Mesenchymal Stromal Cells: Past, Present, and Future

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Adult mesenchymal stromal cells are plastic-adherent cells that are self-renewing and have the capacity to differentiate into various tissue specific lineages. Stromal cells were initially discovered over 100 years ago and substantial insight into stromal cell identification, isolation, characterization, and differentiation has been made, including efforts to elucidate the factors involved in stromal cell differentiation. Stromal cells have immune privilege and thus are attractive candidates for tissue engineering and regenerative medicine applications. Positive results from a number of recent investigations support the use of adult mesenchymal stromal cells for clinical application. This review article provides a brief overview of past, present, and future stromal cell technology.

For stem cell technology, recent investigative focus has been on therapeutic and regenerative stem cell applications. Whereas stem cell applications appear to have substantial promise based on experimental results, studies to confirm repeatability and safety are necessary to support transition from basic research to clinical application. Adult mesenchymal stem cells are undifferentiated cells that are capable of self-renewal and are responsible for adult tissue regeneration.[1] They have the potential to generate various tissue cells in vitro and respond in vivo to biochemical and mechanical stimuli alone or in combination.[2–4] Stem cells have 3 principal characteristics: (1) they are capable of self-renewal, (2) are unspecialized cells, and (3) can give rise to other specialized cell types. Mesenchymal stromal cells (MSC) are cells derived from mesenchymal tissues and they adhere to plastic. These cells are isolated from various tissues including adipose tissue and bone marrow and can differentiate into various tissue lineages (Fig 1).[4,5] There is considerable controversy over whether cells derived from adult tissues are stem cells or stromal cells. For purposes of this review, the acronym MSC refers to mesenchymal stromal cells based upon the current nomenclature guidelines presented below.

STEM CELL HIERARCHY

Stem cells have varying degrees of differentiation potential, which can be described with a hierarchical model (Fig 2).[6]

The fertilized oocyte or zygote is at the base of the model. Zygotes are totipotent because they have the ability to form all tissues, the embryo itself as well as the placental trophoblasts. Embryonic stem (ES) cells are the next level and are pluripotent cells because they can differentiate into almost any type of cell that arises from the 3 germ layers. Next, are induced pluripotent stem (iPS) cells. These are adult somatic cells induced to express pluripotent genes.[7] The next level are multipotent cells such as bone marrow-derived stromal cells (BMSC) and adipose tissue-derived stromal cells (ASC). These cells are diversely distributed and have been found and isolated from various tissues including bone marrow, adipose tissue, muscle, liver, brain, umbilical cord blood, Wharton's jelly from the umbilical cord, placenta, peripheral blood, and pancreas.[8–16] These cells are capable of differentiating into diverse lineages such as cartilage, muscle, and neuronal-like cells.[15,17] At the apex of the hierarchy are unipotent cells that are only capable of producing their own cell type.[18] Glial cells are an example of unipotent cells.

To circumvent the use of controversial ES cells, efforts are underway to identify comparable alternatives. The recently described iPS cells may represent a promising option. Initially reported in 2007, iPS cells are generated by transfecting adult somatic cells with the pluripotent transcription factors; Oct3/4, c-Myc, Sox-2, and Klf4.[7] Takahashi et al[7] reported that human iPS cells were identical to ES cells in morphology, proliferation, gene expression, in vitro differentiation, and teratoma formation. Potential iPS cell therapies have been reported. In a humanized transgenic mouse model of sickle cell anemia[19] treatment with iPS hematopoietic cells significantly improved all
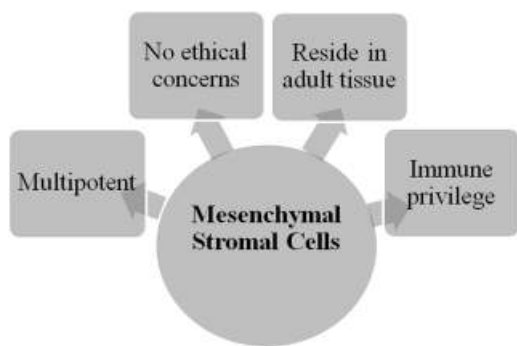
Figure 1 Characteristics of mesenchymal stromal cells. Schematic illustration of mesenchymal stromal cell characteristics.

