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14.1 Stem Cell Biology

14.1.1 Stem Cell Classification

The German biologist Ernst Haeckel first introduced the term “stem cell” in the scientific literature in 1868 with the term “Stammzelle” (stem cell) to describe the unicellular ancestor progenitor of all organisms. In the nineteenth century, Theodor Boveri and Valentin Häcker instead used the same term to describe “cells committed to give rise to the germline” [1], and 4 years later, Edmund B. Wilson made the term “stem cell” universal by reviewing Häcker’s and Boveri’s work in his book entitled *The Cell in Development and Inheritance* [2].

Around 100 years later, Gail Martins of the University of California, Martin Evans and Matthew Kaufman of the University of Cambridge, independently isolated stem cells

from mouse embryos and coined the term “embryonic stem cells” (ESCs). In 2007, Mario Capecchi, Martin Evans, and Oliver Smithies shared the Nobel Prize in Physiology and Medicine for the great achievement in the field of ESCs obtained in the mid-1980s. In 1995, Jamie Thompson of the University of Wisconsin cultured monkey ESCs for the first time and later, in 1999, human embryonic stem cells.

All tissues are composed by highly specialized cells derived from an initial pool of stem cells generated during early embryonic development, which provides a reserve for injured tissue repair and replaces the cells lost daily in the lifespan. Stem cells are unspecialized cells that have two key properties that distinguish them from other types of cells; they have the capacity of self-renewal and the ability of generating differentiated cells [3, 4]. These cells are capable of generating daughter cells for long periods identical to their mother cells (self-renewal). They are also able of differentiating, under specific physiological conditions, into many types of mature cell, which make up totally all our organs and tissues.

This area of interest includes different types of stem cells, which can be isolated during different phases of the development of an organ-

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ism. In the initial stage of the embryo development, stem cells (ESCs) can be found in the blastocyst (50–100 cells), whereas in the adult stage, tissue stem cells can be found almost in all body tissue. These adult stem cells (ASCs) can also be found in the fetus and in babies. Finally, induced pluripotent stem cells (iPS cells) derived from specialized cells (e.g., skin cells) can be “in vitro” engineered, or “reprogrammed,” to become pluripotent cells like embryonic stem cells (Fig. 14.1).

Specifically, embryonic stem cells are derived from preimplanted embryos after the formation of the blastocyst [5]; this is made up of an outer layer of cells, an internal fluid-filled space, and an inner cell mass where the ESCs reside. They are defined “pluripotent” because of their ability to differentiate toward all the different types of body cells and tissues, except for extraembryonic organs such as the placenta, yolk sac, and umbilical cord. On the contrary, embryonic stem cells that immediately arise in the first few divisions of the fertilized egg, and defined “totipotent,” are able to totally generate a viable embryo including extraembryonic organs.

Instead, adult stem cells are committed cells able of differentiating into all mature cell lineages typical of the tissues or organs in which they reside and for this reason described as “multipotent.” For example, stem cells within the adult brain are able to differentiate in neurons and into other two types of cells, astrocytes and oligodendrocytes. Adult stem cells have been found in several organs, mostly those that continuously replenish themselves, such as the blood, skin, muscle, and liver, in large quantity but also in other, less regenerative organs such as the heart and brain.

Finally, induced pluripotent stem cells were “in vitro” produced in 2006 [6] by using viruses for the insertion inside somatic cells of four genes (Oct4, Sox2, c-myc, and Klf4) known to be important for the embryonic stem cell development. These pluripotent stem cells share many characteristics of embryonic stem cells, including the ability to differentiate toward all the cell types in the body. How these four “reprogramming” genes are able to induce pluripotency is not yet well known, and this question is the object of current studies [7]. In addition, recent research is concentrated on finding an alternative way to reprogram somatic

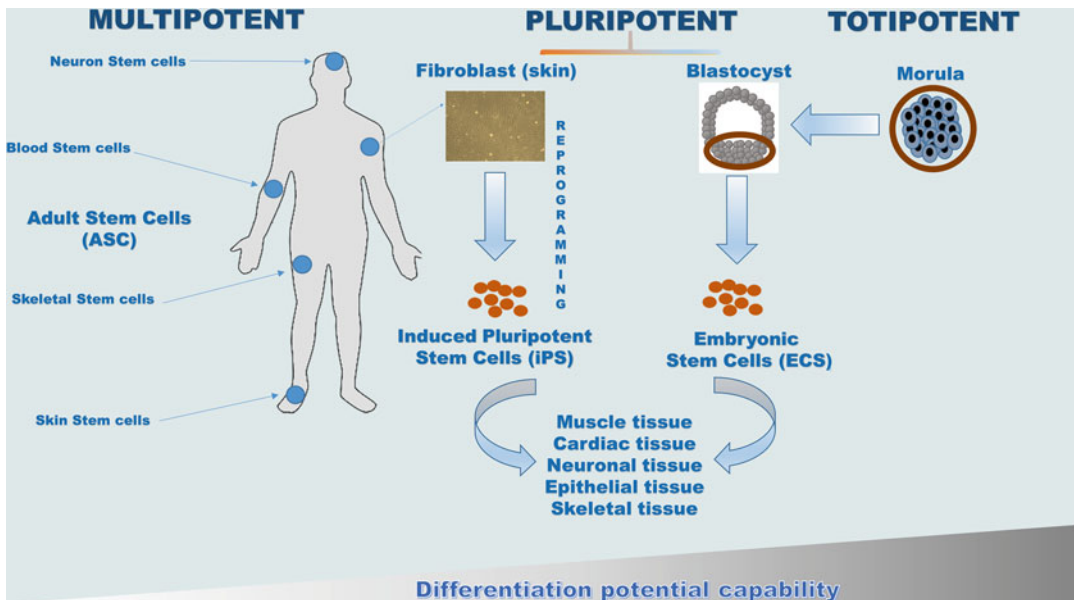


Fig. 14.1 Classification of stem cell sources and definition of differentiation potential capability

cells using safer approaches in clinical sceneries [8, 9].

