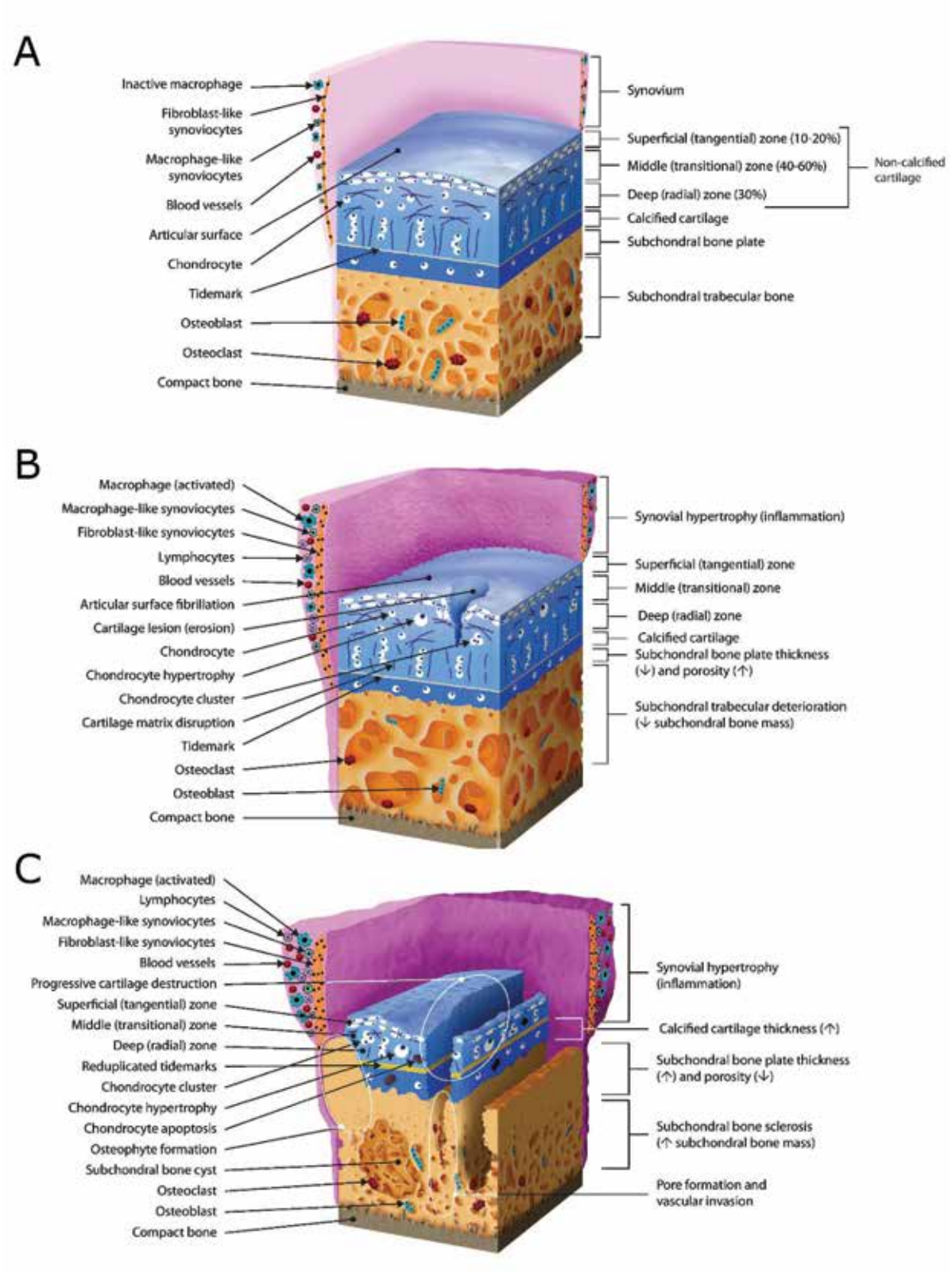

Figure 1. Microarchitectural and histologic changes of articular cartilage, subchondral bone and synovium in osteoarthritis. (A) Normal joint structure; (B) Early-stage osteoarthritis; (C) Late-stage osteoarthritis.

developing hip OA [15]. Any repetitive action that causes frequent injuries and/or cartilage defects such as: often kneeling, heavy lifting and professional sports activities are associated with higher risk of developing OA [12]. Additionally, the previous injury of ligament structures such as meniscal and anterior cruciate ligament tears also increases the risk of OA development [16]. One the other hand, physical inactivity causes higher susceptibility to joint damage, due to less stable and weaker joints [16]. Some studies indicate there is a connection between OA and a slightly increased risk of developing the cardiovascular and atherosclerosis-related disease [12,17].

