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# Stem cells and tissue engineering: an alternative treatment for craniofacial congenital malformations and articular degenerative diseases

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**How to cite this article:** Velasquillo C, Madrazo-Ibarra A, Gutiérrez-Gómez C, Pérez-Dosal MR, Melgarejo-Ramírez Y, Ibarra C. Stem cells and tissue engineering: an alternative treatment for craniofacial congenital malformations and articular degenerative diseases. *Plast Aesthet Res* 2020;7:31. <http://dx.doi.org/10.20517/2347-9264.2020.30>

**Received:** 4 Mar 2020 **First Decision:** 15 May 2020 **Revised:** 2 Jun 2020 **Accepted:** 9 Jun 2020 **Published:** 24 Jun 2020

**Science Editor:** Yi-Lin Cao **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

## Abstract

The life quality of patients with craniofacial malformations is severely affected by the physical disabilities caused by the malformation itself, but also by being subjected to bullying, which leads to a series of relevant psychological and societal effects that have an economic impact on the health sector. Orofacial clefts, notably cleft lip (CL), cleft palate, and microtia, are the most common craniofacial birth defects in humans and represent a substantial burden, both personal and societal. On the other hand, osteoarthritis is a widespread degenerative disease that is becoming more common due to the extension of the human lifespan and to an increase in injuries in young people as a result of their lifestyle. Advances in tissue engineering as a part of regenerative medicine offer new hope to patients that can benefit from new tissue engineering therapies based on the supportive action of tailored 3D biomaterials and the synergic action of stem cells that can be driven to the process of bone and cartilage regeneration. This review provides an update on recent considerations for stem cells and studies on the use of advanced biomaterials and cell therapies for the regeneration of craniofacial congenital malformations and articular degenerative diseases.

**Keywords:** Mesenchymal stromal cells, microtia, cartilage, cleft lip, cleft palate



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## INTRODUCTION

According to the National Cancer Institute, degenerative disease is a pathology in which the function or structure of the affected tissues or organs worsens over time<sup>[1]</sup>. Unfortunately, neither most degenerative diseases nor craniofacial congenital malformation diseases have a cure, so they evolve until patients become severely disabled. Since stem cells became an alternative treatment, they have changed the course of these diseases. Their applications are currently being tested and have shown positive results in several of these diseases.

Stem cells are cells with self-renewal and differentiation abilities. Mesenchymal stem cells (MSC) are adult stem cells that are not hematopoietic and can be found in several tissues, such as adipose tissue, bone marrow, and umbilical cord, to mention some examples. According to the International Society for Cell Therapy (ISCT), MSC must (1) be plastic-adherent; (2) express CD105, CD73, and CD90; (3) lack CD45, CD34; and (4) differentiate into osteoblasts, adipocytes, and chondroblasts<sup>[2]</sup>; however, these criteria do not suffice to justify their therapeutic potential<sup>[3]</sup>. Besides their differentiation ability, MSC have paracrine activity in angiogenesis, cellular activation/proliferation, and immunomodulation<sup>[4,5]</sup>. Since they were first introduced in 1970 by Friedenstein, MSC have changed the treatment of individuals with orthopedic, hematologic, oncologic, ophthalmologic and dermatologic conditions. They have been used mainly to replace cell lines that have been lost or destroyed or to modify the behavior of other cells.

In this paper, we will briefly describe the applications of MSC in common degenerative and congenital diseases in Mexico.

## DEFINING THE REGENERATIVE POTENTIAL OF MSC BEYOND BIOLOGY

For many years, the use of autologous cells isolated directly from biopsies was the only alternative for tissue engineering applications. Fully differentiated cells tend to lose cellular features if they are exposed to a constant cellular division. These cellular features include changes in the extracellular matrix (ECM), protein synthesis, altered metabolism, and dedifferentiation. Regenerative therapies commonly need a high number of cells, leading to the search of cells with high regenerative potential and no risk of morphological features loss. Mesenchymal stromal cells have become a promising alternative since they are one of the first cells in cellular lineage with unlimited fashion propagation and an extensive differentiation ability<sup>[3]</sup>.