14.1.2 Stem Cells' Potential Use: Advantages and Disadvantages

Several challenges must be addressed before stem cells can be used in regenerative medicine applications. The first important issue to be addressed is the identification, isolation, and growth of stem cells which are not easy procedures in the case of rare adult stem cells. The following reports and discusses the positive and negative aspects of the three main promising stem cells types, currently the object of worldwide research and investment.

Pluripotent embryonic stem cells that are easily isolated and have the advantage of an unlimited in vitro growth also have the capability of a great differentiating potential through strictly controlled processes. On the contrary, their clinical use has important limitations, due to their genetic instability, potential tumorigenic risk, and ethical considerations related to their origin [10]. For this last reason, in Europe there are rigorous laws that forbid destructive embryo research, while federal laws in the USA instead allow embryo use only in the case of it being discarded after in vitro fertilization [11].

Induced pluripotent stem cells, deriving from reprogrammed somatic cells with standard protocols, are able to differentiate into the three germ layer cell types [12], but still have a very low reprogramming efficiency. These cells could be a good option in autologous transplant applications, overcoming the tissue rejection; however, like the ES cells, they have an important genetic instability and a high tumorigenic [13] risk. Therefore, the standard and safe use in cell therapies of both stem cell types is still a target to reach which needs extensive research and effort.

Finally, multipotent adult stem cells can be used in autologous transplantation in which the patient's own cells are expanded and differentiated in vitro. They are then implanted in the same person, avoiding the host's immune rejection and

protecting the patients from viral, bacterial, or other types of donor's contamination. The disadvantage of ASCs is a very short life in culturing and expansion and a weaker differentiating potential in comparison with embryonic stem cells.

Although significant progress has been made in the stem cell research field and many preclinical studies have highlighted the great therapeutic potential of these cells, among the stem cells types, only the adult stem cells are currently used in some clinical applications. In particular, the bone marrow stem cells have been employed for more than 50 years, giving excellent results especially in the hematopoietic and immune system pathologies, which are addressed in the next section.

14.1.3 Current Clinical Applications Using Multipotent Adult Stem Cells

Presently, in some cases, this clinical protocol is replaced by autologous transplantation of stem cells; as a matter of fact, in the area of therapeutic implantation, it is very important to have a strong compatibility between the donor and the host tissue, in order to minimize the risk of rejection and at the same time deliver and engraft the stem cells to the target damaged tissue to improve the stem cell integration.

In successful clinical applications, the stem cells used were the blood (hematopoietic) stem cells from the bone marrow for the treatment of leukemia, lymphoma, and several inherited blood disorders. Umbilical cord blood, like bone marrow, is also collected as a source of blood stem cells and then used as an alternative to bone marrow transplantation, especially for the treatment of diseases in children. Other stem cell treatments which proved safe and effective involved bone, skin, and corneal diseases or injuries.

14.1.3.1 Bone Marrow Stem Cells in Transplants

Bone marrow stem cell therapy has been in routine use since the 1970s [14] and is able to

treat a patient's diseased blood. Although it presents a direct complication, due to the donor's immune cells that sometimes can react to the patient's tissues (graft-versus-host disease or GVHD) [15, 16], and an indirect complication, due to a risk of infection in chemotherapy pretreated patients [17], many thousands of people benefit from this kind of treatment every year.

14.1.3.2 Umbilical Cord Blood Stem Cells in Transplants

The umbilical cord blood stem cells (UCSCs) have the advantage of being less rejected by the immune system, compared to conventional bone marrow transplants. UCSCs, adequately cryopreserved in cell banks, are presently used for treating cancer blood disorders in children, such as leukemia, and genetic blood diseases like Fanconi anemia [18, 19].

14.1.3.3 Skin Stem Cells in Transplants

Skin stem cells have been used since the 1980s for the *in vitro* growth of new skin sheets for treating patients with severe burns [20]. However, the new skin has no hair follicles, sweat glands, or sebaceous (oil) glands, so this approach is used only for saving the lives of patients with third-degree burn over very large areas of their bodies [20].

14.1.3.4 Eye Stem Cells in Transplants

Clinical studies have shown that adult stem cells isolated from the limbus area of the eye can be used to repair damaged cornea. As matter of fact, the limbal stem cells can be taken from the patients, *in vitro* cultured and transferred back to their injured eye [20]. The treatment, safe and effective in early stage trials [20], is limited if both eyes have been seriously damaged for the impossibility to obtain the patients' limbal stem cells. The safe and routine use of adult stem cells in clinical therapies needs a considerable research work and for this scope the public funds are required.

14.1.4 Multipotent Mesenchymal Stem Cells

Among the adult stem cells, multipotent mesenchymal stem cells (MSCs) are a promising cell source for tissue engineering and cell-based therapies due to their ability of self-renewal and of differentiating into specific cell lineages. Human Mesenchymal Stem Cells (hMSCs) have aroused great interest in the scientific community since their use in clinical applications does not imply neither ethical problems nor teratoma-risk formation. The number of clinical trials in which hMSCs have been tested has been increasing since 2004 [21], opening up their potential employment in the future treatment of numerous diseases, mainly tissue injuries and immune disorders. These non-hematopoietic adult stem cells, first isolated and studied by Friedenstein in 1971 [22], are able to differentiate into various mesoderm lineages, such as osteocytes, chondrocytes, and adipocytes, as well as ectodermic and endodermic cell lineages [23–27]. MSCs originate from the mesoderm but have a wide distribution in organs and can be isolated from many tissues such as the bone marrow, adipose tissue, muscle, liver, lung, and extraembryonic tissues [28–32].