Patients with lower limb OA are also more likely to suffer from chronic pain, causing a cycle in which pain limits physical activity and physical inactivity contributes to greater knee pain and weight gain, potentially resulting in depressive symptoms as a consequence of OA [15,16]. Therapeutic measures used in treating OA symptoms include both pharmacological and non-pharmacological methods, the choice of which is dependent on disease stage, patient characteristics and comorbidities. Pharmacological treatment includes oral, topical and intraarticularly used analgesics, anti-inflammatory drugs or other substances. Lately, biological therapies such as platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) became widely used in treating patients with OA, even though the leading guidelines advise against their use due to lack of high-quality studies or a straight clinical protocol [18,19] These new agents may slow down the existing condition, alleviate OA symptoms and postpone the need of surgery.

Mesenchymal stem cells

In recent years, due to the increasing use of Mesenchymal stem cells (MSCs) in clinical practice around the world, research on MSCs has become increasingly extensive and relevant. MSCs are adult stem cells present in various tissues throughout the body. They have the potential to differentiate into various cell types and secrete immunomodulatory and trophic signaling molecules that promote local regeneration by secretion of anti-apoptotic, anti-scarring, angiogenic and mitotic signaling molecules, and inhibit bacterial growth by secreting LL-37 [20] (Figure 2). These immunomodulatory and paracrine mechanisms are responsible for their clinical effect, putting them in the focus of regenerative medicine for OA and other medical conditions (Figure 1). The main effects of MSC therapy on knee OA is pain reduction and mobility improvement measured by visual analog scale (VAS), Western Ontario and McMaster Universities Arthritis Index (WOMAC) and Knee Injury and Osteoarthritis Outcome Score (KOOS), while the reported effect on the articular cartilage has not been constant, with studies reporting various end effects regarding both volume and structure, measured by MRI or second-look arthroscopies [21-26]. When considering MSC therapy, factors such as the amount of harvest volume, cell isolation procedure, isolated cell number, regenerative capacity of certain cells and the side effects of therapy have to be assessed, in order to determine the best harvest site for MSCs [27]. Still, key problems associated with MSC therapy include dosing, harvest site, and the number of delivered MSCs, as there is no standard procedure that can answer these questions. Typical harvest sites for MSCs are the bone marrow and adipose tissue. Other sites include the umbilical cord and the placenta [28,29]. The safety of MSCs in the treatment of various musculoskeletal pathologies has been thoroughly studied and confirmed [28,30].