hematologic and systemic signs of sickle cell anemia in the mice. Human iPS cells differentiated into pigmented retina epithelium facilitated short-term maintenance of photoreceptors in a murine dystrophic retina model.[20] Long-term visual function was maintained in the model despite eventual loss of the xenograft cells. Although additional studies are needed to obviate the use of viruses to generate iPS cells, the potential of iPS cells is relatively untapped.

ADULT STEM CELLS/STROMAL CELLS

The discovery of stromal cells dates to the 19th century studies of Julius Friedrich Cohnheim, but most work with stromal cells has occurred over the past few decades.[21,22] Bone marrow stromal cells were first isolated and described in studies by Alexander Friedenstein in the late 1960s.[23] In 1970, Friedenstein isolated bone marrow-derived fibroblast-like cells based on their adherence to plastic.[24] Additional studies by Friedenstein[25,26] and Owen[26,27] demonstrated the osteogenic and adipogenic potential of stromal cells from
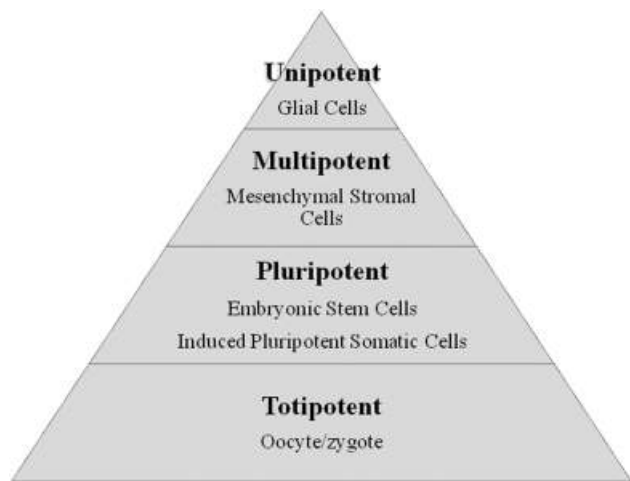

Figure 2 Stem cell differentiation potential. Stem cells have varying degrees of differentiation potential illustrated here as a hierarchy.

bone marrow. Friedenstein also found that guinea pig and rabbit thymus cells could be induced to differentiate into bone by transitional epithelium and decalcified bone matrix, respectively.[28] Marshall Urist showed that demineralized bone or its extracts induced differentiation of mesenchymal-type perivascular cells into cartilage and bone formation when implanted into subcutaneous or intramuscular sites in humans and animal models.[29,30] Similar work was performed independently by Reddi and Huggins.[31] This combined work confirmed the existence of multipotent progenitor cells.

Caplan and colleagues reported the dissociation of embryonic stage 24 chick limb bud mesenchymal progenitor cells and their subsequent differentiation into bone, cartilage, muscle, and other mesenchymal tissues in the 1970s.[32–34] During the early 1980s, Caplan and colleagues discovered that demineralized adult bone contains chemotactic factors that stimulate mesenchymal cells to undergo de novo endochondral ossification. They used in vitro assays with embryonic chick limb bud mesenchymal cells to detect bioactive molecules from demineralized bone matrix.[35–41] They established that the chondrogenic-stimulating activity of demineralized bone was mediated by a 31 kDa protein now known as a heterodimer of bone morphogenic proteins (BMPs).[2] Additional work in the 1980s further validated their findings when cells from bone marrow were used experimentally to accelerate spinal fusion and repair large bone defects in human models.[42] Independent lines of investigation led to the isolation of multiple stromal cell lines capable of supporting the growth and differentiation of hematopoietic stem cells (HSC). Most of the "nurse" cells proved to be multipotent, with the ability to differentiate along adipogenic and osteogenic lineages.[43] The conclusion from these studies was that bone marrow contains a population of cells that contributes to repair of bone defects. These landmark investigations launched the foundation for stromal cell research.