These stem cells, involved in normal human tissue renewal, wound healing, and in physiological responses to injuries [33], have shown repairing effects for the treatment of damaged tissues and degenerative diseases [34–39]. In patients with cirrhosis disease due to hepatitis B, the autologous transplant of mesenchymal stem cells from the bone marrow (BM-MSCs) has showed encouraging results being able to improve the liver function [40, 41]. BM-MSCs have also provided positive responses in the treatment of muscular-skeletal diseases, periodontal tissue defects, diabetic critical limb ischemia, and burnt skin repair [42–44]. In addition, some preclinical studies have reported tissue regeneration through an anti-inflammatory effect of BM-MSCs in myocardial infarction treatment [45], cornea damage, and other tissue injuries, such as the brain, spinal cord [46], and lung [47–49].

For a long time, the main and traditional source of hMSCs for clinical application uses has been the bone marrow, but their employment is still limited not only because the procedures to isolate hMSCs are highly invasive and the cell quantity obtained is low but also because the proliferating and differentiating potential decreases as the donor's age increases [50]. For this reason, the identification of an alternative source of hMSCs has been an important and necessary issue that still needs to be explored, and for this aim, a promising choice could be adipose tissue and neonatal tissues, including placenta.

14.1.5 hMSCs from Neonatal Tissue

Placental tissue is involved in important functions such as nutrition, respiration, and excretion and the maintenance of fetomaternal tolerance. It is made up of the chorionic plate, which is in close contact with the uterine decidua, and the fetal membranes (amnion and chorion), which spread from the borders of the chorionic plate and enclose the fetus in the amniotic cavity. The amniotic membrane (AM) encloses two types of stem cells, epithelial and mesenchymal, which have different embryological origins. The human amniotic epithelial cells (hAECs), derived from the embryonic ectoderm, form a continuous monolayer in contact with the amniotic fluid. The human amniotic mesenchymal stromal cells (hAMSCs), deriving from the embryonic mesoderm, are instead spread in the stromal layer underlying the amniotic epithelium. Stem cells deriving from AM have a great differentiating potential since these two layers originate at day 8–9 after fertilization, in a very early stage of the embryonic development. This has been extensively verified by several studies that report the capability of hAECs and hAMSCs to differentiate toward different cell lineages belonging to all three germ layers [51, 52]. The recovery of these stem cell types does not require any invasive procedures for the donor and does not rise any ethical issue; furthermore, the fact that the placenta is

generally discarded after birth and is available in large supplies makes these stem cells an excellent candidate for their eventual use in cell therapy approaches [51]. The scientific interest for the use of these stem cells in regenerative medicine is also generated by their low immunogenicity characteristics; this is confirmed by the clinical applications that use the AM as biologic bandages in surgical procedures [53] for the treatment of corneal or conjunctival destructive loss [54].

The low immunogenic and the immunomodulatory properties of hAECs and hAMSCs can be explained by their low or limited levels of the HLA-ABC expression and the absence of the HLA-DR expression together with the costimulatory molecules [55–57]. All these immunological characteristics make them particularly suitable for the use in allogenic transplantations for the recovery of the damaged tissue through anti-inflammatory, anti-fibrotic, and pro-regenerative effects, minimizing the risk of rejection. This procedure is less invasive compared to autologous transplants and have all the advantages of allogenic transplantations in which the stem cells can be previously isolated and cryopreserved, making them readily available for possible clinical uses. This fact shortens the time of transplantation, offering the advantage of intervening timely on the damaged tissue before the fibrotic process irreversibly compromises the tissue regeneration.

14.1.5.1 Potential Use of hAMSC in Muscle Repair

The capability of muscle tissues to regenerate in response to injury stimuli represents an essential homeostatic process, in which the cell turnover plays an important role and in the case of small injuries due to contusions, the muscle is able to self-repair its damage through four correlated time-dependent phases: degeneration, inflammation, regeneration, and remodeling repair [58]. The injury of myofibers results in the rapid necrosis in which the influx of extracellular calcium induces the proteolysis of the myofibers [59]. The

necrotic fibers activate an inflammatory response characterized by the recall of specific cell populations into the muscle [60]. The inflammatory response is then followed by a regenerative phase, characterized by satellite stem cell activation and by the presence of regenerating fibers [61]. In the final phase, the maturation of the regenerated myofibers, and the contraction and reorganization of the scar tissue occur, recovering the functional performance of the injured muscle [62]. On the contrary, in the case of severe muscle injuries, the muscle function results permanently damaged for the formation of dense scar tissue (fibrosis) [63, 64] that can diminish the ability of full recovery leading to muscle contracture and chronic pain [65]. Up to date, optimal treatment strategies for severe muscle injuries have not yet been identified, and for this scope, a new strategy needs to be developed. In this context, AM-derived mesenchymal stem cell could be a promising option for their anti-inflammatory, anti-fibrotic, and pro-regenerative intrinsic characteristics.

