TERM

REVIEW ARTICLE

Sources, Characteristics, and Therapeutic Applications of Mesenchymal Cells in Tissue Engineering

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Abstract Tissue engineering (TE) is a therapeutic option within regenerative medicine that allows to mimic the original cell environment and functional organization of the cell types necessary for the recovery or regeneration of damaged tissue using cell sources, scaffolds, and bioreactors. Among the cell sources, the utilization of mesenchymal cells (MSCs) has gained great interest because these multipotent cells are capable of differentiating into diverse tissues, in addition to their self-renewal capacity to maintain their cell population, thus representing a therapeutic alternative for those diseases that can only be controlled with palliative treatments. This review aimed to summarize the state of the art of the main sources of MSCs as well as particular characteristics of each subtype and applications of MSCs in TE in seven different areas (neural, osseous, epithelial, cartilage, osteochondral, muscle, and cardiac) with a systemic revision of advances made in the last 10 years. It was observed that bone marrow-derived MSCs are the principal type of MSCs used in TE, and the most commonly employed techniques for MSCs characterization are immunodetection techniques. Moreover, the utilization of natural biomaterials is higher (41.96%) than that of synthetic biomaterials (18.75%) for the construction of the scaffolds in which cells are seeded. Further, this review shows alternatives of MSCs derived from other tissues and diverse strategies that can improve this area of regenerative medicine.

Keywords Tissue engineering · Mesenchymal stem cells · Regenerative medicine · Biocompatible materials · Tissue therapy

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1 Introduction

There are certain limitations within the therapeutic area focused on the treatment of diseases in which the reparation or replacement of tissue and organs are needed because of the limited capacity of the human body to restore most tissues and organs to their original state [1]. The two principal issues are the lack of donors and transplant rejection by the immune system [2–4]; therefore, and considering that the pharmacological therapy currently available improves the patient's quality of life but fails to restore the functionality of damaged cells, it is necessary to find therapeutic alternatives that allows the reparation and recovery of the damaged tissues.

In the last decades, an alternative known as regenerative medicine, a new branch of medicine dedicated to replace,

regenerate, and repair cells, tissues, and organs damaged owing to factors such as age, chronic illness or congenital defects, has emerged [5, 6]. Currently, this term encompasses diverse areas and technologies, mainly cell therapy (CT), in which stem, progenitor, and primary cells are used to reconstruct structures and restore tissue and organ functions [7–9]; gene therapy, which entails a group of techniques to introduce genetic material and modify the expression of a gene product or alter the biological properties of cells to treat diseases [10]; and tissue engineering (TE), which involves the use of cell sources, scaffolds (or cell supports), and bioreactors to regenerate damaged tissue [11].

The following three basic pillars are necessary for the application of regenerative medicine in TE: cell sources, biomaterials, and culture stimulation. It is important to highlight that the last two pillars provide support for the cell type used, allowing proliferation and differentiation into the tissue of interest and promoting its migration and anchoring [12] to either natural (e.g., derived from components of the extracellular matrix) or synthetic biomaterials [13], along with the creation of an appropriate environment that mimics *in vivo* conditions to enhance tissue development through chemical and electrical mechanisms [14, 15]. Overall, the use of biomaterials has been reported as an option for delivery systems of differentiation and conditioning to induce a desire-specific cell lineage of mesenchymal cells (MSCs). Within this type of signals, it is important to emphasize those known as growth factors, whose function is to signal cells during their development and tissue repair [16], such as some cytokines, which requires support to prolong their half-life in normal physiological conditions, as they are prone to proteolysis, and can be applied for therapeutic purposes. Among this kind of molecules reported in regenerative medicine, especially to TE for various applications, are the following [16]. Transforming growth factor (TGF) for the regulation of cell growth and immune function; vascular endothelial growth factor (VEGF) induces of angiogenesis; Insulin-like growth factor (IGF) acts in endocrine regulation of the somatic growth, induces extracellular matrix synthesis and differentiation of MSCs to different phases in bone tissue repair [17]. Bone morphogenic protein (BMP) is used for promotion of cell regeneration and proliferation as well as metabolic homeostasis and the reduction of pro-inflammatory factors [18]. Fibroblast growth factor (FGF) has an important role in the promotion of angiogenesis and epithelization besides the migration and proliferation of keratinocytes and fibroblast. Epidermal growth factor (EGF) increases the deposition of extracellular matrix and the stimulation of the proliferation of chondrocytes and MSCs as a potent mitogen [19]. Platelet-derived growth factor (PDGF) controls the differentiation of neurons.

Neurotrophic factors (NF) and those factors that are used to stimulate cellular proliferation, morphogenesis, and angiogenesis have been widely used in regenerative medicine. Hepatocyte growth factor (HGF) can be used in combination, such as platelet-rich plasma (PPP), to obtain a platelet lysate (PL) in a singular solution with high concentration of growth factors such as TGB-β, PDGF-AA, PDGF-AB, PDGF-BB, EGF, IGF-1, HGF [20, 21], or independently according to the application of interest. Regarding the first pillar, adult stem cells are one of the principal cell sources in regenerative medicine, and sufficient evidence on their safety has been reported [22]. These cells are responsible for the development, maintenance, and repair of tissues, and numerous clinical trials have demonstrated their high potential in the treatment of diseases; however, until 2018, only three trials had completed phase III [23–25]. Nevertheless, a wide variety of studies in the early stages of clinical trials have used this cell type [26]. MSCs, multipotent stem cells, are considered a key option for tissue repair therapy because they have been studied in clinical trials, and evidence supports their great tissue repair potential in the treatment of numerous diseases, including orthopedic diseases (pseudoarthrosis and craniofacial trauma); degenerative diseases of the skeletal system (osteonecrosis and osteogenesis imperfecta); ocular (glaucoma and macular degeneration); cardiac (ischemic cardiomyopathy); renal, hepatic, and pulmonary diseases; and autoimmune diseases such as rheumatoid arthritis, and multiple sclerosis [24, 27, 28]. Important advances have been made in the TE field using MSCs, for example, the co-culture of these cells with human auricular chondrocytes seeded on collagen have been used to generate cartilage [29], and adipose derived-mesenchymal stem cells (AD-MSCs) have been combined with hyaluronic acid in rat models to regenerate osseous tissue [30]. In addition, the use of same type of MSCs in recombinant human tropoelastin scaffolds resulted in epithelium recovery in a murine model [31]. Apart from the applications of MSCs mentioned previously, these cells have also been used in the cardiac area of TE because cardiac and cardiovascular diseases (CVD) represent the major public health problem, according to the WHO, of which myocardial infarction affects a large part of the population. The WHO estimates that by 2030, almost 23.6 million individuals will die from a CVD and that this type of disease will continue being the main cause of death worldwide [32]. Although there are diverse therapeutic options, these are limited because they can only control the symptoms or prevent disease progress, but without tissue recovery. This review focused on the description of stem cells, specifically MSCs, because they comprise an important cell source in TE, and analyzed published studies that employed these cells in the principal applications of TE.

2 The most promising cell source for TE: mesenchymal/stromal stem cells

2.1 Stem cells

Stem cells (SCs) are defined as non-specialized cells with unlimited or extended self-renewal potential, capable of differentiating into various cell types, and thus, of serving as a reservoir to produce, maintain, repair, and regenerate tissues, while also maintaining their own population [26, 33, 34].

SCs are generally divided into six main categories based on the biology of the source from which they were obtained, which correspond to some stages of the development of a complete organism (Figure 1). These categories include embryonic SCs (ESCs) found in the first stages of embryonic development [35], fetal SCs (FSC) or FSCs from extraembryonic tissues, which can be obtained in the fetal stage, from the tenth week post-fertilization [36]; adult SCs (ASCs), including mesenchymal, hematopoietic, and stromal cells [37]; very small embryonic-like SCs (VSEL), found both in the adult stage and early stages of embryonic development [38]; and finally, those obtained by reprogramming, the induced pluripotent SCs (iPSCs); and nuclear transfer SCs (NTSC) [39].

Table 1 shows the summarized information of studies on the particular biologic characteristic of each SC, published in the last 5 years.

SCs can also be classified based on their cellular potential, in other words, their ability to differentiate. (Table 1) Thus, SCs are classified into totipotent stem cells, which are the least specialized and thus can divide and differentiate into all cell types necessary to form a complete organism [40], pluripotent stem cells, which can form cells of any of the germ layers, with ESCs and iPSC belonging to this group, and multipotent stem cells, which possess one level of specialization lower than the pluripotent SCs and whose function is to generate cells of specific lineages, such as the hematopoietic SCs, which can develop into several types of blood cells [40].

2.2 Mesenchymal cells

MSCs are an *in vivo* multipotent cell population present in adult and neonatal tissues of mesodermal origin as well as in the neural crest; they possess differentiation and self-renewal functions as progenitor cells, and their role is to maintain homeostasis and provide *de novo* specialized cells of mesodermal lineage [14, 28, 43].

According to the International Society for Cellular Therapy (ISCT), this cell type presents three main characteristics: adherence to plastic under culture conditions, expression of specific surface markers (Table 2), and the ability to differentiate *in vitro* into osteoblasts, chondrocytes, and adipocytes [22]. Moreover, it has been observed that they maintain a fibroblast-like morphology and do not generate an immune response [15].

Fig. 1 Stem cell sources in the different stages of human development and stem cell extraction by reprogramming
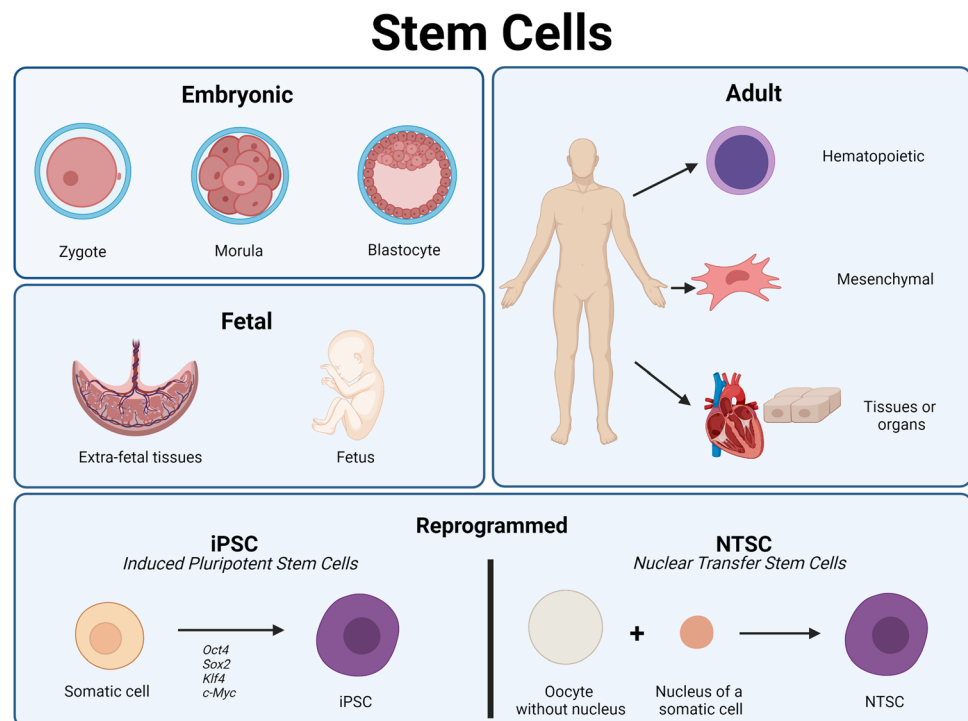
Table 1 General characteristics of stem cells according to their biology