NOMENCLATURE

Stem cell nomenclature has changed several times since its introduction. Caplan[44] popularized the term mesenchymal stem cell in the early 1990s but some investigators have since chosen to omit the stem cell reference when publishing preclinical[45,46] or clinical[47–49] studies using MSC. During the 2000 Annual Meeting of the International Society for Cellular Therapy (ISCT), the Mesenchymal and Tissue Stem Cell Committee concluded that there was inadequate evidence to support the stemness of unfractionated plastic-adhering cells and sought to clarify and standardize the nomenclature related to continued use of the MSC acronym.[50] It was proposed that plastic-adherent cells currently described as MSC be called multipotent MSC.[5] Further, the term mesenchymal stem cell should be used only for cells that demonstrate stem cell activity by standardized criteria (listed below under "Stromal cell characterization");

however, the acronym MSC can be used for either cell type as long as the meaning is clearly defined.

The ISCT reasoning for standardizing terminology is first, to maintain continuity in scientific literature to minimize confusion. Second, it has been demonstrated that the plastic-adherent marrow cells represent a heterogeneous population. There is currently insufficient data to support that unfractionated plastic-adherent marrow cells are stem cells, hence, the Committee recommended that the term multipotent MSC be used for those cells. Third, it is necessary to maintain a uniform consensus between biomedical disciplines. Fourth, current studies suggest that the cells originate from mesenchymal tissue and terminology should designate the cell origin. Lastly, the cells are found within the stromal compartments of native tissue. Thus, the Committee considered that the unfractionated cell population should be termed stromal cells regardless of the tissue source to avoid reference to biologic or therapeutic potential.

MSC are referred to by their tissue of origin. Diverse terminology has been used for stromal cells isolated from bone marrow and adipose tissue. Bone marrow cells are referred to as bone marrow stromal stem cells,[51] marrow stromal cells,[52] and marrow-isolated adult multipotent inducible cells.[53] Commonly used terms for cells derived from adipose tissue include ASC, adipose-derived adult stem cell,[54] adipose stem cell, adipose stromal cell, adipose mesenchymal stem cell, and preadipocyte, among others.[55,56] The term adipose-derived stem cell or ASC was recommended for plastic adherent cells isolated from adipose tissue at the 2004 Second Annual International Fat Applied Technology Society meeting.[56]

STROMAL CELL CHARACTERIZATION

Work to characterize stromal cells dates back over 30 years. To identify MSCs in vitro, cells must have 3 specific characteristics.[21,55,57,58] The first characteristic is the ability of cultured MSC to readily adhere to plastic culture dishes and form fibroblast-like colonies,[59] hence the term colony forming unit-fibroblastic is applied to cultured MSC that are not induced into specific lineages.[59,60] Immunohistochemical analysis of fibroblast-like cells show positive staining for alkaline phosphatase, collagen III, and fibronectin.[59] The second characteristic is that the cells have the capacity to differentiate into various specialized cell lineages. MSC have been induced into adipogenic, chondrogenic, osteogenic, myogenic, and neurogenic-like lineages among others (Fig 3).[15,17,61] The third characteristic is expression of defined cell surface marker profiles. Cluster of differentiation (CD)[62] is a nomenclature system used to aid in the identification and classification of cell surface markers (surface antigens). Surface antigens allow for rapid identification of a cell population.[21] Cell surface markers on cultured MSC have been identified using immunohistochemistry and flow cytometry. The ISCT proposed that specific markers must be expressed to identify MSC.[21] The required positively expressed markers are CD73, CD90, and CD105. The cells
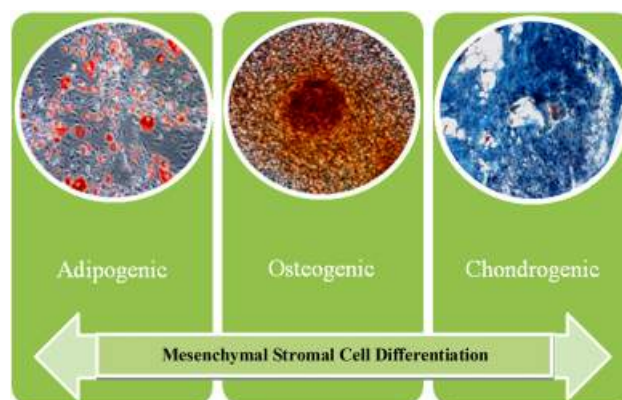