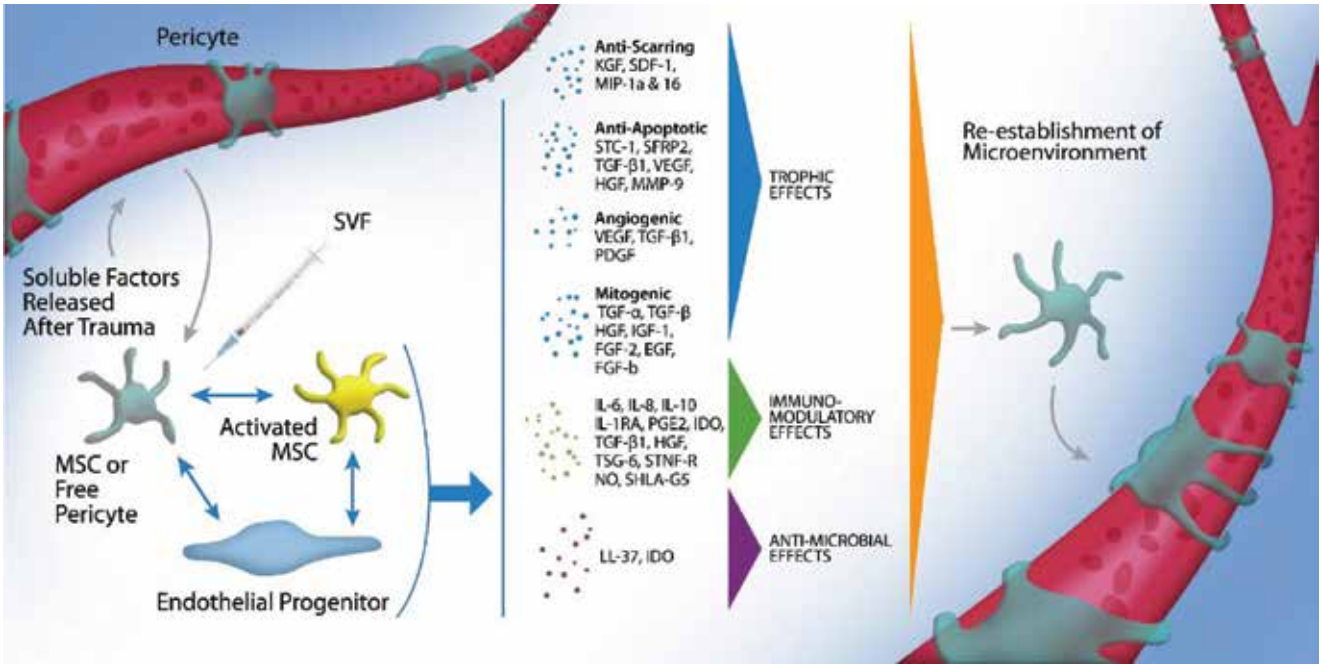

Figure 2. The trophic, immunomodulatory and antimicrobial effects of mesenchymal stem cells or medicinal signaling cells (MSCs). Pericytes are stimulated by soluble growth factors and chemokines to become activated MSCs, and probably in interaction with endothelial progenitors both cell types respond to the microenvironment by secreting trophic (mitogenic, angiogenic, anti-apoptotic, or scar reduction), immunomodulatory or antimicrobial factors. After the microenvironment is re-established, MSCs return to their native pericyte state attached to blood vessels. SVF – stromal vascular fraction. Murphy BM, Moncivais K and Caplan IA. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Experimental and Molecular Medicine (2013) 45, e54; doi:10.1038/emm.2013.94 (Used and adapted with permission of Prof. Arnold I Caplan and publisher's permission).

Bone-marrow mesenchymal stem cells (BM-MSCs)s

BM-MSCs are usually obtained by aspiration from the posterior or anterior iliac crest. Density-gradient centrifugation of the aspirate is needed to produce a bone marrow aspirate concentrate (BMAC), during which the number of MSCs and growth factor containing platelets is increased [31,32]. The application is performed by an intra-articular injection into the target joint. Clinical results of BM-MSCs therapy are generally positive. As previously mentioned, significant improvements in pain levels and function levels were observed in a meta-analysis when compared to VAS, KOOS, WOMAC and Lysholm scores prior to the procedure [33]. In a literature review of research published between 2014 and 2019 an association of cell count and treatment outcomes was observed. In individuals with grade ≥ 2 knee OA on the Kellgren-Lawrence scale, a moderate-high number of cells (40 × 106) was found to achieve an optimal effect, while lower (24 × 106) and higher (100 × 106) cell numbers, were associated with an increase in observed adverse effects, such as persistent knee pain and swelling [34].

Adipose-derived mesenchymal stem cells (AD-MSCs)

Usually obtained from subcutaneous adipose tissue by lipoaspiration, these procedures are less invasive compared to BM-MSCs extraction [35]. Adipose tissue provides a significant, easily accessible source of cells contained in stromal vascular fraction (SVF) and provides a large number of cells from which multipotent AD-MSCs can be isolated, containing 500 times more MSCs compared to the same volume of bone marrow [27,36,37]. As stated previously, the various procedures of MSC harvesting, processing and application are the key limitations of their introduction to standard daily clinical practice and guidelines of professional societies. Therefore, we analyzed the cell populations in the stromal vascular fraction from lipoaspirate (SVF-LA) and stromal vascular fraction from microfragmented lipoaspirate (SVF-MLA). We identified the following cell phenotypes: endothelial progenitor cells (EPC), endothelial mature cells, pericytes, transitional pericytes, and supra adventitial-adipose stromal cells (SA-ASC) (Figure 3). Compared to SVF-LA, SVF-MLA was dominated by a reduction of leukocytes and SA-ASC, and an increase of EPC, suggesting their enrichment by the process of micro-fragmentation, thus indicating their role in the observed effect of MSC therapy on knee OA [38].

Our results

In our institution, we investigated the use of autologous microfragmented adipose tissue (AMFAT) in the treatment of knee osteoarthritis. The effect of intraarticular