The analysis of the potential of MSC for therapeutic purposes can be conducted at different stages. Typically, the mesenchymal phenotype according to the ISCT criteria should be verified; however, additional surface markers have been described, which include being positive for CD29, and negative for CD14, CD11b CD19, CD79 alpha, and HLA-DR surface markers. Differentiation protocols can also be analyzed based on the expression of these markers in chondrogenic, adipogenic, or osteogenic lineages. For example, osteogenic differentiation can be confirmed with alkaline phosphatase activity, calcium release after osteogenic stimulation, catalase (osteoclast inhibitor), and glutathione peroxidase 3 (osteogenic biomarker) expression<sup>[6]</sup>. Transcriptional analysis at mRNA levels is another alternative to track the therapeutic potential of MSC. It is possible to estimate cellular growth and colony-forming potential quantifying the MSC marker STRO-1 and the platelet-derived growth factor receptor A (PDGFR-alpha). A transcriptional increase of Twist-related protein-1 (TWIST-1) and Twist-related protein-2 (DERMO-1) has also been described as crucial for MSC growth and development<sup>[7]</sup>.

There has been a continuous debate about whether autologous or heterologous cells are the most adequate source of MSC in regenerative therapies for congenital and craniofacial diseases. Their immunomodulatory ability is a relevant aspect exerted through the inhibition of T-cell proliferation, which regulates the immune response, and is also involved in the alloimmune response. Autologous MSC have been shown to decrease *in vitro* alloimmune response in host autologous cells in transplanted murine models. It has

been proposed that the homing activity of MSC creates immune-privileged sites that limit the infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in tissues, thus limiting damage and promoting regeneration<sup>[8]</sup>. Meanwhile, heterologous MSC from bone marrow (BM-MSC) have been used for the treatment of pseudoarthrosis and have been proved to promote healing of femoral fractures in a claudication animal model. Heterologous BM-MSC reached the lesion 24 h after being infused, and later promoted a periosteal reaction that lead to fracture consolidation and cartilage formation 120 days after the infusion. In comparison, BM-MSC alone formed a fibro-osteoid tissue<sup>[9]</sup>. These effects lead us to elucidate that the use of autologous versus allogeneic MSC will depend on the required clinical outcome.

In recent years, clinical implications and advantages in the use of stromal vascular fraction (SVF) have opened new alternatives for tissue engineering in craniofacial or degenerative diseases. The differences between SVF and adipose-derived MSC (AD-MSC) are that an SVF is a freshly harvested, heterogeneous population of cells directly isolated from lipoaspirates by mechanical or enzymatic disaggregation that contains stromal cells (15%-30%), erythrocytes, granulocytes, monocytes, pericytes, and endothelial cells<sup>[10]</sup>. AD-MSC are a cultured, more homogeneous subpopulation of cells resulting from a culture selection and *in vitro* expansion. On the other hand, compared to BM-MSC, adipose tissue contains 100-500 fold more MSC, and SVF contains 4-6 fold more MSC, whose therapeutic impact, angiogenic stimulation, T-cell regulation and reduction of IL-10 production represent a feasible source for tissue engineering<sup>[11]</sup>.

Although there are still difficulties to establish the proper dose and clinical safety protocols, there is no doubt of the potential of AD-MSC to accelerate healing processes. Therapeutic efforts for the treatment of degenerative diseases have moved research groups to develop semi-automated, surgically-closed systems to obtain SVF during surgeries with minimal laboratory equipment requirements that will enable the application and implantation of autologous or heterologous MSC for tissue engineering<sup>[12]</sup>.

### Degenerative diseases

A degenerative disease is a pathology in which the function or structure of the affected tissues or organs worsens over time<sup>[1]</sup>. As mentioned earlier, stem cells have changed the course of these diseases and have become an alternative treatment for degenerative disorders.

### Osteoarthritis

Osteoarthritis (OA) is a condition that causes joints to hurt and become stiff. It is the most common cause of arthritis worldwide, and it mainly affects knees (85%), hips, hands, and feet. Approximately 240 million people in the world have OA<sup>[13]</sup>. 5% of adults worldwide have either hip or knee OA. These numbers will increase as the population ages and the obesity rates increases<sup>[14]</sup>. Pain is the main symptom that typically leads patients to seek medical care and guides clinicians into treatment decision-making as well. Pain can be so intense, patients become unable to work, making OA the fourth leading cause of years lived with disability worldwide<sup>[15]</sup>.