Characteristic	Embryonic stem cells (ESC)	Fetal Stem cells	Very small embryonic-like stem cells (VSEL)	Adult stem cells (ASC)	Induced pluripotent stem cells (iPSC)	Nuclear transfer stem cells (NTSC)
Definition	Stem cells derived from the internal mass of the blastocyst or cells available from the morula stage, besides zygote	Pluripotent and multipotent stem cells derived directly from the fetus or extra fetal tissues	Non-hematopoietic pluripotent stem cells that are negative to the lineage marker CD133 that come from adult and fetal tissues	Quiescent stem cells that are present in an adult organism that responds to specific stimuli from the tissues to produce specialized cells	Pluripotent stem cells generated by in vitro reprogramming of somatic adult cells by simultaneous expression to inductor genes	A single cell is produced by the insertion of a nucleus from a somatic cell in an enucleated oocyte
Potential	Totipotent (Zygote) Pluripotent (Morula, blastocyst)	Pluripotent Multipotent	Pluripotent	Pluripotent Multipotent	Pluripotent	Totipotent
Source	Zygote Morula Blastocyst	Fetus Extra fetal tissues (umbilical cord, amniotic fluid, placenta)	Adult bone marrow, umbilical cord blood, peripheral blood	Bone marrow, adipose tissue, skin, skeletal muscle, heart, liver, blood, nerve tissue	Somatic cells	Enucleated oocytes and nucleus from somatic cells
Ref.	[36, 39, 40]	[36]	[37–39]	[34, 39, 41]	[39, 42]	[39, 42]

Table 2 MSCs surface markers according to the ISCT

Surface markers [44]	
Positive	Negative
CD105	CD45
CD73	CD34
CD90	CD14 o CD11b
	CD79α o CD19
	HLA-DR

Two of the criteria for the expression of surface antigens is that ≥ 95% of the total population must express positive markers and only ≤ 2% can express negative markers

However, there is controversy regarding the classification criteria that must be considered to guarantee homogeneity. One of the main controversies is the use of the acronym "MSCs," as some authors use it to describe mesenchymal stem cells and others to refer to mesenchymal stromal cells, but without mentioning the source of extraction, which is the main difference between these cell types. The former population is directly isolated from bone marrow, whereas the latter correspond to any structural or nonstructural tissue [45]. This has led to differences in the expression of the surface cell markers used to differentiate

MSCs, one of them being the negative expression of CD34. In this regard, it has been reported that the CD34 expression depends on the donor and type of tissue, e.g., a percentage of adipose tissue-derived SCs are CD34 positive [43]. Moreover, although other surface markers have been suggested to characterize MSCs (Stro-1, CD271, SSEA-4, and CD146), no consensus has been reached as their expression varies depending on the cell source [25, 46]. Therefore, it is mandatory to know the markers of MSCs obtained from a particular tissue besides those established by the ISCT. Table 3 summarizes the different surface markers reported in the last five years to characterize MSCs based on their cell source.

2.3 Cell source, isolation, and culture

MSCs are present in nearly all the tissues of the body, are located in a perivascular niche [28], and can be obtained from diverse sources, including adult tissue (peripheral blood, adipose tissue, bone marrow, skeletal muscle, brain, dermis, dental pulp, synovial fluid, and tendons) and neonatal tissue (umbilical cord, umbilical cord blood, and placenta) [28, 64].

The proportion of MSCs varies depending on the tissue; they represent approximately 0.00001% of the bone marrow cells and ≥1% of the cells in the adipose tissue. Although the frequency of umbilical cord-derived MSCs is

Table 3 Surface markers present in MSCs based on the source of isolation. Markers presented in bold are reported as tissue-specific markers from the tissue of isolation in various sources

MSCs	Surface Markers	Ref.
BM-MSCs Bone marrow mesenchymal stem cells	CD11a-, CD11b-, CD13-, CD14-, CD19-, CD29 + , CD31 + , CD34 ± , CD45-, CD49a + , CD49b + , CD49c + , CD49d + , CD49e + , CD51 + , CD54 + , CD58 + , CD61 + , CD71 + , CD73 + , CD90 + , CD102 + , CD104 + , CD105 + , CD106 + , CD120a + , CD120b + , CD121a + , CD124 + , CD133-, CD140a + , CD140b + , CD146 + , CD166 + , CD200 + , CD221 + , CD271 + , SSEA-4 + , STRO-1 + , Nestin +	[25, 47–49]
AD-MSCs Adipose tissue mesenchymal stromal cells	CD73 + , CD90 + , CD105 + , CD45-, CD34 ± , CD14- o CD11b-, CD79a- o CD19-, HLA-DR-	[50–53]
	CD13 + , CD29 + , CD10 + , CD49 + , CD26 + , CD3-, CD11b-, CD106-, CD36 + y CD44 + in > 80% of the surface of the AD − MSCs y CD31-, CD45- y CD235a- in less than 2% of the surface. Other: CD271 + , CD200 + , CD273 + , CD274 + , CD146 + , CD248 + , CD140b + , CD163-, CD166 + , CD59 +	
D-MSCs Dental tissue mesenchymal stromal cells	CD90 + , CD166 + , CD44 + , CD29 + , Cd73 + , CD146 + , CD106 + , CD105 + , STRO1 + , SSEA4 + , Nanog + , Oct4 + , Nestin + , Notch1 + , CD34-, CD45-, CD271-, CD71-, CD14-, CD49-, CD11b-, CD133-, HLA-DR-, CXCR4-, CD40 + , CD133-, CD24-	[54]
UC-MSCs Umbilical cord mesenchymal stromal cells	CD29 + , CD44 + , CD73 + , CD90 + , CD105 + , HLA-ABC + , HLA-DR-, Vimentin + , Stro1 + , CD106 + , CD146 + , CD166 +	[55–57]
S-MSCs Synovial tissue or fluid mesenchymal stem cells synovial	CD73 + , CD90 + , STRO-1 + , CD105 + , CD34-, CD45-, HLA-DR-, CD19-, CD40-, CD80-, CD86-, CD11b-, CD14 ± , CD44 + , CD55 + , CD271-, CD55-, CD271 + (according to the region of isolation)	[58–60]
Mu-MSCs Muscle tissue mesenchymal stem cells	CD45-, CD19-, CD14-, CD34-, CD73 + , CD90 + , CD105 + , PDGRFα, CD146 + , CD56 + , CD201 + , CD82 + , CD318 +	[61–63]

similar or even lower than that previously mentioned, they have a better *in vivo* expansion capacity owing to their fetal nature [64].

Table 4 presents the most important characteristics of MSCs based on their isolation source because heterogeneity has been reported in their cellular yield as well as their advantages and disadvantages, which represent the principal criteria for their use in regenerative medicine.

2.4 Therapeutic applications of MSCs in different tissues

According to previous researches, six of the more studied applications of mesenchymal cells in TE were selected, including those in neural, osseous, muscle, cartilaginous, epithelial, and cardiac tissues.

This review covers the use of six sources of MSCs in each of the TE applications. To that end, boolean expressions obtained from the PubMed database over the last 10 years were used. In addition, because more than 500 results were found for some of the applications, the search was limited to the last 5 years in those cases.

Currently, several therapies are under development in the TE field which use MSCs extracted from different sources to treat problems associated with diverse tissues; therefore, a search of the applications with great number of

studies published in recent years was conducted, and the most frequently used cell sources were identified.

Therapies have emerged as an alternative to treat disorders that can no longer be treated with drugs or surgery because the damaged tissue is not able to recover owing to the limited capacity of the organism. The most studied areas include health issues related to the joints, bones, muscle, skin, and cardiac and neurodegenerative diseases, considering their incidence in the global population.

2.5 Applications of MSCs for cartilage and osteochondral diseases

An example of these disorders is osteoarthritis, one of the most common illnesses in middle aged and older individuals, which affects over 250 million people worldwide and is caused by age-related degeneration of the articular cartilage. Although an early intervention can help to prevent the damage from spreading to the healthy cartilage and current treatments are quite promising, there is no definitive treatment to completely restore the auricular cartilage to its normal state [81, 82]. To overcome these limitations, TE has emerged as an alternative to treat this disease. Rahman et al. (2015) created a BM-MSCs rabbit-derived composite construct of poly(lactic-co-glycolic acid) (PLGA)/fibrin scaffold, cultured in a commercial medium

Table 4 Sources of mesenchymal cells and their characteristics

Source	Location	Isolation method	Culture	Cellular yield	Advantages	Disadvantages	Cell formation efficiency	Ref.
Bone marrow	Endostal surface of the iliac crest, trabecular and compact bone, femoral head, humeral head, and vertebral body	Aspiration in the posterior iliac crests. In animal models, it can also be obtained directly from long bones of freshly animals. MSCs are isolated by gradient density centrifugation (Ficoll or Percoll method)	Cell culture in DMEM medium with 10% fetal calf serum (FCS)	Dependent on the characteristics of the donor (e.g., Age). Approximately 100–1000 cells/mL or 10 FCE per 10^5 cells	Easy to isolate within a surgical procedure. High cell growth	Chronic pain at the site of isolation. High risk of infection	52%	[65–67]
Adipose tissue	Adipose tissue	Liposuction or removed fat. Cells are isolated from the white adipose tissue	Cell culture in DMEM containing 10% fetal bovine serum (FBS)	5000 SCs per 1 gr of fat	Easily obtained High initial cell yields. Robust proliferative capacity in vitro	Do not show chondrogenic or myogenic differentiation in all culture conditions. Do not present lineage markers: CD31, CD34, and CD45	60%	[66–68]
Dental tissue	Dental pulp, periodontal ligament, dental follicle, gingiva	Isolation of the tissue from non-invasive surgical tooth extractions	α-MEM with 10% FBS and antibiotic and antimycotic solutions	The frequency of colony formation is 22–77 colonies per 10^4 cells per plate	Easily obtained if the isolation is scheduled. Higher osteogenic differentiation because the memory of its origin. Depending of the source, there is higher or lower generation of mineral compounds	Samples must weigh more than 0.2 gr It cannot survive more than 24 h after extraction Higher restriction of differentiation in vivo	80.4%	[50, 54, 69–72]

Table 4 continued

Source	Location	Isolation method	Culture	Cellular yield	Advantages	Disadvantages	Cell formation efficiency	Ref.
Fetal and extra fetal tissues	Umbilical cord, placenta, Wharton's jelly (WJ), amniotic fluid, fetal tissues from the heart, liver, pancreas, and others	Umbilical cord is the most reported: harvest 10 cm of umbilical cord in DMEM medium. The most common methods of isolation are enzymatic and explants	DMEM medium with 10% human serum/FBS, antibiotic solution (0.1% gentamycin, 0.2% streptomycin and 0.12% penicillin)	Source dependent: blood from the umbilical cord has 1 UFC (Unit forming colonies) per 10^8 cells and WJ have a higher frequency	Does not have ethics limitations. Easy access Colony duplication is faster than BM-MSCs	Current isolation methods generate low cell count. Low capacity of adipogenicity differentiation	Varies with source, from 15% (amniotic fluid) to 80% (WJ)	[55, 67, 73, 74]
Synovial	Membrane and synovial fluids Knee articulations	Arthroscopy with biopsy	MesenGro medium with 10% FBS and 1% penicillin–streptomycin At the third passage, cells can be used for in vitro assays	37 cells/mL in the synovial fluid in patients with osteoarthritis, the number increases with a knee lesion. In healthy persons, cell numbers are 7 times lower to the mentioned above	Higher cell count per colony than other sources Extensive proliferation in culture Maintains its cellular potential in vitro Higher chondrogenic potential	Limited number of cells in the tissue No standard procedure for purifying MSCs from synovial fluid	55%–75% depending on the culture medium (with autologous human serum or FBS respectively)	[58, 66, 75, 76]
Muscle	Postnatal skeletal muscle, below the basal lamina of muscular fibers	Small biopsies. Obtained by isolation of a single fiber or enzymatic digestion of the entire muscle	αMEM 1 ×, + L-glutamine, + 4 ribonucleosides, + deoxyribonucleosides, -ascorbic acid, SH, insulin y 1% penicillin/streptomycin	The yield of viable cells depends on the source of the tissue	Promote angiogenesis Long-term proliferation	Satellite cells are already committed to muscle lineage Passage limit is 10	~ 45%	[66, 77–80]

for chondrocyte differentiation, and observed that the construct was capable of regenerating tissue in an *in vitro* environment [81], thus proving its potential in cartilage TE. In addition, Yin et al. (2016) formed a compound with goat cartilage extracellular matrix (ECM); particles of the fresh cartilage (cartilage ECM-derived particles; CEDPs) were produced through a series of steps; rat BM-MSCs were seeded on the surface of CEDPs and the construct was implanted in rats with defects in the cartilage of the trochlear groove. It was found that the animals showed a good and fast recovery in their joint function [83]. Furthermore, in other congenital diseases associated with cartilage, such as microtia, infants have only few options for atrial reconstruction. Thus, Cohen et al. (2018) generated constructs of human auricular chondrocytes and human MSCs with type I collagen and subcutaneously implanted them in athymic mice, and a cartilage generation equivalent to that of the native auricular cartilage was observed [29].