In our laboratory, the hAMSCs isolated from AM are being studied to investigate their possible use in severe muscle injury also with the goal of sphincter incontinence regeneration. These cells are isolated from the term amnion and dissected from the part connected to the umbilical cord to minimize the presence of maternal cells. Homogenous hAMSC populations are obtained by a two-step procedure: the amniotic membrane is treated with trypsin to remove hAECs and the remaining mesenchymal stem cells are then released by digestion with collagenase [66]. The quantity obtained from the term amnion is about one million hAMSCs [67], a great amount that is possible to cryopreserve. After isolation, the hAMSCs are characterized according to the minimal and univocal criteria indicated by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy [68]. The first cell requirement needed is the plastic-adherent ability when maintained in standard culture conditions; they must also express *CD105*, *CD73*, *CD29*, and *CD90* and lack the expression of *CD45* and *CD31* surface molecules, and finally, they must show a differentiating potential toward osteoblast, adipocyte, and

chondroblast lineages after specific in vitro chemical treatments. Based on this, we demonstrated by phase-contrast analysis on isolated hAMSCs the ability to adhere to plastic Petri dishes (Fig. 14.2a), and by trypan blue assay, they resulted able to exponentially grow from day 1 to day 4 (Fig. 14.2b). Actin fluorescence staining also revealed (Fig. 14.3) their typical fibroblast-like morphology (Fig. 14.3). The presence of MSC markers (*CD90*, *CD44*, *CD73*, *CD54*, *CD105*, and *CD29*) and a very low expression of hematopoietic markers such as *CD31*, *CD34*, and *CD45* were also highlighted by their immunophenotypical characterization (Table 14.1). Moreover, a widespread expression of the mesenchymal ubiquitous *Vimentin* marker was revealed, by fluorescence microscopy analysis (Fig. 14.4) together with the capability to achieve osteogenic, adipogenic, and chondrogenic commitments when growing in appropriate and specific differentiating mediums as highlighted by specific assays (data not shown).

The mRNAs' expression of early and late muscle differentiation markers has been also investigated in hAMSCs after the treatment of chemical and physical differentiating stimuli. The ongoing results confirmed their muscle commitment, suggesting their potential use in cell therapy leading us to suppose that these cells' engraftment could be enhanced compared to other uncommitted transplanted stem cells. This hypothesis is presently under investigation in various muscles injury animal models in order to understand the most efficient differentiating level to be used to improve muscle repair.

14.1.6 hMSCs from Adipose Tissue

The adipose tissue was for a long time considered only for its energy storage function [69, 70], but in 1994 after the discovery of leptin, the first adipokine, it became clear that this tissue is also an endocrine organ playing an important role for several inflammatory diseases in physiopathology [71]. Adipose tissue is widely distributed in the adult human body and is found in the bone marrow; intra-articular, subcutaneous,

and visceral depots; and ectopic sites such as intrahepatic and intramuscular tissues. The worldwide diffusion of obesity has contributed to increase the scientific interest toward this tissue. Even though the mature adipocytes are their main component, it is also composed by

other cell types that contribute to its cellular heterogeneity. These different cell components are usually isolated from surgical specimens or lipoaspirates by collagenase enzyme digestions [72, 73] followed by centrifugation to separate the floating mature adipocytes from the remain-

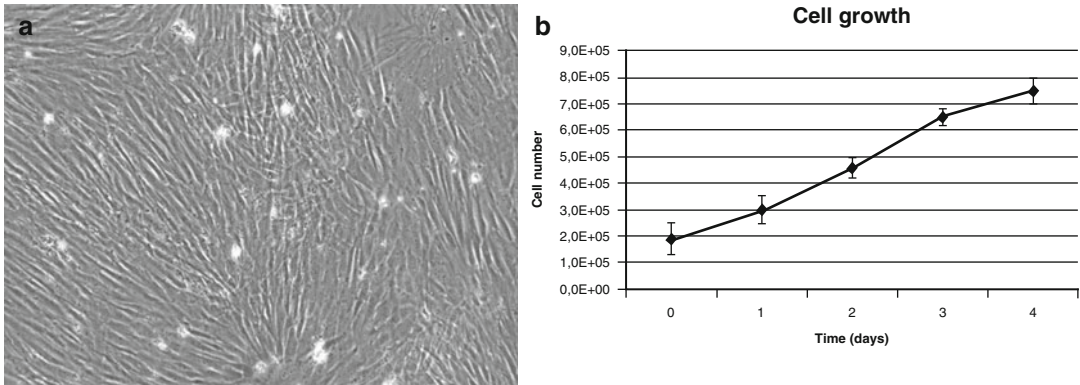


Fig. 14.2 (a) Plastic Petri dishes adherence ability of hAMSCs by phase-contrast microscope analysis (20× objective). (b) hAMSCs exponential growth trend from day 1 to 4, by trypan blue cell assay