OA has been part of the changes of articular cartilage, but that concept has evolved, now considering the whole joint<sup>[16,17]</sup>. Some of the structural damages of joints are (1) loss of cartilage; (2) osteophyte formation; (3) subchondral bone changes; and (4) meniscal alterations<sup>[17]</sup>. Chondral erosions caused by overload or abnormal joint kinematics turn into fissures. In an attempt to repair these lesions, hypertrophic chondrocytes increase their synthetic activity, but, by doing that, they increase the production of proinflammatory mediators and degradation products. These molecules stimulate surrounding synovium, increasing its proliferation, and proinflammatory response as well. All inflammation mediators favor endochondral ossification, causing bone overgrowth and osteophyte formation. Pain comes from the peripheral nociceptors sensing ongoing tissue injury, as well as inflammation in the joint<sup>[16]</sup>.

Nowadays, treatment towards OA is oriented towards minimizing pain, optimizing function, and modifying the process of joint damage. Pain control, as mentioned earlier, is what guides the physician's decision into which treatment to use. Analgesics and anti-inflammatory medications are the mainstay treatment, accompanied by lifestyle modifications such as weight loss and physical therapy/activity<sup>[18]</sup>. Since no medication has been shown to stop the process of OA, measures have been taken to prevent it. Focal cartilage lesions, if left untreated, tend to quickly progress into osteoarthritis.

A retrospective study performed in the National Institute of Rehabilitation in Mexico reported that 61% of the patients undergoing arthroscopic surgery had focal chondral lesions in the knee, with 74% of these being grade III-IV ICRS/Outerbridge<sup>[19]</sup>. Cartilage repair techniques, such as microfractures, autologous chondrocyte implantation, and mosaicplasty have shown to delay the appearance of OA, as well as the need for total joint replacement after chondral injuries in young adults<sup>[20-23]</sup>. Some biological therapies have been researched, including drugs that promote chondrogenesis and osteogenesis<sup>[24]</sup>, matrix degradation inhibitors, apoptosis inhibitors, and anti-inflammatory cytokines<sup>[25]</sup>; however, none of them have demonstrated sufficient symptom improvement to be included in the standard of care<sup>[26]</sup>.

Mesenchymal stem cells have turned into the most explored therapeutic drug in cell-based OA treatment due to their ability to differentiate to chondrocytes and their immunomodulatory properties<sup>[27]</sup>. Furthermore, they have been used in different ways to try and modify the course of the disease.

#### *MSC seeded on scaffolds*

Cartilage implants: by taking advantage of the differentiation capacity of MSC to chondrocytes, MSC have been similarly used for cartilage lesion repair as matrix-assisted autologous chondrocyte implants. Previous studies using chondrocytes seeded on collagen or polyglycolic-acid matrixes have shown good mid- to long-term clinical and magnetic resonance imaging (MRI) outcomes, as well as the ability to delay degenerative changes in the knee<sup>[28-31]</sup>.

A few years ago, the United States Food and Drug Administration approved MACI, a porcine collagen membrane seeded with autologous chondrocytes, for the treatment of focal chondral lesions in the knee<sup>[32]</sup>. Okano *et al.*<sup>[33]</sup> came up with the "cell sheet technology" consisting of multiple cell layers placed on top of another (instead of using a matrix), taking advantage of the intact ECM produced by the cultured chondrocytes and their adhesion factors. This innovative technique has been shown to form hyaline cartilage in preclinical studies and is currently undergoing clinical studies in Japan<sup>[34-36]</sup>. Even though these techniques have had great outcomes, they involve two surgical procedures: one to obtain the cartilage biopsy and the second one for the implantation. This makes the intervention expensive and may increase the risk of surgical complications. MSC seeded on a 3-dimensional scaffold or using the cell sheet technology can help solve this problem. Due to endogenous cell stimulation, MSC differentiate into cartilage, forming a cartilage-like tissue repair<sup>[37]</sup>. Several clinical and preclinical studies using MSC seeded on matrixes have shown positive results in forming cartilage-like tissue and alleviating symptoms<sup>[38,39]</sup>.

In 2015, Kim *et al.*<sup>[40]</sup> conducted a comparative matched paired analysis comparing injected vs surgically implanted MSC in patients with knee osteoarthritis. Patients were evaluated with Patient-Reported Outcome Measures (PROMs), as well as a second-look arthroscopy. After a minimum follow-up of 24 months, patients who underwent MSC implantation showed better clinical and second-look arthroscopic outcomes. Despite the positive findings with this technique, it is usually employed to repair small defects and does not address larger areas related to OA. Problems related to the acquisition of autologous MSC and the risk of graft-versus-host reactions with allogeneic MSC have limited their use in clinical studies.