2.6 Applications of MSCs for musculoskeletal diseases

Another example are the musculoskeletal pathologies, such as fractures, lumbago, and osteoporosis, which have increased owing to a longer life expectancy and the aging global population [84]; thus, it is necessary to find options to treat these disorders. Boeckel et al. (2019) implanted a graft of AD-MSCs with hyaluronic acid (HA) in rats with a critical osseous defect in each femur and observed an increase in the osseous tissue [30]. Moreover, Toosi et al. (2016) created collagen sponges reinforced with biodegradable polyglycolic acid (PGA) fibers, and *in vitro* experiments using these sponges demonstrated that the incorporation of PGA fibers increase cell adherence as well as proliferation and differentiation of BM-MSCs, which make them useful for the treatment of pseudoarthrosis [85]. Zhang et al. (2017) intramuscularly implanted hydroxyapatite/PLGA nanocomposites scaffolds with BM-MSCs and bone morphogenetic protein-2 (BMP-2) in rabbits and found that hydroxyapatite promotes an improvement in bone mineralization and formation [86].

2.7 Applications of MSCs for epithelial covering in traumas

TE has also shown important advances in the field of epithelial tissue regeneration as it is most exposed tissue to damage caused by burns, irritators, traumas, or tumor resection [87]. In addition, when the damage encompasses a large surface, there are limitations in the performance of a skin autograft, leading to complications; thus, researchers have looked for alternatives in graft creation [87, 88]. In preclinical stages, Mashiko et al. (2018) implanted a scaffold composed of recombinant human collagen peptide (rhCP), together with the combination of human AD-MSCs and endothelial cells from umbilical veins, in an ulcer model of irradiated mice and detected improved cicatrization compared with the groups that had only received the biomaterials or AD-MSCs and the controls (without scaffold or cells) [89]. Kellar et al. (2016) also reported the use of AD-MSCs with human tropoelastin in a murine model of excisional wound, in which the scaffold-treated wounds showed a closure rate of 94% as well as a recovery of normal epithelium of 75%, which was higher than that of the controls [31].

2.8 Applications of MSCs for muscle pathologies

Furthermore, TE is a great alternative for the treatment of certain muscle pathologies, such as muscular dystrophy or diseases caused by lesions or congenital defects [90]. Ansari et al. (2016) created RGD microspheres (common peptide motif of cell adhesion: arginine-glycine-aspartic acid) coupled to alginate and used them to encapsulate gingival mesenchymal stem cells (GMSCs). This construct was subcutaneously implanted in immunocompromised mice, together with a cocktail for myogenic differentiation, showing that after 4 weeks of *in vitro* differentiation, the GMSCs exhibited morphology similar to that of muscle cells and that the RGD-alginate-coupled scaffold regulated their myogenic differentiation. Furthermore, in *in vivo* experiments, the generation of small muscle-like structures was observed through hematoxylin-eosin (H&E) staining [90].

2.9 Applications of MSCs for regenerations of nervous tissue

Owing to the specific properties of nervous tissue-derived cells (excitability and conductivity) and their low regenerative capacity, it is highly complicated to administer treatments to cure disorders of the central nervous system, such as neurodegenerative diseases, traumatic lesions of the brain or spine, and cerebral infarction. Therefore, cell replacement through regenerative medicine has been an innovation in neuronal engineering, in which only devices to treat nervous system dysfunctions had been designed [91, 92]. The viability and neurogenesis of MSC have been examined *in vitro* in different scaffolds. In this regard, Quintiliano et al. (2016) studied a 15% PLGA scaffold with D-MSCs obtained from dental pulp and reported a fast degradation, 41% by day 28, which represents an advantage as this attenuates the progressive damage sustained by the nervous fibers after a lesion and provides sufficient time for the cells to appropriately proliferate, migrate, and differentiate into mature nervous tissue; these results support

the execution of preclinical tests in animal models [93]. For neurodegenerative diseases, Jamali et al. (2017) proposed the use of a poly-L-lactic acid scaffold with MSCs collected from the trabecular meshwork to obtain dopaminergic neurons, which constitute an appropriate source for the treatment of Parkinson's disease [94].

2.10 Applications of MSCs for mechanical cardiac pathologies

Finally, another area of interest for TE is the treatment of cardiac diseases, such as myocardial infarction, as the currently available treatments have diverse limitations. Nali et al. (2017) created a construct using UC-MSCs over a scaffold comprising decellularized umbilical artery, implanted it in a myocardial infarction rat model, and observed an improvement in the heart function [95]. Chen et al. (2018) created nanofiber patches of chitosan/silk-fibroin-modified cellulose with mice AD-MSCs, implanted them in rats with myocardial infarction, and later observed myocardial fibrosis attenuation [96]. Moreover, Prat-Vidal et al. (2020) created a scaffold called PeriCord comprising a decellularized pericardial matrix colonized with WJ-MSCs, which was implanted on a non-revascularizable scar in the inferior wall of a 63-year-old male patient. The three-month follow up showed optimal results, and the magnetic resonance indicated a ∼9% reduction in the scar mass of the treated area [97]. Currently, the PeriCord scaffold is commercialized for its use in similar researches.

The tables below summarize the most recent studies on TE regarding osseous (Table 5), cartilaginous (Table 6), osteochondral (Table 7), muscle (Table 8), dermal (Table 9), nervous (Table 10), and cardiac (Table 11) tissues. These tables provide detailed information on the cell source, extraction tissues, and type of isolated MSCs, in addition to the analyses performed, markers used for cell characterization by different techniques (Immunofluorescence (IF), Flow cytometry (FC), Immunohistochemistry (IHC), Immunostaining (IS), Western-Blot (WB), Enzyme-linked immunosorbent assay (ELISA) and Polymerase chain reaction (PCR)), cellularized scaffolds, type of scaffold used, and findings.

2.11 Clinical trials in TE with MSCs

There are several clinical trials in the TE field where MSCs are used, as shown in Figure 2. Some of the clinical trials are focused on treating cartilage and osteochondral diseases. One example is the clinical trial that seeks to treat knee osteoarthritis (NCT00850187) using BMMSCs

on a collagen I scaffold [198]. Another one is using ADMSCs seed on a human amniotic membrane (NCT04670302) as a treatment for supraspinatus tear [199].

On the other hand, there are clinical trials focused on bone regeneration. One of them consists of treating bone loss using BMMSCs on a scaffold called BioMax (NCT01389661). It was reported that in the nine patients who participated in the study, there was bone formation in the space where maxillary cyst was located, which had already been extracted and there were no severe adverse reactions reported [200, 201]. Another clinical trial attempted to treat the cleft lip and palate condition using DPMSCs seeded on a collagen and hydroxyapatite scaffold (NCT01932164). The study reports that bone formation was quantified in five patients with this condition using TC. Six months after the intervention, final completion of the alveolar defect, with an 89.5% mean bone height was detected [202]. Furthermore, another clinical trial is based on treating periapical periodontitis using UCMSCs encapsulated in a plasma-derived biomaterial (NCT03102879). The study was reported as an efficacy trial. One year after the intervention, the treated tooth remained in the mouth without pain [203].

Other clinical trials are focused on the recovery of epithelial tissue. One example is a clinical trial that consists of treating diabetic foot ulcer (NCT03865394) using ADMSCs suspended in a fibrin solution [204]; however, no results were presented.

Finally, there is a clinical trial that consists of evaluating patch with WJMSCs seeded on a decellularized human pericardial matrix to treat myocardial infarction (NCT03798353). The study reported that the construct with the scaffold called Pericord and the WJMSCs was implanted on a non-revascularizable scar in the inferior wall of a 63-year-old man. The three-month follow up showed optimal results, and magnetic resonance indicated ∼9% reduction in the scar mass of the treated area [97, 205]. Currently, the PeriCord scaffold is commercialized for its use in similar research.

As can be seen, TE using MSCs seeks to treat a wide variety of pathologies and uses various types of MSCs according to the purpose of the clinical trial.

By November 2021, 15 clinical studies had reported the use of MSC in TE in accordance with the CrinicalTrials.gov web page of the United States. Among those 15 results, eight have been already completed, two are in the recruitment phase, two are in an unknown state, three aren´t in recruitment phase yet and one clinical trial doesn´t have specific information about the phase of the study

Table 5 Applications of MSCs in bone tissue engineering

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Human	BM-MSCs	Unspecified		COL I, IBSP, BMP2, OCN	H&E staining, Masson trichrome staining	ALP activity assay (alkaline phosphatase)	Silk fibroin (SF) with bioactive mesoporous glass (MBG)	MBG/SF scaffolds had superior strength and good biocompatibility and stimulated bone formation ability superior to a polycaprolactone/MBG scaffold. In vivo gene expression was common to osteogenic markers on this scaffold	2019	[98]
	BM-MSCs	Unspecified				ALP activity assay	Silk fibroin scaffolding and bioactive glass of nanoparticle silk fibroin	50% more fixation of MSCs compared with scaffolding with microparticles with greater promotion of ALP activity	2019	[99]
	UC-MSCs + hUVEC	Lonza	IF: PECAM1	ALP, OCN, COL I, VEGF, CDH5, vWF	H&E staining, Alizarin-red stain		Calcium phosphate cement scaffolding (CPC)	The scaffold was implanted in a rat model with cranial defect. Excellent osteogenic and angiogenic capacity were observed	2018	[100]
	UC-MSCs	Umbilical cord		ALP, OCN	H&E staining, Alizarin-red stain		Films of 3-hydroxybutarate, 3-hydroxyhexanoate and silk fibroin (P(3HB-co-3HHx)/SF)	The union of these biomaterials promotes osteogenic proliferation and differentiation which makes it a candidate in bone tissue engineering	2020	[101]
	D-MSCs	Dental pulp and dental follicle	FC: CD44+, CD90+, CD73+, CD34−CD45−	RUNX2, SPPI, BMP2, OCN	Masson's trichrome stain, Alizarin-red stain		Collagen-nanohydroxyapatite/phosphoserine cryogel with osteogenic factors in the culture medium	The construct was implanted subcutaneously in a mouse model and a bone-like structure was observed at the periphery of the scaffold (with both DP-MSCs and DF-MSCs)	2020	[102]
	D-MSCs	Dental pulp	FC: CD73+, CD105+, CD90+, CD34+, CD45+	SPPI, ALP, RUNX2, COL I				In a rabbit model with bilateral mandibular critical size defect, the construct was implanted, and new bone formation and angiogenesis were observed	2020	[103]
	AD-MSCs	Lipoaspirate	IHC: OCN		Alizarin-red stain	ALP activity assay	Collagen hydrogel, elastin-like polypeptide (ELP) and bioglass	In vitro, there was an increase in ALP activity, osteocalcin content. Formation of calcium and phosphorus mineral deposits were observed	2020	[104]
	AD-MSCs + endothelial cells from the umbilical cord	Abdominal fat tissue	IS: CD105, PECAM1, ACTA2		H&E staining, Masson's trichrome stain, Alizarin-red stain	ALP activity assay	Hydroxyapatite granules with fibrin	Confirmed bone formation, with a complete vascular network in vitro	2020	[105]
	SF-MSCs	Synovial fluid	FC: CD44+, CD90+, CD105+, CD73+, CD45−CD34−, CD11b−, CD19−, HLA-DR-	SPPI, OCN, COL1A1, RUNX2			Ketone–ketone polyether (PEKK)	A rabbit model with calota's skeletal damage had the construct implanted and increased osteogenic ability was observed	2019	[106]