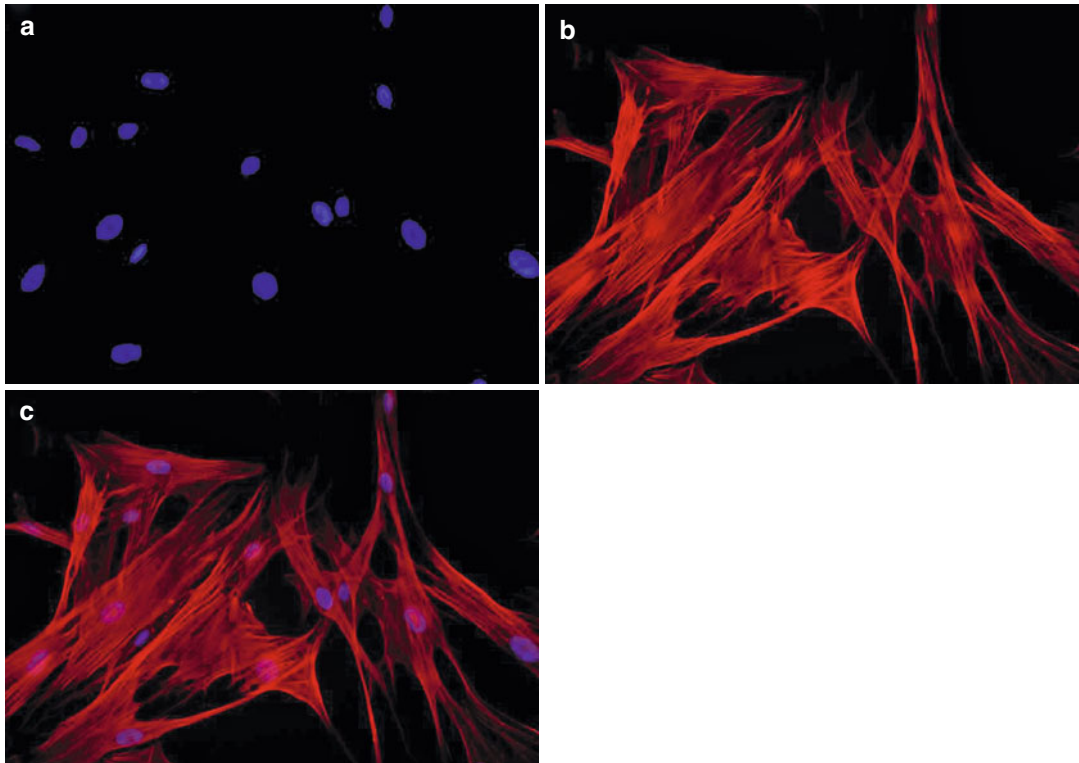


Fig. 14.3 hAMSCs typical fibroblast-like morphology by actin fluorescence analysis (a) Hoechst nuclei staining, (b) phalloidin actin staining, and (c) merged image (20× objective)

ing cells, forming a stromal vascular fraction (SVF) pellet [73]. It contains endothelial cells, macrophages, fibroblasts, B- and T-lymphocytes, myeloid cells, pericytes, smooth muscle cells, pre-adipocytes, and adipose-derived stem cells (ADSCs). After about 1 week of expansion in specific medium, it is possible to obtain from one milliliter of human lipoaspirate between 0.2 and 0.4×10^6 of ADSCs which are able to differentiate toward the adipocyte, chondrocyte,

and osteoblast lineages [74, 75]. Since many patients routinely undergo liposuction annually, it is easy to isolate hundreds of million ADSCs from a single donor, making them particularly interesting for regenerative medicine applications. Recently, the International Federation for Adipose Therapeutics and Science (IFATS) have provided minimal criteria for the characterization of ASC based on functional and quantitative features [76], and many companies have developed closed system devices designed for ADSC isolation [77]. These automated devices have improved the methods to obtain reproducibility of results and their safety in clinical application uses.

The clinical translation of ADSCs still remains object of intensive research [78], but some very promising findings have been already reported. Finnish and collaborators at the Universities of Helsinki and Tampere, for example, used autologous human ADSCs to repair hard palate defect

Table 14.1 hAMSC mesenchymal and hematopoietic markers immunophenotypical characterization by FACS Cytometer analysis

Mesenchymal markers		Hematopoietic markers	
CD90	96 %	CD31	0 %
CD44	97 %	CD34	3 %
CD54	99 %	CD45	0 %
CD29	95 %		
CD105	96 %		
CD73	98 %		

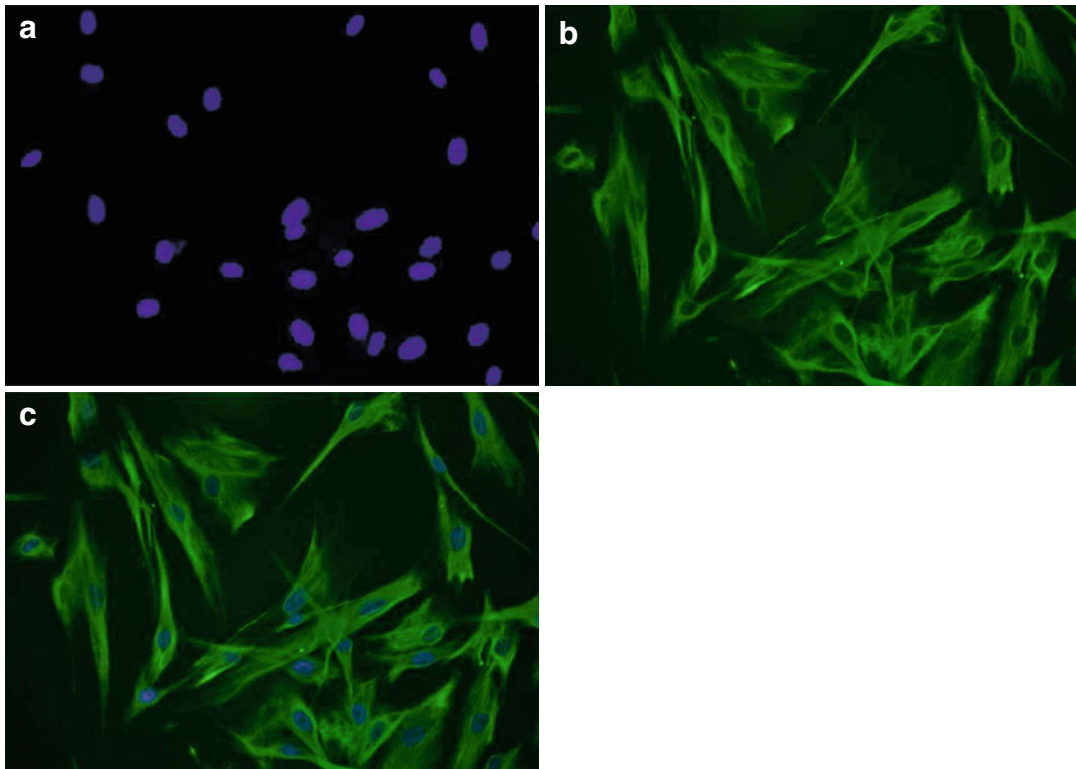


Fig. 14.4 hAMSCs mesenchymal Vimentin expression by fluorescence microscopy analysis: (a) Hoechst nuclei staining, (b) Vimentin fluorescence analysis, and (c) merged image (20× objective)

[79], reporting the encouraging results of a full recovery of the oral function and independent groups have shown similar results [80, 81].