Figure 3 Photomicrographs of mesenchymal stromal cell differentiation. Mesenchymal stromal cells can be induced into adipogenic, osteogenic, and chondrogenic lineages, among others. (A) Canine adipose-derived stromal cell (ASC) adipogenesis. Lipid droplets are stained with Oil Red O. (B) Canine bone marrow-derived stromal cell (BMSC) osteogenesis. A bone nodule is stained with Alizarin Red. (C) Canine ASC chondrogenesis. Collagen fibers are evident in a chondrogenic pellet stained with Masson's Trichrome.

lack the markers CD11b and CD14 (monocytes and macrophages), CD34 (primitive HSCs and endothelial cells), CD45 (pan-leukocytes), CD79α or CD19 (B cells), and HLA-DR. Cultured MSCs are also identified by a number of other surface markers including positive expression of CD13, CD29, CD144, CD166, and HLA-ABC and lack of expression of CD3 (T-cell receptor complex component), CD31 (neutrophil removal), CD117 (HSCs), and CD62L (leukocytes).[63–72]

Cell surface marker expression can vary because of different isolation techniques and as a function of culture time.[73,74] There is also variation associated with species, cell origin (embryonic or adult), and site of origin. Zuk et al[75] demonstrated that cell surface markers differ between ASC and BMSC. ASC were positive for CD49d (α4 integrin) and vascular cell adhesion molecule or CD106, and there was no CD49d expression on BMSC. Pericytic markers like CD146 (Muc18) have been identified on the surface of ASC and BMSC with flow cytometry.[65,76–78] To date, no single marker or set of markers has been determined to permit the unequivocal isolation and purification of MSC.[4]

Extensive research has been dedicated to isolation and phenotypic characterization of MSC in a number of species including cattle[79]; dogs[80,81]; horses[82]; cats[83]; people [15]; non-human primates[84]; mice[85] and rats.[86] Primary BMSC have a heterogeneous morphologic phenotype of spindle-shaped cells and distinct colonies. After expansion, the phenotype becomes a homogenous population of spindle-shaped cells. Several species have morphologic differences between ASC and BMSC. Canine and equine ASC and BMSC share some similar morphologic phenotypes, but there are differences among cell passages.[87,88] Morphologically, primary ASC form a more homogeneous spindle-shaped population of cells. This homogenous spindle-shaped morphology is

retained through expansion. Efforts to characterize the morphologic and cytochemical properties of MSCs are on-going.

IMMUNOGENICITY OF ADULT STROMAL CELLS

Adult stromal cells are an alternative to ES cells because they are ethically and socially acceptable and have immune privilege. The immunosuppressive profiles of ASC and BMSC are well described.[89–93] BMSC are immune privileged or even immunosuppressive.[94,95] Le Blanc et al[95] conducted extensive research on human MSC immunologic properties, and one of their first studies was to determine the effect of MSC on lymphocyte proliferation in mixed lymphocyte culture (MLC). Cell proliferation was suppressed after the addition of 10,000–40,000 MSC, but low cell doses stimulated lymphocyte proliferation. In another study, osteogenic, adipogenic, and chondrogenic differentiated MSC had no lymphocyte alloreactivity and had greatest inhibition in MLC after osteogenic differentiation.[94] Based on these investigations, it appears that differentiated and undifferentiated MSCs do not elicit alloreactive lymphocyte proliferative responses and, in fact, modulate immune responses.