Meniscus repair: menisci play an important role in load-bearing and load transmission to the cartilage and subchondral bone. Approximately 15% of knee lesions are associated with damage to the meniscus<sup>[41]</sup>.

Meniscal lesions generate knee instability and further cartilage damage favoring the development of OA. Treatment for meniscal lesions is decided depending on the complexity and the location of the damage. Repair strategies are used when the rupture is small, located in the vascular areas, and the meniscus can be stabilized intra-articularly. However, partial meniscectomy or complete meniscectomy is required in complex lesions. Meniscectomies cause an increase of 235% contact pressure<sup>[42]</sup>, as well as an increase in OA incidence<sup>[43-45]</sup>. The use of meniscal substitutes after partial meniscectomy has shown symptom relief, as well as a slow decrease of articular degeneration; however, they do not prevent it<sup>[46,47]</sup>.

Leroy *et al.*<sup>[46]</sup> reported a decrease in scaffold dimensions leading to a concern about the scaffold's capacity in the long term. The use of MSC in combination with meniscal substitutes have become of great interest due to the evidence of meniscal-like tissue formation after implantation in rats, pigs, and rabbits<sup>[48,49]</sup>. Olivos-Meza *et al.*<sup>[50]</sup> conducted a comparative study between patients who received meniscal substitution with acellular polyurethane meniscal scaffolds (APS) vs. polyurethane scaffold enriched with peripheral blood MSC (MPS). They evaluated femoral and tibial articular cartilage status using MRI T2-mapping 3, 6, 9, and 12 months after surgery, as well as clinical evaluation using PROMs. No differences were observed between APS and MPS during the 12-month follow-up; however, a longer follow-up is needed to see the scaffold degeneration and tissue formation.

MSC exosomes: exosomes are extracellular vesicles that function as intercellular communication vehicles transferring lipids, nucleic acids (mRNA and microRNAs) and proteins to generate a response in recipient cells<sup>[51]</sup>. Exosomes are rich in microRNA, which can bind specific sites in transcribed mRNA, modifying their expression and transduction<sup>[51,52]</sup>. These properties have been studied to promote cartilage regeneration and decrease pro-inflammatory molecules in OA<sup>[53-57]</sup>. Tao *et al.*<sup>[56]</sup> and Toh *et al.*<sup>[58]</sup> reported several microRNAs (140-5p, 23b, 92a, 125b, 320, 145, 22 and 221) derived from human synovial MSC, which promote cartilage regeneration, OA suppression, and cartilage/extracellular matrix homeostasis in preclinical studies. The exosomes' potential for OA treatment, good tolerance, and minimal risk of immunogenicity and toxicity has made them one of the most important hotspots for future research. However, further studies describing how to obtain large-scale purified exosomes as well as their clinical efficacy and biosecurity are still needed.

Intra-articular injections: intra-articular injections of MSC have become the main modality of cell therapy research for OA treatment due to their simple application thanks to their anti-inflammatory, immune-regulatory, and regenerative abilities. MSC can be either injected with no other components or mixed with hyaluronic acid (HA), platelet-rich plasma (PRP), or saline solution, to mention some examples. Preclinical studies have shown cartilage repair, reduction in proinflammatory cytokines, and improved imaging, morphology, and histology<sup>[59,60]</sup>. Mixed injections with PRP/MSC or HA/MSC have shown significantly better results on the repaired cartilage than individual uses of any of them.

Several clinical trials have been developed worldwide using MSC derived from the stromal vascular fraction (SVF), umbilical cord (UC-MSC), adipose tissue (AD-MSC) or bone-marrow (BM-MSC), the latter being the most common site. BM-MSC have shown a better chondrogenic ability compared to AD-MSC<sup>[61]</sup> and have shown an improvement in cartilage quality and knee function, as well as a decrease in pain and other symptomatology<sup>[27]</sup>. Most clinical trials that use AD-MSC and SVF have been conducted using mixed injections combined with PRP. Results have been positive, showing an increase in cartilage thickness, significant positive changes in MRI, and symptomatology improvement<sup>[62]</sup>. Few trials have been done using UC-MSC. Cartistem<sup>®</sup> is the first approved allogeneic cell treatment for OA in the world. It was approved by the Ministry of Food and Drug Safety in Korea and is now commercially available<sup>[63]</sup>. It uses UC-MSC combined with sodium hyaluronate. Up to 5000 patients have been treated with Cartistem<sup>®</sup> and around 97.67% of them have shown improved quality of life<sup>[63,64]</sup>.