Table 5 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Rat	BM-MSCs	Bilateral Femur		ALP, SPP1, OCN, RUNX2	H&E staining, Masson's trichrome staining, Alizarin-red, Von Kossa stain	ALP activity assay	Scaffold composed of a bovine serum polyvinylpyrrolidone/albumin/BMM2-derived peptide nucleus with a polycaprolactone shell with collagen I	In vitro scaffolding was favorable for cell adhesion and survival. In vivo, they promoted bone formation and locking of the calvary defect in rat model	2019	[107]
	BM-MSCs	Femur	IF: VCL and OCN. WB: ERK1/2, p-ERK1/2, HIF1A, PECAM1 and EMCN	ACTB, ALP, OCN, BMP2, SP7, HIF1A, VEGFA, CXCR4	Alizarin-red staining, Calcein stain		Mesoporous active glass scaffolding (MBG) of coglycolic lactic acid pole (PLGA) particles with bioactive lipid FTY720 using supercritical CO_2 foaming technique	In vitro facilitated osteogenic differentiation with an overexpression of Hif-1a: osteogenic and proangiogenic effects. In vivo promoted vascularized bone regeneration	2019	[108]
	D-MSCs	Dental pulp from incisor teeth	IF: SPP1	SPP1, OCN, COL 1			Self-assembled peptide hydrogel (SPG-178-Gel)	Osteogenic differentiation was achieved in the hydrogel	2017	[109]
	D-MSCs	Dental pulp	FC: CD31$^-$, CD45$^-$, CD73$^+$, CD90$^+$		Sirius-red stain and toluidine blue, TRAP stain	ALP activity assay,	Heavy collagen gel	Rat model with bone defect in calota to which the construct was implanted demonstrating an improvement in the healing of cranioencephalic bone	2016	[110]
	AD-MSCs	Inguinal adipose tissue			H&E stain, picrosirius red		Decellularized human amniotic membrane	In vitro osteoinduction promoted bone-like matrix deposition mineralized by AD-MSCs. In vivo, the stimulation of bone deposition compared with controls, in addition to the healing of calvary defects with large graft incorporation	2021	[111]
	Mu-MSCs	Hind limb and back muscles		OCN, COL 1, TGFB1, RUNX2, ACTB	H&E stain, alizarin-red	ALP activity assay	2D alginate hydrogel with platelet-rich plasma	Osteoblastic phenotype was induced in MSCs in vitro and in vivo (scaffolding with MSCs was implanted subcutaneously in mice)	2012	[112]
Mice	AD-MSCs	Inguinal fat pads			H&E stain, Masson's Trichrome staining		Macroporous hydrogel of gelatin micro ribbons and encapsulation of BMP-2	Using low doses of BMP2 accelerated bone mineralization regeneration	2020	[113]

Table 5 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers					Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry					
Pig	UC-MSCs	Umbilical cord			H&E staining		Electrospinning of poly(lactic-coglycholic) copolymer (PLGA) seeded with green fluorescent protein (GFP)	In a model of alveolar cleft of pork, the construct was implanted and proved to be a good candidate for the repair of this condition; UC-MSCs in vitro may contribute to bone regeneration	2017	[114]
	AD-MSCs	Unspecified	WB: ACTB, ERK1/2, p-ERK1/2 IHC: SPP1	COL1A2, OCN, BMP2, SP7, CXCL8, RUNX2	H&E staining, Alizarin red, Von Kossa stain	AlamarBlue Trial	Mineralized collagen scaffold/ amniotic membrane	Promotes osteogenesis without inflammatory response	2020	[115]
Rabbit	BM-MSCs	Femur	IHC: SPP1		Rhodamine B		Fibrous mesh of polycaprolactone and lactic acid	Increased capacity for biomineralization, infiltration, and cell proliferation. In vitro osteoblastic differentiation	2020	[116]
	BM-MSCs	Bone marrow				ALP activity test	Collagen sponges derived from duck skin with hydroxyapatite with silymarin	The scaffold with concentration of 100 µM of Smn had greater efficiency for the adhesion of MSCs, growth and expression of osteogenic markers	2019	[117]
	AD-MSCs	Adipose tissue			H&E staining		Gelatin and tricalcium phosphate (TCP) scaffolds	Average mineral density higher than that of the control group in in vivo model. Scaffold osteoconductivity managed to fill in and cure the model's host defect in mice	2021	[118]

. **Immunodetection techniques markers**: PECAM1 = Platelet and endothelial cell adhesion molecule 1, ACTA2 = Actin alpha 2, smooth muscle, aorta, VCL = Vinculin. OCN = OCN/ BGLAP (Osteocalcin), ERK1/2 = Mitogen-activated protein kinase, p-ERK1/2 = Mitogen-activated protein kinase phosphorylate, HIF1A = Hypoxia inducible factor 1 subunit alpha, EMCN = Endomucin, SPP1 = Secreted phosphoprotein 1. **PCR markers:** COL 1 = Collagen type I, IBSP = integrin binding sialoprotein, BMP2 = Bone Morphogenetic Protein 2, ALP = Alkaline Phosphatase, VEGF = Vascular endothelial growth factor, CDH5 = cadherin 5, vWF = von Willebrand factor, RUNX2 = RUNX family transcription factor 2, COL1A1 = Collagen type I alpha 1 chain, ACTB = Actin beta, SP7 = SP7/OSX (Sp7 transcription factor), VEGFA = Vascular endothelial growth factor A, CXCR4 = C-X-C motif chemokine receptor 4, TGFB1 = Transforming growth factor beta 1, COL1A2 = Collagen type I alpha 2 chain, CXCL8 = C-X-C motif chemokine ligand 8

Table 6 Applications of MSCs in cartilage tissue engineering

Source	Type of MSCs	Extraction tissue	Type of analysis and markers		Histology	Spectrometry	Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR						
Human	BM-MSCs	Knee joints	FC: CD90+, CD44+, CD45−. IF: COLII	COLI, COLII, ACAN, POU5F1, SOX2, NANOG, ZFP42		DMMB assay and Blyscan assay	Gelatin microspheres in bioreactor with F12: DMEM + 10% FBS and chondrogenic induction medium	Increased chondrogenic differentiation in dynamic culture and 3D culture compared with a 2D culture	2020	[119]
	BM-MSCs	Femoral head		ACAN, COL2A1, SOX9, COL10A1, MMP13, RPL13A	Alcian blue stain/fast green	Blyscan assay and hydroxyproline assay	Graphene oxide nanosheets with L-lactic/polyethylene glycol poly-D-acid hydrogel	Continuous release of TGF-β3. In vitro: increased chondrogenesis, dependent on TGF-B3. In vivo: greater production of cartilaginous matrix than scaffolds without graphene nanosheets	2020	[120]
	UC-MSCs	Umbilical cord	IF: Calcein assay AM		H&E stain		ECM biomimetic scaffolding of adipose tissue with hydroxyapatite gradients (HA-G)	A rat model with rotator cuff defect was implanted with the construct and an improvement in the repair of the lesion was noted	2020	[121]
	WJ-MSCs	Wharton's jelly			H&E stain, and toluidine blue		Extracellular matrix of acellular cartilage	Model of goat with defect in the articular cartilage of the femoral condyle to which the scaffold was inserted with the cells showing repair and regeneration of the cartilage	2018	[122]
	DP-MSCs	Third molar	FC: CD44+, CD90+, CD106+, CD11b−, CD45−.IHC: COLII	ACAN, COL10A1, COLII	H&E stain		Nanocellulose-based thermosensitive hydrogel (NC) by adhesion of beta-glycerophosphate (GP) and chitosan (CS) (NC-CS/GP-21)	THE DPSC implanted in the NC-CS/GP-21 hydrogel showed proliferative capacity in addition to chondrogenesis	2020	[123]
	AD-MSCs	ATCC	ELISA: COLII	COLIA1, SOX9, ACAN	Alcian blue stain, Sirius red and Safranin-O	DMMB assay	Rosette nanotubes functionalized with lysine and covered with gelatin methacrylate and polyethylene glycol diacrylate	Increased expression of chondrogenic markers significantly (59%, 71%, and 60% of Col II, GAG, collagen synthesis)	2020	[124]

Table 6 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
AD-MSCs	Infrapatellar fat pad		ELISA: COLII	COL1, COL2, ACAN, SOX9	Masson's trichrome stain and toluidine blue	Bioprinted scaffold of polyurethane elastomer 1,4-butanediol thermoplastic	They maintained the proliferative potential of MSCs, maintaining chondrogenesis. In vivo, extracellular matrix deposition and integration with adjacent tissue	2020	2020	[125]
SF-MSCs	Synovial liquid		FC: CD73$^+$, CD90$^+$, CD105$^+$, CD34$^-$, CD45$^-$, HLA-DR$^-$ IHC: COL2A1	ACAN, SOX9, COL2A1, COMP, COL2B	H&E stain	Collagen sponge	SF-MSCs seeded in collagen sponges demonstrated good chondrogenic capacity	2018	2020	[126]
SF-MSCs	Synovial liquid		FC: CD13$^+$, CD29$^+$, CD44$^+$, CD49C$^+$, CD73$^+$, CD90$^+$, CD105$^+$, CD151$^+$, CD34$^-$, CD45$^-$, CD49f$^-$, CD184$^-$ IF: COLI, COLII, ACAN	ACAN, COL1A2, COL2A1, SOX9, COL10A1	Safranin-O	Meniscus-derived decellularized matrix	SF-MSCs seeded on the scaffold with TGF-beta3 and IGF-1 have a beneficial effect on fibro chondrogenesis of cells	2018	2020	[127]
Rat	BM-MSCs	Femur	IHC: COLII	COLII, COLI, COL10A1, SOX9, ACAN	H&E stain, Alcian blue, Safranin-O	Blyscan assay	Gelatin methacrylate hydrogel bioprints (GelMA)	In vitro and in vivo regeneration of mature cartilage	2020	[128]
BM-MSCs	BM-MSCs	bone marrow	IHC: COL II, ACAN, COL10A1	COLI, COLII, GAG, SOX9|	Toluidine blue and Safranin-O		Glycerol poly-sebacate scaffold with poly (1,3-propylene sebacate) and carthagen	In vitro they promoted chondrocyte differentiation and inhibited osteogenic differentiation	2020	[129]
Rabbit	BM-MSCs	Femur and tibia	IHC: PRG4, CILP, COLII, COLI, SOX9. ELISA: COLII. IF: COLII, COLI, PRG4, SOX9	PRG4, CILP, COL2A1, COL1A1, SOX9, ACTB	Sirius red		Printed polycaprolactone (PCL) or PCL/hydroxyapatite (HA) scaffolds with cytosine-containing microspheres	Heterogeneity of chondrocytes and secreted proteins that promote the regeneration of functional cartilage in vivo and in vitro	2020	[130]

Table 6 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
BM-MSCs	Femur and tibia	IF: COLII, ACAN	COLII, COLI, SOX9, ACAN, COMP	Safranin O/red Picrosirius y de F-actin	DMMB assay and hydroxyproline assay	Poly-ε-caprolactone fibers coated with extracellular matrix derived from decellularize chondrocytes	MSCs tended to spread more on scaffolding with PCL than scaffolding without this cover. The expression of chondrogenesis markers was higher	2021		[131]
AD-MSCs	Adipose tissue of the abdomen	IF: COLII	COL2A1, ACTB	H&E stain, Safranin-O		Scaffolding of soluble poly-L-lysine/carthagegenine nanoparticles with polylactic-coglycolic acid/methacrylate hyaluronic acid	In vivo, a condroncial defect was recovered with the presence of intact and smooth cartilaginous tissue. Results greater than just using NP or scaffolding separately	2020		[132]
SF-MSCs	Synovial fluid	IHC: COLII		Toluidine blue		Chitosan-based hydrogel	The construct was implanted in a rabbit model with cartilage defect in femoral patellar grooves and cartilage regeneration was observed	2019		[133]
Goat	BM-MSCs	Nape	IHC: COLI, COLII	COLI, COLII, SOX9	H&E stain, Safranin-O/Fast Green		Two-layered scaffold: polyethylene glycol (PEG) with poly (glutamic L acid)-g-polycaprolactone (PLGA-g-PCL)	After 2 months in vivo in goat, there is neocartilage formation in the model of defect in the temporomandibular junction	2021	[134]

Immunodetection techniques markers: COLII = Collagen type II, COL2A1 = Collagen type II alpha 1 chain, ACAN = Aggrecan COL10A1 = Collagen type X alpha 1 chain, PRG4 = Proteoglycan 4, CILP = Cartilage intermediate layer protein, SOX9 = SRY-box transcription factor 9. **PCR markers:** POU5F1 = POU class 5 homeobox 1, SOX2 = SRY-box transcription factor 2, NANOG = Nanog homeobox, ZFP42 = ZFP42 zinc finger protein/REX1, MMP13 = Matrix metallopeptidase 13, RPL13A = Ribosomal protein L13a, COMP = Cartilage oligomeric matrix protein, COL2B = Collagen type II alpha 1 chain isoform B, GAG = Glycosaminoglycan

Among all of these results, only nine are examining the use of MSCs with scaffolds, showing that this therapeutic option represents a great area of opportunity.