14.2 Clinical Applications

14.2.1 Stem Cells and Sphincters Dysfunctions of the Pelvis

At present there are more than 350 clinical trials involving human MSCs for very different entities (www.clinicaltrials.gov). Most of these studies involve the use of mesenchymal stem cells from the bone marrow and adipose tissue, and no significant adverse effects were observed in all studies. Relatively few studies were performed to treat a degenerated sphincter muscle in humans with MSCs or MSC-like cells.

Based on the promising preclinical “in vitro” and “in vivo studies” [82–84], MSCs have been also investigated for their potential therapeutic applications in sphincter dysfunctions of the pelvic floor, both in the proctologic and in the urogynecological field [21, 85–88]. As a matter of fact, the use of MSCs for fecal and urinary incontinence treatments may be a major step forward in clinical efficacy with minimal risks, especially compared to surgical repair treatments [89–96].

The incontinence, both fecal and urinary, may result from the loss of the sphincter function due to muscle damage and peripheral nerve lesions, with various combinations of both. The rebuilding of the muscle fibers and nerve endings by a regenerative therapy employing mesenchymal stem cells is then an ideal treatment concept, especially because clinical use of these stem cells appears free of ethical concerns and risk of tumor formations [97].

Autologous and heterologous MSCs are used for “in vivo” studies on animals, while only autologous MSCs are employed in human trials. The most widely used technique for the production of adequate amounts of MSCs provides for their harvesting from several adult tissues, such as the bone marrow, muscle, and adipose tissue. Subsequently, MSCs are “in vitro” expanded, until desired cell numbers are achieved ready to transplantation to regenerative therapy [98].

However, the use of MSCs in clinical practice is still not widespread, and the clinical application in patients remains an important goal. Certainly, this is not due to the low consideration of physicians for this procedure but rather to the complex production method of expanding some types of stem cells and to their high costs [99].

The gynecological field of urinary incontinence was the first that received the attention of the scientific community, and only later researchers focused their interest on fecal incontinence. The first MSCs application in a rat urinary incontinence model was published in 2000 by Chancellor et al. [100], and only 8 years later Carr published the first study on patients affected by stress urinary incontinence [101].

14.2.2 Urinary Incontinence

14.2.2.1 Animal Models

Different animal models have been used to mimic the injuries that can produce urinary incontinence. The first model, introduced by Lin in 1998 [102], utilized vaginal distension in female rats to simulate the trauma of childbirth with damage to surrounding muscles and nerves. Subsequent other models have been developed to investigate the incontinence mechanism, including nerve injury (transection of pudendal or sciatic nerve), direct urethral injury (urethral transection or cryo-chemo injury), and pelvic ligament injury. However, female Sprague–Dawley rats are the most used in these experiments [103–105].

In preclinical studies reported, two main approaches have been used for MSC transplants: the systemic administration by intravenous injection and local injections by direct puncture [21, 106–108]. The advantage of the first method is characterized by the simplicity of the technique and the ability of MSCs to migrate “in vivo” to specific inflammatory tissue and concentrate at the site of the lesion, thanks to the capacity of MSCs to “homing” into the site of injury in several disease models. Cruz et al. in 2012 [109], and Dissaranan et al. in 2014 [110], showed, by intravenous injection of MSCs in a rat model, the homing of these cells in the urethra and a

facilitated recovery of continence. However, additional studies have shown limits to this technique as reported by Rombouts and Ploemacher in 2003 [111] due to the fact that the increasing age and passage number in stem cell culture reduce the homing effect and the efficiency of MSCs engraftment. Furthermore, Fischer et al. in 2009 [112] reported that systemically infused MSCs often suffer from a first-pass effect where the larger cells become trapped in capillary beds of various tissues, decreasing their therapeutic bioavailability and functionality. Therefore, for increasing the number of mesenchymal stem cells and the efficiency of differentiation into the damaged sites, researchers have used local injections of MSCs, as reported by several pilot studies [43].

Functional analysis and histological examinations were performed to evaluate the therapy outcome, before and after treatment. Measurements of leak point pressure and bladder capacity were monitored to detect changes in urinary incontinence. To confirm the survival and differentiation of transplanted cells, histological sections of animal urethra were studied by immunohistochemistry–immunofluorescence analysis.

Although not all evaluation methods were uniform, in almost all studies performed on animal models of urinary incontinence, positive results are reported that showed both the improvement of the functional sphincter activity and the regeneration of new muscle and neuronal cells in the injured area.

Some of the most significant studies on animal model of urinary incontinence are summarized in Table 14.2.

14.2.2.2 Clinical Study

The clinical trial studies were performed using autologous muscle-derived mesenchymal stem cells (MDSCs) or adipose-derived mesenchymal stem cells (ADSCs) which can be obtained in large quantities from patients with an easy and low-invasive biopsy under local anesthesia. In these trials, the direct injection of MSCs was the most widely used procedure, performed by a local intrasphincteric injection by transurethral or periurethral approach using cystoscopy or ultrasonography guidance, both in females and

in males. Clinical outcome was commonly based on 3-day leakage diary, 24 h pad test, quality-of-life score, and urodynamic test by urethral pressure measurement at rest and in squeezing.

In 2008, Carr et al. from the University of Toronto [101] reported the first clinical trial and published the results of 1-year follow-up on eight women in which urinary incontinence was treated with local injection of MDSCs. In this study, autologous muscle cells obtained from the thigh of patients, using a percutaneous needle technique, were expanded in culture and concentrated into a single-use dose containing $18\text{--}22 \times 10^6$ cells for injection in patients. This pilot study reported an improvement in urinary incontinence, especially between 3 and 8 months after the initial injection. Moreover, this study proved to be safe and with the absence of adverse events related to MDSCs transplant.