Analysis of MHC II molecules and costimulatory molecules showed that ASC have a low immunogenic profile after extended culture time.[90–92] Lopez et al[96] evaluated the use of allogeneic (different individuals but same species) and syngeneic (genetically identical) ASC in a rat spinal fusion model. Adult rat adipose-derived stem cells loaded onto bioabsorbable scaffolds were implanted to promote lumbar spinal fusion.[96] There was no difference between allogeneic and syngeneic ASCs to promote spinal fusion, and both were superior to scaffold alone. A parallel immune study showed that there was a noncytotoxic humoral response to fetal bovine serum and no T-cell response to allogeneic or syngeneic ASC.[97] The results from these studies highlight the use of allogeneic and syngeneic adult stromal cells on biocompatible scaffolds for therapeutic or regenerative purposes and also further confirm the immune privilege of MSCs. Moreover, the immunosuppressive properties of MSC may potentially reduce the incidence of graft-versus-host disease after allogeneic transplantation.

STROMAL CELL MICROENVIRONMENT

Specific cellular and matrix interactions are necessary for stromal cells to proliferate and differentiate.[4] The concept of a stromal cell niche (microenvironment) introduced by Schofield in 1978 and expanded upon by Weiss, Kincade, Weismann, and others has gained wide support.[67,98–101] The niche is comprised of all the elements including adjacent nonstromal cells, the local extracellular matrix (ECM), and soluble molecules, immediately surrounding the naïve stromal cells.[67] The microenvironment is thought to influence gene expression and stem cell self-renewal and commitment to specific lineages.[102] There are several theories surrounding stromal cell lineage determination.[103] One theory is that stromal cells support the formation of a niche for other cell types.[104] For examples, the cells and ECM of bone marrow stroma form a microenvironment that controls quiescence, self-renewal, and commitment of stem cells and proliferation, maturation, and apoptosis of more mature cells.[105] Stromal cells in the microenvironment control the differentiation and proliferation of progenitor cells through cytokines and other factors.[104] Majumdar et al[74] found that primary and osteogenic differentiated BMSCs expressed essential hematopoietic cytokines and supported hematopoiesis in long-term culture. These findings support the theory that stromal cells initiate and support the formation of the niche.

A major focus is on signaling factors and intercellular communication in the local environment and interactions between stromal cells and their neighboring ECM.[106] It has been suggested that a highly orchestrated system maintains the stromal cells in their undifferentiated state until specific signals activate their differentiation potential for reparative or regenerative purposes. MSC secrete soluble factors that stimulate proliferation or differentiation and decrease inflammatory and immune responses.[4,102,107] Some soluble factors secreted by stromal cells are antiapoptotic, angiogenic, and chemoattractants.[108] To inhibit apoptosis, MSC must secrete antiapoptotic bioactive molecules, including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), stanniocalcin-1, and granulocyte-macrophage colony stimulating factor (GM-CSF) among others.[108] Under hypoxic culture conditions, ASCs expressed HGF, VEGF, GM-CSF, transforming growth factor-beta (TGF-β), and basic fibroblast growth factor (bFGF).[109] Secretion of critical proteins minimizes cell death in areas adjacent the site of injury. The MSC secretion of ECM molecules insulin-like growth factor-1, placental growth factor, monocyte chemoattractant protein-1 (MCP-1, CCL2), interleukin-6 (IL-6), bFGF, and VEGF induces angiogenesis.[108] Hung et al[110] found MSC conditioned media promotes the in vitro formation of capillary-like structures through high levels of IL-6, VEGF, and MCP-1 among others. Cultured MSC produce numerous chemoattractants including CCL2, 3, 4, CXCL2, 5, 10, and 12 that induce the migration of leukocytes to injured tissue.[108]