3 Discussion

The use of TE in the treatment of several diseases has proved to be a promising alternative for many patients. This review is particularly based on the analysis of recent studies that use MSCs in TE and also considers the type of scaffold employed and the application in TE.

3.1 MSCs terminology

However, there is a lack of homogeneity in the use of terminology in this research field. As previously described, the ISCT proposed three minimal criteria to define MSCs, a subtype of SCs, for consistency purposes. Nevertheless, its statement also mentions that one of the main issues found is that the "MSCs" acronym has been used to refer to mesenchymal stem cells as well as mesenchymal stromal cells. To avoid confusion, the offered solution was to specify the cell source from which the cell culture was obtained, making it possible to continue using the "MSCs" acronym to describe either of the two populations without having to directly indicate the differences between them [44]. Despite this effort, there is still controversy on how to characterize and name MSCs, which hinders the search of information in databases. Consequently, given the extended use of the acronyms without mentioning the biologic origin of the cells, it is considered important to highlight the differences between both concepts. The term "mesenchymal stem cells" refers to the fraction of SCs derived from bone marrow with *in vivo* multipotent ability, and thus would be equivalent to the term "BM-MSCs," whereas "mesenchymal stromal cells" are found in tissues different from the bone marrow, also showing *in vitro* multipotent features [45], and thus encompassing, by definition, all the other tissues in which SCs can be found. Although both populations present the same proposed basic characteristics, defining the acronyms helps to restrict the search of information. Additionally, this lack of specificity creates a problem on one of the proposed identification criteria of MSCs, the surface markers, as the ISCT has only accepted a few markers to describe MSCs based on the premise that positive markers should be present in 95% of the population; however, as shown in Table 4, surface markers tend to change, and their specificity depends on the cell source or even the donor. Therefore, it is necessary to explain whether one is referring to a mesenchymal stem cell or a mesenchymal stromal cell, and if appropriate, the source of isolation.

Another factor to consider is the notorious lack of standardization and characterization of the cell cultures of mesenchymal cells, as there are several studies in which the surface markers are not verified by immunodetection techniques, but through a test of differentiation into the three main linages (osteoblasts, chondrocytes, and adipocytes), which is verified through histological staining and without confirming the homogeneity of the culture. Figure 3 shows the methodology proposed to work with the MSCs populations using the minimum markers necessary to characterize them based on their extraction source.

3.2 Limitation between TE and CT

Moreover, the search of TE applications showed that consensus on the information encompassed by the TE and CT fields and maybe even on the implant of biomaterials is still necessary probably because TE possesses the same fundamentals as CT, but it is characterized by the use of biocompatible scaffolds and bioreactors or physical and chemical stimuli that improve the differentiation of the cell cultures. This issue was evinced after the first search filter, in which the results obtained classified the exclusive use of MSC differentiated in special culture media such as TE only because of the use of a special inductor media. In this particular research, frequent use of scaffolds from different origins derived from tissues derived from MSCs, known as MSCs-derived decellularized extracellular matrices (MSCs-ECM), serve as cell support. During the publication review, some of the studies were found to classify the use of MSCs-ECM scaffolds implanted in animal models as TE [206], but no culture of MSCs was performed in the scaffold; thus, these studies were regarded as regenerative medicine therapies and were not included as part of the specific TE field.

3.3 First pillar of TE: Cell source

Furthermore, it was observed that most studies address osseous, cartilaginous, and osteochondral TE (Tables 5, 6, and 7); the last being a combination of the first two. In addition, a higher use of MSCs derived from bone marrow can be observed, as shown in the tables previously mentioned, followed by those derived from adipose tissue, and lastly those derived from umbilical cord. This is because the bone marrow possesses a better differentiation capacity, and it was also the first source from which MSCs were isolated and identified. Nonetheless, its use for cell collection is limited as it involves performing a procedure that is dangerous for the patient, which makes bone marrow a complex source for the isolation of MSCs. In contrast, the adipose tissue, which is an accessible cell source and is largely present in the organism, is the second most used

Table 7 Applications of MSCs in osteochondral tissue engineering. **Immunodetection techniques markers:** IL1 = Interleukin 1, TNF-alpha = Tumor necrosis alpha. **PCR markers:** CDH2 = Cadherin 2, ITGA5 = Integrin subunit alpha 5

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Human	BM-MSCs	Lonza	IF: COLII, SOX9, COLI, RUNX2		Alizarin-red stain		Poly (lactic-coglycolic acid) with porous gradient and hydrogel of hyaluronic acid and hydroxyapatite with BMP-2 and TGF-B1	Spatial differentiation facilitated to osteocytes and chondrocytes with the use of growth factors in the scaffold	2020	[135]
	BM-MSCs	Cyagen	IHC: COLII, GAG, COLI, OCN. ELISA: IL1, TNF-alpha	ALP, OCN, RUNX2, COL II, ACAN, SOX9, COL1	Toluidine blue	ALP activity assay	Poly hydrogel (N-acryloyl 2-glycine) and methacrylate gelatin	Increases the expression of genes related to chondrogenesis and osteogenesis in hBM-MSCs, with concurrent regeneration of cartilage and subchondral bone in rat model	2019	[136]
	UC-MSCs	Umbilical cord	FC: CD73$^+$, CD90$^+$, CD105$^+$, CD44$^+$, CD29$^+$, HLA-ABC$^+$ CD34$^-$, HLA-DR$^-$. IHC: SOX9, COLII				Hyaff-11 (FIDIA Advanced Biopolymers, Italy) o Chondro-gide (Geistlich Biomaterials, Italy S.r.l.)	The study shows the commitment of UC-MSCs seeded on the scaffold to chondrogenic differentiation after 4 weeks and to osteogenesis after 30 days	2017	[137]
	AD-MSCs	Lipoaspirate	IHC: COLII, COLI	COLI, CDH2, ITGA5	Toluidine blue	DMMB assay	Bilayer scaffold: poly-L-glutamic acid and chitosan cartilage layer and PLGA bone layer with nanohydroxyapatite/chitosan	Chondrogenic differentiation (expression of glycosaminoglycans and COL II). AD-MSCs migrated from the chondrogenic layer through the scaffold to the osteogenic zone and differentiated	2020	[138]
	AD-MSCs	Subcutaneous adipose tissue	IS: COLI	ACAN, SOX9, COL2A1	H&E stain, Masson's trichrome stain, Safranin-O/ Fast Green, toluidine blue		Decellularized extracellular matrix of bovine cartilage with Poly-ε-caprolactone nanofibers	Increased chondrogenic expression; in addition, they managed to fill osteochondral defects in vivo in rats, obtaining a long-term regeneration of cartilage	2021	[139]

Table 7 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Rat	BM-MSCs	Unspecified	IF: F-actin, SOX9, COLII, ACAN, RUNX2, OCN	ALP, OCN, RUNX2, SOX9, COLII, ACAN	Alizarin-red stain, toluidine blue and Safranin-O	ALP activity assay	Two layers: emulsions of osteogenic peptide/B-tricalcium/polyphosphate (lactic-coglycolic acid) and water in pole oil (D,L-lactic-co-trimethylene carbonate acid) in a thermosensitive cartilage frame with another layer of Cabbage I hydrogel with TGF-B1	The scaffold allowed the formation of three layers: osteogenic, subchondral, and cartilaginous; high viability and proliferation in the subchondral and cartilage layer, but also in the osteogenic layer	2020	[140]
	BM-MSCs + chondrocytes	Femur and tibia	IHC: COLI, COLII		H&E stain y Masson's trichrome stain		Chitosan bilayer scaffold with chitosan/phosphate-B-Tricalcium	Chondrocytes maintained their lineage while MSCs differentiated to the osteogenic lineage. In vivo study showed that the scaffold had characteristics similar to native tissue: hyaline cartilage and subchondral bone	2019	[141]
Rabbit	AD-MSCs	Subcutaneous adipose tissue of the neck	IHC: COLII. ELISA: GAG, COLII		H&E stain, Toluidine blue		Silk fibroin and hydroxyapatite scaffolding with a layer of calcified cartilage	In vivo model in osteochondral defects in rabbit knees, the scaffolded group with AD-MSCs had greater integrity and rigidity. There was cartilage and bone formation	2020	[142]

Table 8 Applications of MSCs in muscle tissue engineering

Source	Type of MSCs	Extraction tissue	Immunodetection techniques	PCR	Histology	Spectrometry	Scaffold	Discovery	Year	Ref.
Human	BM-MSCs	Bone marrow		ACTA2, CNN1, SMNT, PECAM1, vWF, KDR			Pectin hydrogel nanofibers with 25% and 50% oxidations	They allow the differentiation of MSCs into vascular smooth muscle cells and endothelial cells	2019	[143]
	BM-MSCs + células troncales hematopoyéticas	Lonza	IHC: CD34		H&E stain y Masson's trichrome stain		Poly-(1,8 octamethylene citrate) with small intestine submucosa	Increasing cell density with coculture allowed to increase the regeneration of bladder muscle tissue	2021	[144]
	UC-MSCs	Wharton's jelly	IS: ACTA2, α-actinin, MYH1, ACTA1	MYH1, ACTN3			Fibrin hydrogel with rapidly degrading microbeads	MSCs planted on the scaffold gave excellent cell viability and proliferation as well as good myogenic differentiation	2012	[145]
	UC-MSCs	Wharton's jelly	IHC: α-actinin, MYH1	ACTN3, MYH1			Alginate-fibrin microbeaters packed in an alginate matrix modified with Arg-Gly-Asp (RGD)	The use of UC-MSCs in the scaffold demonstrated an improvement in cell viability and myogenic differentiation	2012	[146]
	G-MSCs	Unspecified	FC: CD73+, CD105+, CD146+, CD34−, CD45−. IF: MYH2, MYOD, MYF5	MYOD1, MYF5, MYOG	H&E stain		Alginate scaffold coupled to RGD	An immunocompromised mouse model was implanted with the construct subcutaneously and the formation of muscle-like structures was observed	2016	[90]
	G-MSCs	Unspecified	FC: CD29+, CD44+, CD73+, CD90+. IF: MYOD, MYF5, PAX7. WB: MYOD, PAX7, MYF5		H&E stain y Masson's trichrome stain		Acellular extracellular matrix of porcine intestinal mucosa (SIS-ECM)	In a rat model with a myomucosal defect of the tongue, tissue regeneration was observed with the use of the construct	2017	[147]
	AD-MSCs	Abdominal adipose tissue	IHC: ACTA2		H&E stain		PLGA Triple Layer Sheet	In in vivo model of rats with subtotal bladder resection, those who received the cellularized implant with smooth muscle cells derived from AD-MSCs, the bladder was completely regenerated with a full recovery of function	2020	[148]
	AD-MSCs	Liposuction of abdominal subcutaneous fat	FC: CD29, CD44, CD73, CD90, CD105, HLA-ABC. IHC: DES, ACTA2. SMMHC. IB: ACTA2, CNN1, TGFB1		H&E stain, Masson's trichrome stain		Poly-L-lactic-co-ε-caprolactone multilayer scaffold	Biocompatibility with scaffolding. In the rat model, there was an improvement in contractibility, suggesting an improvement in bladder function. Uniform distribution between the leaves as well as differentiation of the AD-MSCs to smooth muscle	2019	[149]