Later, Sebe et al. in 2011 [126], Gotoh et al. in 2013 [127], and other authors reported that MSCs are able to reduce urinary incontinence symptoms and improve quality of life of patients. Results of 11 clinical trials in a total of 456 women and 241 men, published in peer-reviewed journals [128], showed that MDSCs are safe for the treatment of urinary incontinence, suggesting their potential use in cell therapies. However, only a restricted number of studies have focused on the number of stem cells to be used, and in this context, a multicenter study of Carr, Chancellor, and colleagues, published from 2008 to 2014 [88, 100, 101, 129–133], reported that treatment outcomes depend on the number of transplanted cells. These authors reported that in all groups, there was a statistically significant reduction in stress leaks within 1–3 months of treatment that was maintained through the 12-month follow-up, suggesting that the efficacy of MSCs is related to cell dose. In particular patients who received higher doses (200×10^6 cells) appeared to have better efficacy outcomes than those who received lower doses (10×10^6) [133].

The most significant clinical studies on urinary incontinence are reported in Table 14.3.

Table 14.2 Summary of stem cell studies in animal models of urinary incontinence

	Author	Stem cell type	Animal	Injury/injection	Outcome	Conclusion
1	Chermansky et al. [113] (2004)	MDSCs	Female rat	Urethral cauterization Periurethral injection 1 week after injury	Increases in LPP at 2, 4, and 6 weeks MDSC integration in urethral striated muscle	Periurethral MDSC injection improves sphincter function in rats with sphincter deficiency This procedure can be used to treat stress incontinence, with the advantages of minimal invasion and fast recovery
2	Fu et al. [114] (2010)	ADSCs	Female rat	Vaginal distension Periurethral injection 4 weeks after injury	Increases in max bladder capacity and LPP. Muscle thickness increased at 1 and 3 months	Transplanted cells survived and differentiated into striated muscle cells and peripheral nerve cells
3	Kinebuchi et al. [115] (2010)	BMSCs	Female rat	Combined urethrololysis and toxin injection Periurethral injection 1 week after injury	During the following 12 weeks LPP increased but not significant vs control group Skeletal muscle and ganglia were significantly greater than in the control group	
4	Lim et al. [116] (2010)	HUCB	Female rat	Periurethral electrocautery Periurethral injection 1 week after injury	Increases in LPP at 2 and 4 weeks Sphincter muscle restored	HUCB is a easy and safely stem cells therapy applicable with noticeable therapeutic efficacy
5	Lin et al. [117] (2010)	ADSCs	Female rat	Vaginal distension Periurethral or intravenous injection 1 week after injury	Improvement in LPP with either transplantation method Higher urethral elastin and smooth muscle content in treated animals	Transplantation of ADSC via urethral or intravenous injection is effective in the treatment of SU1 in a preclinical animal model
6	Xu et al. [118] (2010)	MDSCs (-/+ fibrin glue)	Female rat	Bilateral pudendal nerve transection Intraurethral injection 4 week after injury	Increases in LPP at 1 and 4 weeks MDSCs with fibrin glue increased muscle and microvessel density	MDSCs with fibrin glue may restore the histology and function of the urethral sphincter

(continued)

Table 14.2 (continued)

	Author	Stem cell type	Animal	Injury/injection	Outcome	Conclusion
7	Zou et al. [119] (2010)	BMSCs	Female rat	Bilateral proximal sciatic nerve transection Implantation of tissue-engineered sling seeded with BMSCs, sling, or no sling. 4 week after injury	The tissue-engineered sling group had normal LPP, higher collagen content, and higher failure force at 12 weeks after treatment	MSC have potential for use in the next generation of prosthetic devices
8	Kim et al. [120] (2011)	BMSCs	Female rat	Bilateral pudendal nerve dissection Periurethral injection 2 weeks after injury	LPP were restored to the control values at 4 weeks Injected MSCs positive for muscle-specific markers	MSCs might differentiate into muscle lineage cells and may contribute to the repair of damaged muscle tissue
9	Corcos et al. [121] (2011)	BMSCs	Female rat	Bilateral pudendal nerve transection Periurethral injection 4 week after injury	LPP were restored to the control values at 4 weeks New tissue organization with striated skeletal muscle cells	MSCs significantly improved LPP and partially restored damaged external urethral sphincters
10	Imamura et al. [122] (2011)	BMSCs	Female rabbit	Urethral cryoinjury Intraurethral injection 1 week after injury	LPP significantly higher vs. control group at 1 and 2 weeks Growing of striated and smooth muscle-like cells	BMSCs can reconstruct functional urethral sphincters
11	Wu et al. [123] (2011)	ADSCs	Female rat	Bilateral pudendal nerve transection Periurethral injection 3 weeks after injury	Improvements in MBV, LPP, CP, FUL vs. control group at 2 weeks Significantly improvement of striated urethral muscles	ADSCs significantly strengthened local urethral muscle layers and significantly improved the morphology and function of sphincters
12	Zhao et al. [124] (2011)	ADSCs +/- nerve growth factor (NGF)	Female rat	Bilateral pudendal nerve transection Periurethral injection	ADSCs+NGF resulted in significant improvements in LPP and RUPP as well as the amount of muscle and ganglia, at 2, 6, and 8 weeks	NGF could enhance the therapeutic efficacy of ADSC transplantation