### **Congenital anomalies**

Congenital anomalies, also known as birth defects, are structural or functional anomalies that occur during intrauterine life<sup>[65]</sup>. These defects can be identified prenatally, at birth, or even during later infancy. They occur in 2%-4% of live births<sup>[66]</sup> and are more common in stillborn spontaneous miscarriages. Approximately 50% of all congenital anomalies are not linked to a specific cause<sup>[65]</sup>; however, they are commonly caused by genetic abnormalities and/or environmental exposures. Genetic abnormalities include chromosomal alterations (e.g., Down syndrome) or single-gene/monogenic disorders. The latter have different modes of inheritance such as autosomal dominant, autosomal recessive, or X-linked<sup>[67]</sup>. On the other hand, environmental exposure to a teratogen, any agent that causes abnormalities in the form or function of the fetus, can produce cell death, alter normal growth of tissues, or interfere with normal cellular differentiation, resulting in a congenital anomaly<sup>[68]</sup>.

Birth defects are divided depending on the pathophysiology of the defect: (1) malformation when the intrinsic development is abnormal; (2) deformation when extrinsic mechanical forces modify a normally formed structure; (3) disruption when a vascular defect causes a malformation; or (4) dysplasia when there is an abnormal organization of cells into tissues<sup>[68]</sup>. These defects can be isolated or present in syndromes or associated patterns that may affect one or more organ systems. A lot of preventive measures, as well as treatment measures, have been focused on these anomalies due to their medical, surgical, psychological, and cosmetic significance.

### **Congenital microtia**

Congenital microtia is the incomplete formation or growth of the auricle, leading to the small or deformed auricle. It may occur as an isolated condition or as part of a syndrome or spectrum of anomalies. Microtia severity ranges from a complete absence of the auricle (anotia) to a mild size discrepancy. Most of the time, microtia occurs unilaterally (79%-93%), the right side being the most affected side<sup>[69]</sup>. It is associated with hearing loss of the ipsilateral ear, but normal hearing in the unaffected ear. Speech and language development are usually normal. Individuals with microtia, however, are at a higher risk of communication delay and attention deficit disorders<sup>[70,71]</sup>.

The etiology of microtia is poorly understood, though there is strong evidence supporting the importance of environmental causes such as altitude, and gestational exposure to certain drugs<sup>[72-75]</sup>. Ethnicity has been reported to be an important consideration due to the high incidence and prevalence of microtia among Asians, Hispanics, and Native Americans. In Mexico, the World Health Organization and the Mexican Registry and Epidemiological Surveillance of External Congenital Malformations (RYVEMCE) reported a prevalence of 6.15-7.37 cases per 10,000 childbirths, being one of the countries with the highest prevalence of microtia worldwide<sup>[72,75]</sup>. Due to the psychological and functional implications related to microtia, there have been several studies focusing on the surgical treatment and biotechnology measures needed to recreate an auricle as similar as possible to the native one.

Auricle reconstruction with autologous rib cartilage remains the gold standard for patients with microtia/anotia. Tanzer *et al.*<sup>[76]</sup> and Brent *et al.*<sup>[77]</sup> described this technique as an alternative to allogeneic implants in the late 1950s, overcoming several problems associated with these implants. Sculpted autologous costal cartilage graft is one of the most challenging procedures in plastic and reconstructive surgery since the surgeon has to handcraft the cartilage trying to create an ear similar in appearance to the contralateral one. Grafts have good long-term durability and grow concomitantly as the patient ages<sup>[77]</sup>. However, costal cartilage grafts are not as consistent as synthetic implants: they require long operative time, harvesting results in donor-site morbidity, and, occasionally, there is an insufficient source of cartilage.