Table 8 continued

Source	Type of MSCs	Extraction tissue	Immunodetection techniques	PCR	Histology	Spectrometry	Scaffold	Discovery	Year	Ref.
	Mu-MSCs	Hind limb	IS: MHC, PECAM1,COLI, alpha-Btx, laminin, SYP, NF-M, LAMA1 e integrin				Extracellular muscle matrix of lower limbs decellularized from mice in perfusion bioreactor	The scaffolds planted with Mu-MSCs showed growth and survival in vivo as well as myofiber formation and neovascularization in a model of volumetric muscle loss. Ex vivo *analysis* showed restoration of muscle strength treated	2017	[150]
	Mu-MSCs	Muscle biopsies of children	FC: CD34, CD56, 7-aminoactinominD WB: laminin, Ki67, fibronectin, PAX7, MYOD, MHC, MYH3, ACTA2, TE7, laminin,	PAX7, MYF5, MYOD, MYH1, DMD, LAMA1, B2M		Fluo-4 AM	Mouse diaphragm decellularized extracellular matrix	The cellularized scaffold was tested with cardiotoxin and the in vitro regenerative response of self-renewal and remodeling of the extracellular matrix was activated	2019	[151]
Rat	BM-MSCs	Femur and tibia	IHC: cTnI		H&E stain		Polylactic acid and polyglycolic acid scaffolding (50:50)	Adhesion to the scaffolding at 24 h. It contains normal arrangements of myofilaments, desmosomes, gap slits, and Z-lines	2019	[152]
	BM-MSCs	Femur		NKX2.5, TNNI2, MYH7			Polyvinyl alcohol (PVA), chitosan (CS) and carbon nanotube (CNT1) at different concentrations	Voltage stimulation was performed, with the PVA-CS-CNT1 scaffold being the most viable (PVA 66%, CS 25%, 8% CNT)	2019	[153]
	AD-MSCs	Inguinal region	IHC: ACTA2. IF: ACTA2		H&E stain		Poly-ε-caprolactone/chitosan scaffold	In in vivo rat model, there was smooth muscle of a strong bladder regeneration, with a large capacity and a greater number of veins	2018	[154]
	AD-MSCs	Adipose tissue of inguinal or subcutaneous region	IHC: MYOG, MYOD1, DBS. PECAM1, MHC, SYP	BDNF, NTF3, NGF	H&E stain and Masson's trichrome stain		Decellularized human amniotic membrane	In in vivo model, combined with interval-based high-intensity training, there was increased muscle regeneration and improved tissue remodeling regeneration after volumetric muscle loss in rats	2020	[155]
	Mu-MSCs	Hind limb	IHC: ACTA2, VEGF	PAX7, MYOG, FGF2 y VEGFB	H&E stain and Masson's trichrome stain		Biodegradable elastomer of urea poly (urethane ester)	Scaffolds planted with Mu-MSCs had a higher number of blood vessels and smaller residual scaffolding area and multinucleated giant cells	2017	[156]
Mouse	BM-MSCs	Femur			H&E stain, Alizarin red, Oil I, Safranin-I		Pluronic F-127	Increased the number of myofibers in regeneration and improved muscle strength in a mouse muscle contusion model	2020	[157]

Table 8 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
	BM-MSCs	Unspecified	IHC: VEGF, SFC, IL6 WB: MYOD, DES, MYOG, ACTA1, iNOS, Arginase, TGFR-2, SPP1				Gelatin and decellularized extracellular matrix of skeletal muscle	The scaffold supports survival, growth, and production of trophic factors and expression of myogenic proteins of MSCs in vitro	2019	[158]
	AD-MSCs	Abdominal adipose tissue	FISH: chromosome Y in transplanted cells. BCA Protein Assay. Mouse Cytokine Array		H&E stain		Hybrid 3D polypropylene fumarate resin (PPF) scaffolds: diethyl fumarate (DEF) with gold nanoparticles	In vivo, there was low presence of immunomodulators and increased tissue regeneration of muscle	2017	[159]
	Mu-MSCs	Neonatal stage skeletal muscle (satellite cells)	IS: TnI y CDH15	PAX 7, MYOD, ACTA2, MYH, CDH15, CD34			Polyaniline nanofibers (PANI) and polyacrylateanitrile (PAN) with graphene and graphene oxide	Cells cultured on PANI-CSA/G scaffolds had greater proliferation and differentiation than other scaffolds	2016	[160]
	Mu-MSCs	Skeletal muscle of the front and back legs (satellite cells)		PAX7, MYOD, ACTA2, MYH, CDH15, CD34			Polyacrylonitrile and polyaniline membrane	They showed greater cell proliferation and differentiation, and due to the conductivity of the material, the cells were induced to a more mature state	2016	[161]
Pig	AD-MSCs	Adipose tissue	IHC. ACTA2, elastin, CD3, CD25, c-Kit, MEF2, cTnI NKX2.5, cTnT, vWF, PECAM1, Ki67		Masson's trichrome stain, Picrosiruis red and Gallego stain		Decellularized human pericardium	In animals with scaffold in in vivo model of myocardial infarction, the area of infarction at one month was lower than the controls, and there was an improvement in cardiac function	2017	[162]

Immunodetection techniques markers: MYH1 = Myosin heavy chain 1, ACTA1 = Alpha actin 1, MYH2 = Myosin heavy chain 2, MYOD = Myoblast determination protein 1, MYF5 = Myogenic factor 5, PAX7 = Paired box 7, DES = Desmin, SMMHC = Myosin heavy chain, smooth muscle isoform, CNN1 = Calponin 1, MHC = Myosin heavy chain, muscle, alpha-Btx = Alpha-bungarotoxin, SYP = Synaptophysin, NF-M = Neurofilament medium polypeptide, LAMA1 = Laminin subunit alpha 1, Ki67 = Proliferation marker protein Ki-67, MYH3 = Myosin heavy chain 3, TE7 = Fiobroblast marker TE7, cTnI = Cardiac troponin I, DBS = Guanine nucleotide exchange factor DBS, SFC = Stem cell factor, IL6 = Interleukin 6, iNOS = Nitric oxide synthase, inducible, TGFR-2 = TGF-beta receptor type-2, TnI = Troponin I, CDH15 = Cadherin 15, c-Kit = Mast/stem cell growth factor receptor Kit, MEF2 = Myocyte-specific enhancer factor 2, cTnT = Troponin T, cardiac muscle. **PCR markers:** SMNT = Survival of motor neuron 1, telomeric, KDR = kinase insert domain receptor, ACTN3 = Actinin alpha 3, MYOD1 = Myogenic differentiation 1, MYOG = Myogenin, DMD = Dystrophin, B2M = Beta-2-microglobulin, TNNI2 = Troponin I2, fast skeletal type, MYH7 = Homo sapiens myosin heavy chain 7, BDNF = Brain derived neurotrophic factor, NTF3 = Neurotrophin 3, NGF = Nerve growth factor, FGF2 = Fibroblast growth factor 2, VEGFB = Vascular endothelial growth factor B, CD34 = CD34 molecule

Table 9 Applications of MSCs in dermal tissue engineering

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Human	BM-MSCs	Lonza	IHC: IVL and laminin	VEGF, PECAM1, ACTA2. RT-PCR: ACTB, VEGF, HIF1A	H&E stain, Masson's trichrome stain		AuFe nanoparticles with conditioned medium	Injection in mouse model with skin wound presented angiogenesis, re-epithelialization, and tissue remodeling	2019	[163]
	WJMSCs	Wharton's jelly	FC: CD73$^+$, CD90$^+$, CD105$^+$, CD14$^+$, CD20$^-$, CD34$^-$, CD45$^-$, HLA-DR$^-$, CD80$^-$, CD86$^-$. IHC: ACTA2, DES		H&E stain, Masson's trichrome stain		Silk fibroin (SF) scaffolding	In a mouse model of splinting excision wounds, the construct was implanted. Re-epithelialization and a reduced formation of scar tissue in the healing process were observed	2019	[164]
	GMSCs	Gum tissue	FC: CD90$^+$, CD105$^+$, CD73$^+$, CD34$^-$, CD45$^-$	KRT18, KRT14, KRT5, IVL, FLG, SFN			HyStem®-HP in addition to the use of Acalypha indica	The use of scaffolding and A. indica promotes epidermal differentiation of G-MSCs	2018	[165]
	AD-MSCs	Unspecified	IHC; KRT10, KRT14	KRT10, KRT14, ACTB			Silk bio-complex/hyaluronic acid modified with polyethylene glycol/chitosan/poly-ε-caprolactone (PCP)	Scaffolding with 20% HA demonstrated better interactions in adhesion, response, proliferation, and cell differentiation in the absence of growth factors	2021	[166]
Rat	BM-MSCs	Bone Marrow	IHC: KRT10, KRT12		H&E stain, Masson's trichrome stain		Silk fibroin film with polydopamine	In vitro promoted adhesion and migration of MSCs; in vivo the wound healed completely with the formation of new skin and hair 14 days after trauma, without generating significant inflammation. There was epithelialization and good organization of collagen deposition	2019	[167]
	AD-MSCs	Abdominal adipose tissue	IHC: PECAM1		H&E stain, Masson's trichrome stain		Alginate hydrogel	Improved wound healing in vivo, collagen synthesis and angiogenesis in the affected area	2019	[168]

Table 9 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Mouse	BM-MSCs	Bone marrow	IF: ACTA2, VEGF y TGFB1		H&E stain, Masson's trichrome stain		Graphene oxide nanoparticles with a dermal acellular matrix	Diabetic murine model of total thickness skin wound in the back, where there was angiogenesis and collagen deposition as well as rapid re-epithelialization	2019	[169]
	AD-MSCs	Subcutaneous adipose tissue	IHC: GFP IF: PECAM1	VEFGA, PDGFRB, FGF2, HGF, TGFB1, GAPDH	Masson's trichrome stain		Glycosaminoglycan liquid collagen scaffold (GAG)	In vivo increased capillary formation, collagen content, epidermal thickness, and expression of essential growth factors	2019	[169]
Miniature pigs	BM-MSCs	Bone marrow	IHC: ACTA2		H&E stain, picrosirius red		Electrolyzing nanofibers of poly-ε-caprolactone and polyvinyl alcohol (PVA)	The scaffold supports proliferation of MSCs, human fibroblasts, and keratinocytes. Improved wound healing in in vivo model	2019	[170]
Yorkshire Pigs	AD-MSCs	Subcutaneous fat	IHC: ACTA2, PECAM1, VEGFA. WB: PECAM1, VEGFA		Picrosiruis red	Hidroxiproline assay	Pegilated Fibrin Hydrogel (FPEG)	In porcine burn model, scaffold with AD-MSCs showed increased angiogenesis and avoided contraction of adjacent tissue, minimizing scarring	2018	[171]
Rabbit	AD-MSCs + mouse skin fibroblasts	Subcutaneous fat			AM Calcein		Extracellular matrix gel derived from culture of stem cells derived from adipose tissue	Minimal cytotoxicity, the scaffold showed high concentration of proteins such as collagen, fibronectin, biglicane, TGF-β	2020	[172]

Immunodetection techniques markers: IVL = Involucrin, KRT10 = Keratin 10, KRT14 = Keratin 14, KRT12 = Keratin 12, GFP = Green fluorescent protein. **PCR markers:** KRT18 = Keratin 18, KRT5 = Keratin 5, FLG = Filaggrin, SFN = Stratifin, PDGFRB = Platelet derived growth factor receptor beta, HGF = Hepatocyte growth factor, GAPDH = Glyceraldehyde-3-phosphate dehydrogenase