MSC MULTIPOTENTIAL

Stromal cells are characterized by their ability to differentiate into various lineages. Selective differentiation and then maintenance of differentiated adult stromal cells in vitro is stimulated by specific growth factors and cytokines.[67] Osteogenic differentiation can be achieved by the addition of dexamethasone, ascorbic acid, and β-glycerophosphate (DAG) to culture media for 2–3 weeks and confirmed with Alizarin Red staining of calcium in mineralized colonies. Additionally, there is increased expression of

bone-specific genes and proteins associated with the bone phenotype such as alkaline phosphatase and collagen type I (ColI) can be seen.[111] Jäger et al[112] reported that combinations including DAG only, DAG+BMP2, and DAG+BMP2+porcine collagen scaffold induced human BMSC osteoblastic differentiation. Furthermore, they reported that BMSCs cultured with DAG-induced ColI secretion, which was increased by DAG+BMP2. This study gives further insight to MSC osteogenic differentiation and the use of various osteogenic induction agents.

To promote MSC differentiation into the adipogenic lineage, stromal cells can be incubated with insulin, dexamethasone, isobutyl methyl xanthine, and rosiglitazone. Over a 10–14 day culture period, lipid vacuoles accumulate within the cells. The cells express adipogenic specific genes like peroxisome proliferation-activated receptor-γ, leptin, lipoprotein lipase, and fatty acid-binding protein, and stain positive for intracellular lipid accumulation with Oil Red O.[15,113] Vallee et al[113] demonstrated the adipogenic differentiation potential of ASCs on three-dimensional (3D) substitutes. Specifically, ASCs were induced into adipogenesis and used to generate 3D tissue constructs that had greater surface area than ASCs alone. Results from this study show promise for the potential use of MSC technology to repair soft tissue defects.

Chondrogenic differentiation can be achieved by culturing MSC in high-density pellet cultures in the presence of TGF-β. After 21–28 days, pellets stain positive for sulfated glycosaminoglycan with Safranin O/fast green and have increased mRNA levels of ColII and aggrecan.[114] Murdoch et al[114] showed that human MSC chondrogenic differentiation can be achieved in micromass pellets as well as in transwell cultures, but transwell cultures had 50% greater proteoglycan content per cell and a more homogenous distribution of Col II. These findings provide essential information regarding MSC chondrogenic differentiation and highlight the potential for chondrogenesis without scaffolds.

Sago et al[17] reported that canine ASC can differentiate into early and mature neuronal cells in vitro. Investigations have also evaluated the expression of neural makers on BMSCs.[115–119] Studies of human and murine BMSC show that MSC express neuronal markers without induction and show elevated expression of neuronal markers under specific culture conditions. In 2006, canine BMSC isolated from the iliac crest and induced with dibutyryl cAMP and isobutylmethylxanthine constitutively expressed neuron or astrocyte specific proteins in vitro.[117] In contrast, researchers found that BMSCs differentiated into the neurogenic lineage via chemical induction may resemble neuronal cells, but lack their function. Neuhuber et al[120] found that when BMSC were chemically induced into neurogenic differentiation, rapid morphologic changes occurred within the cells but neither cell motility nor process extension was observed. Additionally, there was an absence of essential neuronal proteins required for neurogenesis. Electrophysiological analyses of BMSCs differentiated into neurons are necessary before conclusions can be made about their neuronal function. MSC applications for future neurological treatments are promising; however, more work is needed to validate the capacity of MSCs for neurogenic differentiation.

Use of stromal cells for treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease is being investigated.[121] Parkinson's disease is characterized by the loss of dopaminergic and nondopaminergic neurons. In vitro, MSC can be induced to differentiate into cells that possess characteristics of dopaminergic neurons including expression of neuron-specific genes.[122,123] Additionally, MSC survived, expressed hydrolase activity, and promoted functional improvements when transplanted into a MPTP mouse model.[124] The use of animal models aid in understanding these neurologic disorders so that future research can be applied to people and animals.