Tissue engineering techniques emerged as an alternative treatment. The idea of preformed ear structures seeded with cells goes back to the 1940s when Peer *et al.*<sup>[78]</sup> started using diced cartilage placed inside an

auricle shaped mold. Research started focusing on scaffolds that could promote cell proliferation, as well as matrix production. Decades later, research focused on finding the ideal scaffold that would induce cellular proliferation and cartilage tissue formation. This was proved by Vacanti *et al.*<sup>[79]</sup> and Rodriguez *et al.*<sup>[80]</sup>, who conducted several preclinical studies showing that polyglycolic acid (PGA) + polylactic acid (PLA) would promote *in vitro* cell proliferation and matrix production, and *in vivo* cartilage formation after implantation. Mice were implanted with 3D ear-shaped scaffolds seeded with chondrocytes. After 12 weeks, scaffolds were almost entirely degraded; however, the neo-tissue maintained the original 3D structure and demonstrated histological cartilage appearance. These studies were the introduction of biotechnology to regenerative medicine<sup>[81]</sup>.

The combination of seeded auricular chondrocytes (AuCs) to scaffolds and the computer-assisted design/computer-aided manufacturing (CAD/CAM) technology<sup>[82-84]</sup> led to the start of clinical studies. The first clinical application was done in Shanghai in 2018 by Guangdong Zhou *et al.*<sup>[84]</sup>, where 5 patients with unilateral microtia were implanted with 3D printed PCL + PGA scaffolds seeded with autologous chondrocytes from the cartilage remnants of the microtia. 2.5 years later, they reported the follow-up of one patient showing the formation of cartilaginous tissue after histologic evaluation, the transition from a stiff graft to a more flexible one over the time, and the degradation of the scaffold without losing the original ear shape.

Currently, autologous chondrocytes from the microtia auricle are being isolated, expanded, and seeded onto the constructs, showing normal elastic cartilage on histology<sup>[85]</sup>. However, monolayer expansion of chondrocytes results in dedifferentiation<sup>[80,86]</sup>, limiting the capacity to generate robust cartilage, and needs extensive 3D construct culture before implantation<sup>[84,87]</sup>. Mesenchymal stem cells have the potential of massive expansion and the ability to differentiate into chondrocytes through co-culture or co-implantation<sup>[88]</sup>.

Studies have been done using articular cartilage co-cultures with MSC, though little is known about AuCs and MSC. Pre-clinical *in vivo* studies have shown the formation of cartilage, but the impact of these studies is limited due to the use of non-human cells, the lack of specific markers for elastic cartilage, and the absence of mechanical evaluation<sup>[89-94]</sup>. Cohen *et al.*<sup>[95]</sup> conducted a comparative preclinical study evaluating cartilage formation in constructs using human AuCs vs human AuCs and MSC in a 1:1 ratio. The study showed that the auricular cartilage generated in the 1:1 constructs was similar in structure, histology, biochemical development, and mechanical properties to discs containing only AuCs and native human auricular cartilage after 3 months *in vivo*. To date, no clinical study using AuCs in combination with MSC has been conducted. However, these findings suggest MSCs could solve several problems related to cartilage culture and could bring other benefits related to their immunomodulatory/anti-inflammatory potential.

### **Cleft lip and palate**

Cleft lip (CL) and palate (CLP) are common congenital malformations in Mexico, with an incidence of 1 in 800 births<sup>[96]</sup>. Up to 2003, CLP had a prevalence of 139,000 affected children throughout the country, with approximately 10 new cases identified daily<sup>[97]</sup>.

Patients with CLP undergo (on average) 4 surgical procedures during their lifetime: (1) lip closure and primary nasal repair; (2) palate closure; (3) alveolar bone graft; and (4) rhinoseptoplasty<sup>[98]</sup>. The alveolar bone graft is the placing of bone in the primary palate to restore the continuity of the maxillary arch and separate the oral and nasal cavity<sup>[99,100]</sup>. This allows adequate dental hygiene, promotes harmonic facial growth, and provides the necessary bone matrix for the eruption of the lateral and canine incisors<sup>[101,102]</sup>.

The best donor area for the bone graft is the iliac crest, where approximately 3-8 cm<sup>3</sup> of bone are obtained. Several problems are associated with this procedure, such as recipient area alterations (lack of integration,

bone sequestration, infection or bone resorption), donor area complications (hematoma, infection, abnormal scar, pain and temporary inability to walk)<sup>[103]</sup>, and insufficient bone graft<sup>[104,105]</sup>. In these cases, the possibility of synthetic bone substitutes (silicone, polytetrafluoroethylene, polyethylene, polyester, polyamides, acrylic, metals, cyanoacrylate, resins)<sup>[106-111]</sup>, or natural bone substitutes (calcium phosphate, granules) has been suggested<sup>[112-119]</sup>.