Table 10 Applications of MSCs in neural tissue engineering

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Human	BM-MSCs	Unspecified	ICC: MAP2	MAP2, B2M			Electrolyzed nanofibers of polyvinyl alcohol and sulfated alginate	Biocompatible scaffolds that support cell proliferation and neurogenesis of hBM-MSCs in cell culture without using growth factors. Neuroinductive effects without addition of materials for differentiation. They also support the growth of Schwann cells	2020	[173]
	UC-MSCs	umbilical cordon	IF: vWF, MBP, NF-M, MAP2, Syn		Masson's trichrome stain		Silk collagen and fibroin scaffolding	The construct was implanted in a hemiplegic model of traumatic brain injury in canines and it was seen that in addition to repairing the anatomical structure of the lesions, it also restores the gait of the extremities after a brain injury due to trauma	2021	[174]
	DPSCs	Third molar	FC: CD73+, CD90+, CD166+, CD14−, CD19−, HLA-DR−; IF: Nes, NeuN, GFAP, TUBB3; WB: Bcl2, Bax, CASP3, MBP, GAP-43		H&E stain		Thermosensitive hydrogel heparin-poloxamer	Rat model to which the construct was implanted in addition to fibroblast growth factor (bFGF) promoting neuronal regeneration	2018	[175]
	AD-MSCs	Lipoaspirate from abdominal fat	FC: CD73 CD105. IF: Nes, Ki67, SOX2, CD105, CD73. WB: Nes y SOX2				Decellularized dermal matrix	Neuronal differentiation by the expression of nestin and SOX2 in scaffold	2019	[176]
Rat	BM-MSCs	Femur and tibia	IS: GFP, NF-H, CASP3, NeuN, TUBB3, GFAP, Claudin-11	CASP3, CSPG4, MMP2, NEFH, VEGFA, GAPDH	H&E stain, NeuN		Polypyrrole/polylactic acid nanofibers	Recovery of the spine injury model with a decrease in scar tissue compared with the control group. Abundant NF and NeuN in stains as well as myelination	2019	[177]
	AD-MSCs	Perirenal adipose tissue	IHC: CD105, CD90, CD49d, CD31, CD106, CD45, Nes, NF-L, SOX2, Islet-1, HB9, ChAT, SYP		FM1-43 and RH 795 stain		BD™PurMatriz™ peptide hydrogel (BD Biosciences)	Increased expression of Islet1 (immature) in 3D cultures. HB9, ChAT, and synaptophysin increased in 2D (mature); therefore, 3D demonstrates more proliferation for neuron progenitor cells	2020	[178]

Table 10 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers				Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology	Spectrometry				
Mouse	BM-MSCs	Cell Bank, Chinese Academy of Sciences, China	WB: NSE, DCX, MAP2, BDNF, NTF3, Bax, Bcl-2	MAP2, ENO2, DCX, BDNF, NGF, NTF3	H&E stain		Chitosan hydrogel, hydroxyethyl cellulose, collagen and B-phosphoglycerate	In a mouse model of spinal cord injury, the hydrogel had a thermosensitive behavior similar to neural tissue with biocompatibility. Promoted the recovery of motor function of the hind limbs probably by suppression of apoptosis and increased neurotrophy of nerve cells	2020	[179]

Immunodetection techniques markers: MAP2 = Microtubule associated protein 2, MBP = Myelin basic protein, Syn = Synapsine, Nes = Nestin, NeuN = RNA binding protein fox-1 homolog 3, GFAP = Glial fibrillary acidic protein, TUBB3 = Tubulin beta-3 chain, Bcl-2 = Apoptosis regulator Bcl-2, Bax = Apoptosis regulator BAX, CASP3 = Caspase 3, GAP-43 = Neuromodulin, NF-H = Neurofilament heavy polypeptide, NF-L = Neurofilament light polypeptide, Islet-1 = Insulin gene enhancer protein ISL-1, HB9 = Motor neuron and pancreas homeobox protein 1, ChAT = Choline acetyltransferase, NSE = Enolase, DCX = Doublecortin, B2M = Beta-2-microglobulin, CSPG4 = Chondroitin sulfate proteoglycan 4, MMP2 = Matrix metallopeptidase 2, NEFH = Neurofilament heavy chain, ENO2 = Enolase 2. **PCR markers:**

Table 11 Applications of MSCs in cardiac tissue engineering

| Source	Type of MSCs	Extraction tissue	Type of analysis and markers			Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology				
Human	BM-MSCs	Unspecified	FC: CD73$^+$, CD90$^+$, CD105$^+$, CD11b$^-$, CD14$^-$, CD19$^-$, CD34$^-$ CD45- y HLA-DR2$^-$; IHC: MYH3, cTnT	ACTA2, BMP4, CCL5, COL1A1, CCN2, FN1, HGF, IDO1, IL6, IL8, IL10, LIF, NFKBIA, PDGFA, PTGS2, TNFAIP6, VEGF		Collagen scaffold	The recovery of cardiac function may not be due to the direct contractional contribution of the cells but to the fact that other cellular pathways that promote the repair process by the MSCs can be activated	2017	[180]
	BM-MSCs	Unspecified	IF: CD90, GATA4, cTnT, cTnI, Cx43, MHC, ACTA2			Mat of type I collagen nanofibers, oriented at random	Cells cultured in scaffolds show a time-dependent evolution of cardiomyogenesis, an improvement in the expression of cardiac markers and a decrease in cell stem markers	2018	[181]
	hMSCs	PT-2501; Lonza	IF: cTnT, ACTA2, PECAM1			Poly (glycerol sebacate)/gelatin fibers	The scaffold has the mechanical strength to support the MSCs seeded as well as ability of differentiation into cardiomyocytes to repair the myocardium and improve cardiac function. It is shown to be biocompatible and have mechanical properties for myocardial restoration	2011	[182]
	hMSCs	PT-2501; Lonza	IF: ACTA2, TnT, CD44			Poly(ε-caprolactone)-gelatin nanofibers coupled with VEGF	The incorporation of VEGF showed an improvement in the proliferation of MSCs as well as an increase in the expression of specific cardiac proteins	2017	[183]
	hMSCs	PT-2501; Lonza		GATA4, MEF2C, NKX2.5, CACNA1C		Elastic polyurethane nanofibers	Construct stimulates cardiac differentiation of MSCs	2011	[184]
	hMSCs + cardiomyocytes	PT-2501; Lonza	IF: actinin			Polycaprolactone scaffolds with gold nanoparticles	An increase in proliferation and differentiation of MSCs with gold nanoparticles on the scaffold was shown	2015	[185]
	UCB-MSCs	Umbilical cord blood	FC: ACTA2, Tn, Tm, ANP			Fibrin/PLGA-based electrolyzing scaffolding	The scaffold supports cell anchorage, viability, and proliferation as well as provides a favorable environment for the differentiation of cells to a cardiac phenotype	2013	[186]
	UC-MSCs	umbilical cord	IHC: PECAM1		H&E stain	Decellularized umbilical artery	Scaffold seeded with cells showed an improvement in cardiac function in a rat MI model	2017	[95]
	WJ-MSCs	Wharton's jelly	FC: CD73$^+$, CD90$^+$, CD105$^+$, CD31, CD45$^-$ y HLA-DR$^-$			PeriCord, decellularized pericardial matrix	PeriCord was tested in a first implantation in humans, and magnetic resonance imaging after 3 months showed a reduction of ~ 9% of scar mass in the treated area	2020	[97]
	UC-MSCs + cardiomyocytes	umbilical cord	IF: ACTA2, cTnI			Graphene oxide/alginate microgel	Implanted scaffolding in rats showed decreased infarcted area and improved cardiac function	2019	[187]
	WJ-MSCs	Wharton's jelly	IHC: PECAM1, ACTA2, MHC	MYH6, MYH7, TNNT2 y NKX2.5		Polyethylene glycol, hyaluronic acid and chitosan	hWJ-MSCs were mixed with the scaffold and injected into rabbits with MI. Angiogenesis and cardiogenesis were observed	2017	[188]

Table 11 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers		Histology	Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR					
Rat	BM-MSCs	Tibia	FC: CD44$^+$, CD90$^+$, CD34$^-$, CD45$^-$ IHC: DES y ACTA2			Collagen-I scaffold	MSCs patch can promote reverse remodeling of the infarcted area	2012	[189]
	BM-MSCs	Unspecified			Sirius red stain	Alginate and chitosan anti-charge polyelectrolyte complexes	The scaffolds with the cells were implanted in rats with MI. Increase in ejection fraction, improvement in neovascularization, and attenuation of fibrosis were seen	2014	[190]
	BM-MSCs	Unspecified	FC: CD90$^+$, CD29$^+$, CD44$^-$, CD106$^-$, CD25$^-$, CD11b$^-$, CD45$^-$ IHC: DES, cTnT			Decellularized cardiac tissue	MSCs isolated from rats can be used to generate bioartificial hearts along with isolated endothelial and cardiac cells on a decellularized cardiac scaffold	2019	[191]
	BM-MSCs	Unspecified	IF: PECAM, cTnT, Cx43		H&E stain, Masson's trichrome stain	Poly(ε-caprolactone/gelatin) nanofibers (PG)	In a rat model with myocardial infarction, the construct was implanted and demonstrated to provide mechanical support to induce angiogenesis and accelerate cardiac repair	2014	[192]
	AD-MSCs	Cervical adipose tissue	IHC: DES, CD68, CD45, CD34		H&E stain, Masson's trichrome stain y Sirius red	Acellular pericardium	MI was induced in rats and the patch was implanted and neovascularization was observed	2018	[193]
	AD-MSCs	Edpididymis	IHC: ACTA2, CD31$^+$, Sca-1, CD117			Platelet-rich fibrin (PRF)	AD-MSCs grafted into PRF implanted in rats with MI has a benefit in preserving left ventricle function	2015	[194]
Mouse	BM-MSCs	Femur and tibia	FC: CD34$^-$, CD45$^-$, CD90$^+$ y CD73$^+$ IF: Cx43, cTnT			Polyurethane, 3-hydroxybutyrate-co-4-hydroxybutyrate (P(3HB-co-4HB)) or polypropylene carbonate	Cells were more abundant in polyurethane and p(3HB-co-4HB)	2013	[195]
	AD-MSCs	Unspecified	FC: CD44$^+$, CD90$^+$, CD34$^-$, CD45$^-$ IHC: PECAM1		Masson's trichrome stain	Patches of cellulose nanofibers modified with chitosan/silk fibroin	Patches with AD-MSCs were implanted in rats with MI which demonstrated survival of grafted cells as well as attenuation of myocardial fibrosis	2018	[96]
Pig	AD-MSCs	Unspecified			Movat stain, Galician stain	A myocardial decellularized scaffold and a pericardial scaffold	Scaffolds with the cells were implanted in pigs and both grafts showed a recovery of ventricular function; however, the pericardial scaffold showed greater structural integrity as well as greater penetration and retention of cells	2018	[196]

Table 11 continued

Source	Type of MSCs	Extraction tissue	Type of analysis and markers			Scaffold	Discovery	Year	Ref.
			Immunodetection techniques	PCR	Histology				
Rabbit	AD-MSCs	Adipose tissue of the nape	IHC: DES, CD34		H&E stain, Masson's trichrome stain	Decellularized pericardium	The scaffold with the cells was implanted in rabbits with MI and was shown to improve the contractile function of the left ventricle	2017	[197]