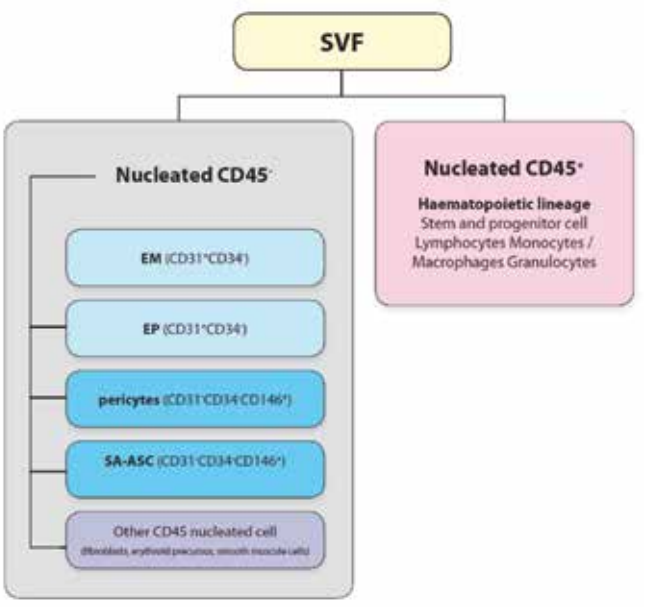

Figure 3. Summarized results of main immunophenotypes in the stromal vascular fraction (SVF) nucleated cell populations from microfragmented adipose tissue isolated from 20 osteoarthritic patients. EMC – endothelial mature cells, EPC – endothelial progenitor cells, SA-ASC – supra-adventitial adipose stromal cells.

injection of AMFAT on a series of 17 patients with late-stage knee OA (Kellgren-Lawrence grades III and IV) was studied, using quantitative MRI assessment of glycosaminoglycan (GAG) content in cartilage by using delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) index, as well as clinically by a standard orthopedic physical examination and VAS assessment. A standard lipoaspiration technique was performed, and the harvested fat was micro-fragmented and applied intraarticularly into the patients' affected knee joints. Pain estimates measured by VAS decreased significantly, both for resting and movement estimates at 3, 6 and 12-month follow-ups. Cartilage GAG content, measured by delayed gadolinium-enhanced magnetic resonance imaging of cartilage dGEMRIC index, significantly improved in 52.9% of measurements and deteriorated in only 11.2% of measurements, which would be a normal disease course for the late-stage OA [39]. In our second study, functional scores were assessed at 12 months follow-up for 20 patients. Seventeen patients (85%) showed a significant improvement in KOOS and WOMAC scores. KOOS improved from 46 to 176% when compared with baseline, WOMAC increased from 40 to 45%, while VAS rating increased from 54% to 82%. Three patients (15%) were subjected to total knee replacement surgery and were excluded from the study [40]. The last trial included ten patients (18 knees) suffering from knee OA grades III and IV who were assessed for GAG content and clinical outcome after a single intraarticular injection of AMFAT in a 2-year period. Study results indicated an improvement of GAG content, measured by the dGEMRIC index, with more than half of the measurements signifying a relevant

improvement in a 2-year follow-up, which is in contrast to GAG content reduction over the natural course of the disease. VAS pain score also significantly decreased over the 24-month period, both in resting and movement [41]. Taken together, these studies suggest that the application of autologous microfragmented adipose tissue with SVF in patients with knee OA increases GAG levels in hyaline cartilage, consequently reducing pain and improving movement abilities, while also postponing the need for total joint replacement surgery in patients with more advanced OA stage.

Conclusions

Despite the current negative stance of the official guidelines on MSC treatment, the minimally invasive, one-step, economic procedure of their application and positive patient outcomes indicated both by pain reduction and increased GAG content cannot be neglected. Promising results and rare adverse events encourage future studies that would determine exact dosing, harvest site, and the number of delivered MSCs in a standard procedure. While joint replacement surgery still represents the gold standard in the treatment of OA, MSCs therapy provides a possibly great alternative and it is assumed that it will take a major role in future OA treatment, especially in patients that are not yet candidates for joint replacement surgery.

Note: Mesenchymal stem cells in orthopedics - current perspective is an excerpt from the texts prepared for: Genes, scientific journal (Primorac D, Molnar V, Rod E, Jeleč Ž, Čukelj F, Matišić V, Vrdoljak T, Hudetz D, Hajsok H, Borić I. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. Genes (Basel). 2020 Jul 26; 11(8):854. doi: 10.3390/genes11080854.), Journal of Stem Cells Research, Development & Therapy (Perić V, Kottek T, Molnar V, Matišić V, Čukelj F, Primorac D. Mesenchymal Stem Cells in the Treatment of Knee Osteoarthritis. J Stem Cell Res Dev Ther 6: 050.) and Croatian Medical Journal (Hudetz D, Borić I, Rod E, Jeleč Ž, Kunovac B, Polašek O, Vrdoljak T, Plečko M, Skelin A, Polančec D, Zenić L, Primorac D., Early results of intra-articular microfragmented lipoaspirate treatment in patients with late stages knee osteoarthritis: a prospective study. Croat Med J. 2019 Jun 13;60(3):227-236. doi: 10.3325/cmj.2019.60.227.) in which the comprehensive review of osteoarthritis pathophysiology and MSC treatment were prepared and presented in full.

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