The use of cell-based therapy represents one of the most advanced methods to approach craniofacial abnormalities. Several animal models have been used to test alveolar cleft-grafting materials including mice, rabbits, cats, dogs, goats, sheep, and monkeys. Studies have shown heterogeneous results in terms of biocompatibility, bone regeneration capacity, integration, resorption, and mechanical resistance due to the physicochemical characteristics of each material<sup>[120,121]</sup>. Existing systematic reviews support the ability of bone regeneration on these materials for the treatment of small periodontal bone defects, but recommend further studies on major bone defects such as palatal fissures<sup>[122-124]</sup>.

Scaffolds, as in all biotechnology-related applications, have been a major research topic regarding CLP. The ideal scaffold should have macro-geometry, micro-architecture, bioactivity, and appropriate mechanical properties<sup>[125]</sup>. The first two characteristics have been addressed with the introduction of 3D printed scaffolds. A head CT scan is performed in patients with CLP, and a scaffold with the patient's exact macroscopic geometry is created. Bioactivity and mechanical properties are determined by the scaffold material. Several different materials like polycaprolactone (PCL) with hydroxyapatite and platelet-derived growth factor-BB<sup>[125]</sup>, cryogels<sup>[126]</sup>, demineralized bone matrices, PLA, among others, have been tested to evaluate bone regeneration and cellular migration<sup>[127-131]</sup>. Today, the use of bioceramics, such as calcium phosphate, in combination with biomimetic polymer scaffolds, folic acid derivatives, morphogens, and stem cells are currently considered the most promising alternatives for CLP regeneration<sup>[127]</sup>.

The use of mesenchymal stem cells is emerging as an alternative treatment or in combination with previously-described therapies for patients with CLP. As mentioned earlier, MSC can be obtained from different parts of the body such as adipose tissue, bone marrow, and umbilical cord. The generation of an artificial alveolar cleft and the implantation of teeth in the regenerated bone region have been accomplished in dog models using BM-MSC<sup>[132-134]</sup>. Ahn *et al.*<sup>[135]</sup> reported the first case of regeneration of an alveolar cleft defect. Patient-specific 3D-printed bioresorbable polycaprolactone (PCL) scaffolds were seeded with iliac BM-MSC and showed 45% defect regeneration 6 months after transplantation, with a 75% bone mineral density compared to the surrounding bone. AD-MSC, due to their availability and easy handling, are excellent candidates for tissue engineering in CLP patients. Preclinical studies comparing bone regeneration between AD-MSC and autogenous bone graft in canine maxillary alveolar cleft models showed no significant differences, meaning AD-MSC can be an acceptable alternative<sup>[136]</sup>. However, clinical studies are needed to confirm their efficacy and reproducibility in humans.

Unlike other alternatives, MSC derived from dental tissues have been studied for CLP patients due to their higher accessibility and less invasive retrieval. Lee *et al.*<sup>[137]</sup> reported that stem cells from human exfoliated deciduous teeth (SHEDs) have mineralization potential after expressing bone-specific osteogenic markers following insertion into *ex vivo*-cultured embryonic palatal shelves and *in novo* culture. Furthermore, Nakajima *et al.*<sup>[138]</sup> compared the bone regeneration ability of SHEDs, BM-MSC, and dental pulp stem cells in mice. They concluded that after 12 weeks of transplantation, the ratio of new bone formation was not significantly different among these groups. However, SHED produced the largest osteoid and widely distributed collagen fibers. Up until now, no clinical studies have been conducted using SHEDs. Although a huge effort has been devoted to the use of tissue engineering as a solution for treating bone defects, more evidence is still needed.



## CONCLUSION

Mesenchymal stem cells are an emerging alternative for tissue engineering therapies. Besides their differentiation ability, they also express paracrine functions, which have shown to be immunomodulatory and anti-inflammatory. Taking advantage of these functions, MSC have been studied in different fields for the medical treatment of degenerative and congenital diseases. Despite favorable findings in preclinical studies, more clinical studies following all the steps described in translational medicine are needed to address their efficacy, safety, and clinical application. The complexity of these technologies must be considered carefully, and every country must follow a single regulatory pathway.

## DECLARATIONS

### Acknowledgements

The authors would like to thank Mariana Rodríguez for copy-editing this paper.

### Authors' contributions

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### Availability of data and materials

Reviewed several papers already published.

### Financial support and sponsorship

This research work was supported by SECTEI under the project SECTEI-/183/2019 (10160c19).

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

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