ORTHOPEDIC APPLICATIONS OF ADULT STEM/STROMAL CELLS

Use of MSC for orthopedic applications has shown variable efficacy to treat fracture nonunion, ligament and tendon injuries, osteogenesis imperfecta, and spinal cord injuries.[47,48,125–127] Research is still needed before MSC technology can be clinically applied. In 1999, Horwitz and colleagues used allogeneic bone marrow transplantation to treat children suffering from osteogenesis imperfecta. Patients had increased total mineral bone content, decreased fracture rate, and new dense bone formation 3 months after treatment.[48] A clinical study in people with acute spinal cord injury treated by intralesional injection of bone marrow mononuclear cell fraction and subcutaneous GM-CSF showed both motor and sensory improvement.[127] Hiyama et al[128] found that MSC transplantation was partially effective in inhibiting canine disc regeneration and MSCs may be responsible for maintaining immune privilege in intervertebral discs.

Recently, adult stromal cell-based therapies to treat injuries, growth defects, and degenerative diseases have been introduced.[125,127,129–131] Adult stromal cells are currently being investigated for treatment of tendon and ligament injuries in several animal models[132] and osteoarthritis (OA) in dogs.[133] Young et al[134] demonstrated the use of MSC to repair an Achilles tendon injury in a rabbit model. After 12 weeks, regeneration of new tendon-like tissue in the 1 cm defect was evident. Adult stromal cell treatment developed for tendon injuries in horses is slated for human clinical trials.[135] This treatment has been used in over 1500 horses and resulted in a 50% reduction in the reinjury rate.[136,137] The results from the equine study stress the importance of animal models in making adult stromal cell application for treatment and regenerative purposes a reality.

TISSUE ENGINEERING

The concept of tissue engineering was pioneered by Bisceglie in 1933 when he discovered that mouse tumor cells survived in a biocompatible polymer membrane implanted

into the abdominal cavity of chick embryos.[138] Decades after Biscgelie's experiments, Green performed a series of cartilage generation experiments by implanting chondrocytes on bone spicules into nude mice.[139] The research was largely unsuccessful, but it was a part of the movement that paved the way for later studies in tissue generation. A few years later, Chick et al[140] found that pancreatic β cells cultured on synthetic capillaries and perfused with media, released insulin in response to changes in glucose concentration. In 1985, Fung coined the term tissue engineering based on the traditional definition of tissue as a fundamental level between cells and organs in living organisms; however, it was not until ~1990 that the term was generally accepted.[141]

Biocompatible scaffold carriers are crucial for stromal cell tissue reconstruction. In general, scaffolds are biological constructs that closely mimic the tissue to be replaced. Stromal cells adhere to the scaffold, replicate, differentiate, and then organize into new tissue. Criteria for an ideal scaffold include: (1) biocompatible and absorbable; (2) supports cell growth; (3) high porosity; (4) possesses chemical factors that promote cell attachment and differentiation; and (5) reproducible. To optimize stromal cell bone repair, a scaffold should possess the characteristics of osteoconduction, osteoinduction, and should promote cellular adherence and recruitment.[2] Human ASCs produced more osteoid when seeded on hydroxyapatite (HA)/tricalcium phosphate (TCP) scaffolds than cells cultured on collagen/HA–TCP composite.[142,143] Studies have evaluated the use of HA scaffolds combined with other biologic components such as collagen or calcium phosphate (TCP) for bone regeneration.[96,144–148] Other types of scaffolds currently in use as stromal cell carriers include: coral, natural bovine bone mineral, polyglycolic acid, collagen, ceramic, poly(lactic-co-glycolic acid), and hydrogel among others.[144,146,149–153] In 2008, Macchiarini et al[154] reported that a deproteinized human trachea colonized with epithelial cells and MSC-derived chondrocytes was successfully implanted to replace the left main bronchus of a patient with end-stage bronchomalacia.