Immunodetection techniques markers: GATA4 = GATA binding protein 4, Cx43 = Gap junction alpha-1 protein, TnT = Troponin T, Tn = Troponin, Tm = Tropomyosin, ANP = Atrial natriuretic peptide, Sca-1 = Complex scaffold protein scaA, CD117 = Mast/stem cell growth factor receptor Kit. **PCR markers:** BMP4 = Homo sapiens bone morphogenetic protein 4 (BMP4), transcript variant 1, CCL5 = Homo sapiens C–C motif chemokine ligand 5 (CCL5), transcript variant 2, CCN2 = Homo sapiens cellular communication network factor 2, FN1 = Fibronectin 1, IDO1 = Indoleamine 2,3-dioxygenase 1, IL8 = C-X-C motif chemokine ligand 8, IL10 = Interleukin 10, LIF = LIF interleukin 6 family cytokine, NFKBIA = , NFKB inhibitor alpha PDGFA = Platelet derived growth factor subunit A, PTGS2 = Prostaglandin-endoperoxide synthase 2, TNFAIP6 = TNF alpha induced protein 6, MEF2C = Myocyte enhancer factor 2C, CACNA1C = Calcium voltage-gated channel subunit alpha1 C, MYH6 = Myosin heavy chain 6, TNNT2 = , Troponin T2, cardiac type
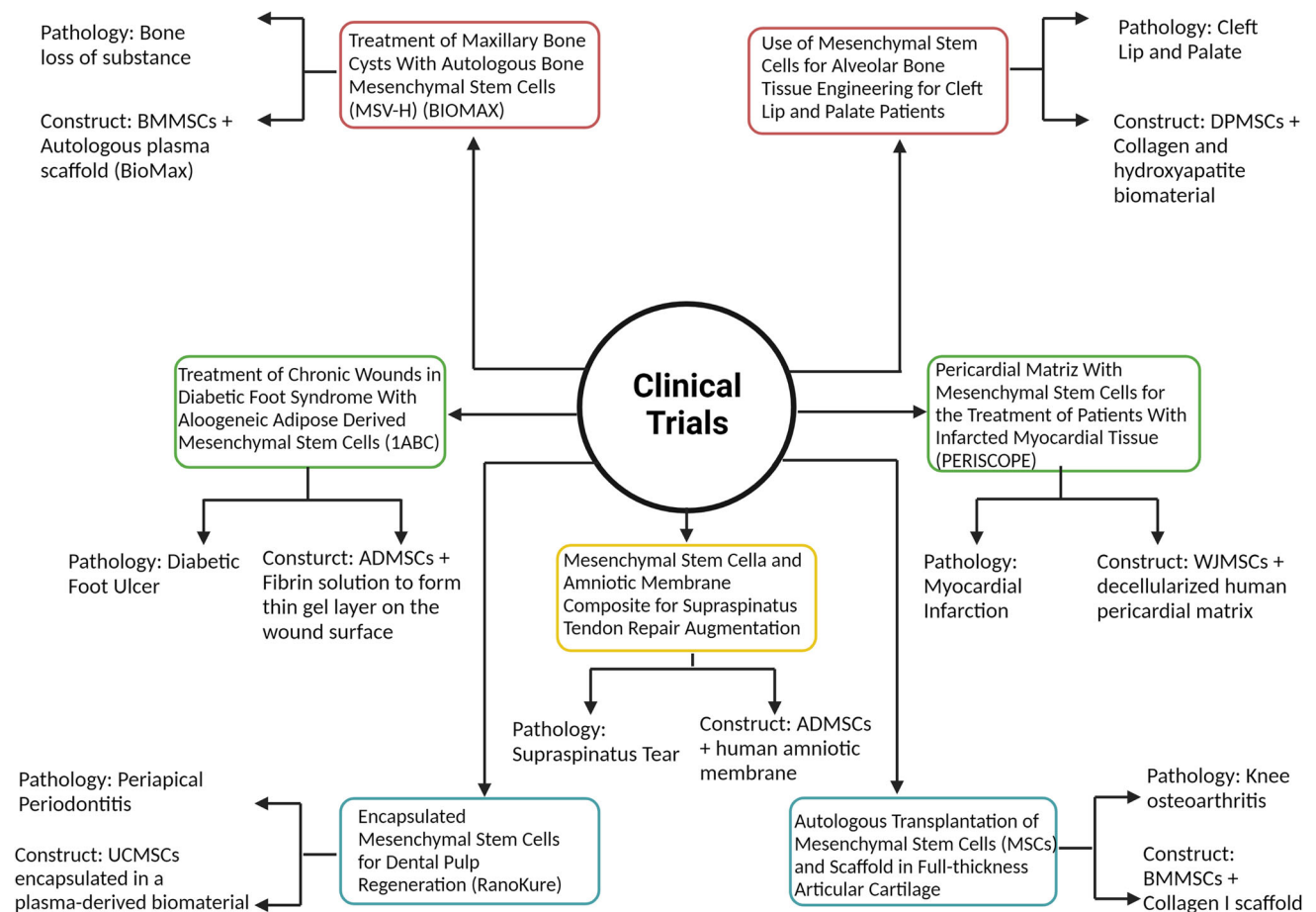
Fig. 2 Clinical trials from the last ten years in TE using MSCs as the principal cell source for different pathologies

tissue for the collection of MSCs and has the highest extraction yield of MSCs; thus, it represents an autologous transplant alternative for patients (Table 4).

In the case of the umbilical cord, although it represents an easily accessible source and the MSCs are closer to the first stages of development, presenting more primitive properties [207], there is not much research on this subject because it is not a common practice to save this type of extraembryonic tissue at the moment of birth, particularly owing to the associated costs, and the genetic and chromosomal studies required to verify that the donor (the neonate) will develop normally [208].

3.4 Second pillar of TE: Biomaterials

In accordance with TE's second pillar, including biomaterials, it can be observed that the use of scaffold synthesized using natural biomaterials or of those with a combination of natural and synthetic biomaterials, predominates in most studies, with the synthetic biomaterials the least used ones. Of the total 112 studies analyzed (Tables 5–11), 41.96% used natural biomaterials, 32.14%

employed a combination of natural and synthetic biomaterials, and only 18.75% used exclusively synthetic biomaterials for the construction of scaffolds. This is because the use of natural or combined materials allows us to better mimic the natural environment of cells, favoring their differentiation and adhesion to the scaffold. The five biomaterials most widely used are collagen, chitosan, gelatin, hydroxyapatite, and silk fibroin; however, alginate, HA, and fibrin, among others, are also employed. Decellularized extracellular matrices have also been reported as scaffolds for the seeding of MSCs [111, 122, 127] as well as the use of other compounds, such as growth factors or functionalization with nanoparticles to improve the scaffolds' functionality [183, 209].

3.5 Third pillar of TE: Culture stimulation

Finally, the last pillar addressed by TE, external stimuli are applied to improve cellular growth and differentiation, which constitutes an important aspect to successfully mimic the tissue of interest through the use of bioreactors. The use of bioreactors has been reported in the different
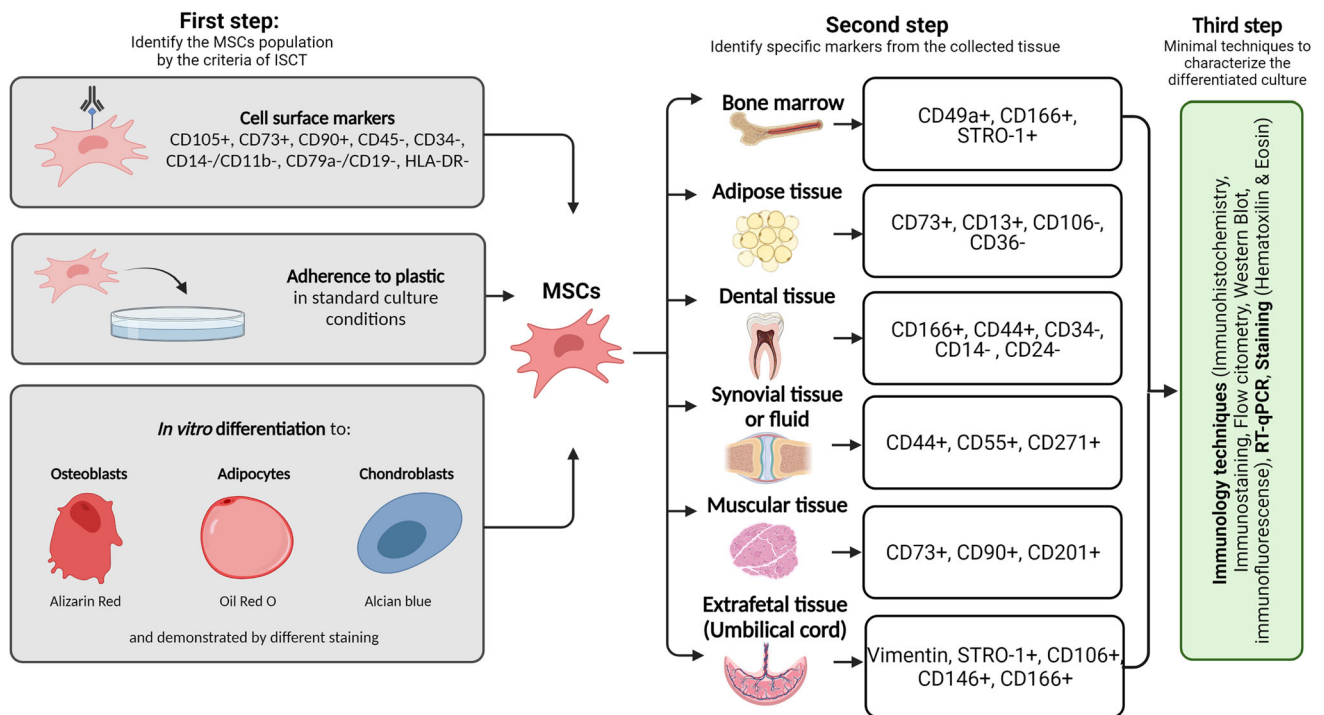
Fig. 3 Methodology proposed to characterize mesenchymal and stromal cell cultures based on their site of collection (specific surface markers according to the literature) and the three minimum general techniques to characterize MSCs after differentiation into the target cell population

applications discussed in this revision, either with scaffolds or cell cultures only; the most relevant studies are discussed in the following text. Quarta et al. (2017) employed Mu-MSCs and other muscle-resident cells and a perfusion bioreactor as an effective treatment for muscle volume loss, which enhanced the efficacy of the Mu-MSCs in the constructs [96]; Sulaiman et al. (2020) observed that the culture of BM-MSCs in gelatin microspheres in a dynamic reactor increased the differentiation of chondrocytes *in vitro*, along with cell proliferation as well as RNA and protein expression when compared with the static cultures [97]. Moreover, Xu et al. (2019) reported that the use of a bioreactor in a 3D culture of human BM-MSCs with alginate and gelatin microspheres presented higher proliferation than 2D cultures using only alginate or alginate and gelatin for the generation of cartilage [210]. In osseous TE, Zhang et al. *(*2021*)* demonstrated that the mechanical stimulation of human UC-MSCs cultured in a porous hydroxyapatite scaffold that was created using a bioreactor led to the modulation of the inflammatory response and osteogenic differentiation [211]. Finally, Agabalyan et al. (2017) used dermal SCs (DSCs) from hair follicles in parallel DASGIP (Eppendorf) reactors to produce skin-derived precursors and regenerate the dermis and reported that the expansion in the bioreactor cultures was five times higher than that of the static cultures [212]. Although the use of bioreactors is associated with better differentiation

results and larger cultures, it is used in a reduced percentage of the applications of MSCs in TE.

For the MSCs to be stable during the culture in bioreactors, they have to be taken care of and stimulated right from the moment of isolation from the tissue to increase their survival, differentiation efficiency, and homing to the damaged tissue [213]. Growth factors are important for this conditioning because during this phase, the use of biomaterials avoids the degradation of these inductor molecules, maintains their stability and bioactivity, and ensures the release of a low and continuous concentration of these factors without causing toxic damage to the organism [214].

4 Conclusion

MSCs can be considered the main cell source in regenerative medicine and TE as they are easy to obtain (multiple cell sources) and possess multipotentiality; especially AD-MSCs, which are obtained through procedures that are safer and less painful for the patient, present a high cell yield, facilitating their application in therapy.

As a perspective, this review proposes the need to homogenize the terminologies related to regenerative medicine, including the concepts of CT and TE as well as the use of the acronym "MSCs" to facilitate bibliographic search. In addition, it is necessary that the markers–

morphology–differentiation correlation proposed by the ISCT becomes a basic requirement to characterize MSCs and achieve the homogenization goal. Furthermore, standardization for the selection of tissue-specific markers that allow a more specific cell characterization is necessary.

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Declarations

Conflict of interest The authors have no potential conflicts of interest with respect to the research, authorship, and / or publication of this article.

Ethical statement We do not report ethical issues for human and animal right.

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