Numerous canine studies have demonstrated the importance of stromal cell/scaffold interactions. Jafarian et al[144] compared BMSCs on HA/TCP and NBM carriers for alveolar bone regeneration in a canine mandibular defect model and concluded that HA/TCP enriched with BMSC provided better conditions for bone regeneration compared with the NBM. Cui et al[149] demonstrated that an engineered bone composed of autologous ASCs on natural coral scaffold promoted repair of critical sized cranial defects in canine models. These studies highlight the fact that ASC osteogenic capacity is significantly influenced by the carrier scaffold. The use of MSC on biologic scaffold carriers is a promising therapeutic entity for tissue regeneration. Optimization and customization of stromal cell-scaffold interactions are critical to derive the most benefit from clinical application.

Tissue engineering has not only been investigated for the repair of bone defects, but also for regeneration of adipose tissue and cartilage. Tissue engineering strategies have been proposed for breast tissue reconstruction and other soft tissue defects with methods encompassing stromal cells, growth factors, and/or scaffolds to generate adipose tissue.[155] Cartilage has poor regenerative capacity, so MSC technology provides promising treatment options. Wakitani et al[156] reported successful repair of caprine chondral defects using MSC in a type I collagen gel. Later, he applied his work clinically to treat human patients with full-thickness articular cartilage defects and knee OA.[157,158] Twelve patients treated with BMSC injections in conjunction with a periosteal grafts for cartilage defects on the medial femoral condyle had better arthroscopic and histologic outcomes. These studies show that MSC technology has potential for regenerative and reparative purpose, although more work is needed before clinical application.

THERAPEUTIC USES

Regenerative medicine is a rapidly growing specialty. Recently, Daar and Greenwood[159] defined regenerative medicine as the field of research focused on the repair, replacement or regeneration of cells, tissues, and organs. The primary goal is to augment the body's natural ability to replace tissue damaged or destroyed by injury or disease. Stromal cell research has proven invaluable in the development of cellular therapy because of enormous contributions to knowledge of the mechanisms of cellular proliferation and differentiation.[160] Many current treatments may be augmented or replaced by regenerative medicine, and a number of therapies are currently under investigation.

In addition to orthopedics, other medical disciplines will benefit from stromal cell technology. Current investigations include stem cell treatment for myocardial infarctions.[130] Orlic et al[161] reported that bone marrow cells injected into the myocardium resulted in myocardial regeneration consisting of cardiomyocytes and vascular elements. Mangi et al[162] reported that murine BMSCs injected into damaged myocardium over expressed Akt, a prosurvival gene. The Akt-MSC inhibited ventricular remodeling and restored cardiac function 2 weeks after myocardial infarction. Another study by the same investigators showed that cardiac function was restored within 72 hours after injection of the rat Akt-BMSCs.[163] It is believed that this effect occurred by paracrine actions of the cells and demonstrated that Akt-MSCs release factors that exerted beneficial effects on isolated cardiomyocytes in vitro when exposed to hypoxia. Bi et al[164] investigated the endocrine actions of murine BMSC and found that they reduced the severity of cisplatin induced acute renal failure whether administered by intraperitoneal injection or intravenous infusion. They concluded that BMSC secrete specific factors that protect the kidney from toxic injury. Stromal cells have also been used to treat liver disease. Implantation of in vitro expanded murine MSC significantly reduced

experimentally induced chronic liver injury and fibrosis.[165] Continued research is underway to incorporate stromal cells into standard treatments.

CONCLUSIONS

There have been great strides in stromal cell technology to treat a variety of diseases and injuries since early studies laid the foundation for stromal cell therapies and tissue regeneration. Standardization of MSC isolation, characterization, and differentiation is necessary to derive the greatest benefits from them. Biocompatible scaffolds to facilitate cell proliferation, differentiation, and tissue development are also critical for their use. Investigations are underway to identify scaffold properties that will optimize stromal cell activity. Additional investigations and clinical trials to establish safety and efficacy are also necessary before routine clinical application. Stromal cell technology is a rapidly emerging discipline that has significant potential to shift the paradigm surrounding restoration of tissue lost to trauma or disease.

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