	Author	Stem cell type	Animal	Injury/injection	Outcome	Conclusion
13	Cruz et al. [109] (2012)	BMSCs – GFP labeled	Female rat	Vaginal distension Intravenous injection 1 h after injury	Significantly more MSCs home to the pelvic organs than control group at 4 and 10 days	The study provides basic science evidence that intravenous administration of MSCs could provide an effective route for cell-based therapy to facilitate repair after injury
14	Chun et al. [125] (2014)	hAFSCs early differentiated into muscle, neuron, and endothelial progenitor cells	Female ICR mice	Bilateral pudendal nerve transection Periurethral injection 2 weeks after injury	Increases in LPP and CP at 2 and 4 weeks High levels of myogenic, neuronal and endothelial marker expression	Early-differentiated hAFSCs may have synergic effects on myogenesis, neuromuscular junction formation, and angiogenesis in the regeneration of the damaged urethral sphincter

ADSCs adipose-derived mesenchymal stem cells, *BMSCs* bone marrow-derived mesenchymal stem cells, *CP* closing pressure, *FUL* functional urethral length, *GFP* green fluorescent protein, *hAFSCs* human amniotic fluid stem cells, *HUCB* human umbilical cord blood, *MDFSCs* muscle-derived mesenchymal stem cells, *LPP* leak point pressure, *MBV* maximum bladder volume, *NGF* nerve growth factor, *RUPP* retrograde urethral perfusion pressure

14.2.3 Fecal Incontinence

14.2.3.1 Animal Models

Based on animal and clinical experiences of MSCs therapy for the treatment in urinary incontinence, in 2008 Lorenzi et al. and Kang et al. published the results of the first two in vivo studies with induced anal lesion.

In his study, Lorenzi treated an experimental rat model of anal sphincters injury followed by surgical repair with intrasphincteric injection of expanded rat bone marrow mesenchymal stem cells (BMSCs). The results indicate that, 30 days later, the injection of BMSCs led to an increase of muscle tissue in the injured area of the external and internal anal sphincter in which it is possible to observe abundant muscle cells of different sizes and irregularly disposed. Furthermore, functional studies highlighted an improved contractility of muscle fibers [135].

Kang injected 3×10^6 MDSCs into the anal sphincter in rats with cryoinjured anal sphincters, as a fecal incontinence model. One week after treatment, the anal sphincter was trimmed and functional tests and microscopic examination were evaluated. In the MDSCs injection group, contraction amplitude was higher than in the control group but not significantly. By immunohistochemical staining, regenerating muscle fibers were observed in variable orientation, both smooth and skeletal [136].

Two years later, White et al. published their study on 120 rats to estimate the effect of myogenic stem cells on contractile function of the external anal sphincter after transection with or without surgical repair. He noted that, in the sphincter repair group, injection of myogenic stem cells induced the enhancement of the contractile function [137]. Aghaee-Afshar et al. in 2009 [138] and Kajbafzadeh et al. in 2010 [139] focused their study on the use of stem cell transplantation in sphincter injury model without subsequent surgical repair. A stem cell injection was performed respectively 2 and 3 weeks after injury, and results were obtained at different intervals from the treatments, showing significant improvement in the electrical activity and in the mean resting pressure of the anal canal.

Furthermore, histopathologic evaluation showed regenerated myotubes and a significant decrease in interstitial fibrosis.

In 2012, Pathi et al. [140] published an interesting randomized study on 204 female rats with external anal sphincter laceration and repair. Animals were treated with direct or intravenous injection of 4×10^6 heterologous BMSCs. The contractile function of sphincters and the parameters of wound healing were analyzed up to 21 days after injury, showing that the direct injection of MSCs into the injured anal sphincter leads to full functional recovery, while the intravenous injection did not fully rescue the compromised sphincter. Direct injection of BMSCs also increased, in the injured area, the expression of the RNA level of lysyl oxidase and TFG-Beta1, two genes involved in collagen, elastin, and matrix synthesis.

Some other significant studies were published in the last years. Salcedo and colleagues [141, 142], in two consecutive articles in 2013 and 2014 on animal model, with induced lesion of the anal sphincter, tested the efficacy of BMSCs via intramuscular or intravenous injected. In both studies, he reported a significant improvement in the anal pressure in BMSCs transplanted groups. However, in the first study, in which BMSCs were also injected into a group of rats with nerve injuries by pudendal crush, he did not report any functional improvement. Nevertheless, he also describes a marked decrease in fibrosis and scar tissue as effects of MSC transplantation.

The articles published in 2015 by a study group of Texas University examine the relationship between the muscular disruption and contractile force of sphincters after transection and repair of external anal sphincter. In intramuscular injection of the myogenic stem group, there was a substantial improvement in the generation of the contractile force, but there was no difference in the anal sphincter volume compared to the control. He suggests that stem cells might improve the contractile function through other cellular processes [143]. Montoya paper [144] reported an original study in which, after 2 weeks from a complete sphincter transection, rats were injected

Table 14.3 Summary of clinical trials of stem cells for urinary incontinence

	Author	Stem cell type	Patients number	Injection/cell number	Outcome	Conclusion
1	Carr et al. [101] (2008)	Autologous MDSCs	8 Women	Intrasphincteric (transurethral injection) ($18\text{--}22 \times 10^6$ cells)	Improvement in SUI at follow-up of 12 months (mean/median 16.5/17) No adverse events	Therapy is safe and clinically feasible
2	Sebe et al. [126] (2011)	Autologous MDSCs	12 Women	Intrasphincteric (transurethral injection) ($1\text{--}5 \times 10^7$ cells)	8 subjects improved symptoms of SUI at follow-up of 12 months No local complication were reported	Cell therapy seems clinically feasible and safe and shows promising results
3	Gotoh et al. [127] (2013)	Autologous ADSCs not expanded (Celution system)	11 men	Intrasphincteric (transurethral injection) $7.5 \times 10^6\text{--}3.3 \times 10^7$ cells	SUI improved progressively in eight patients during the 1-year follow-up No significant adverse events were observed	Cell therapy might represent a safe and feasible treatment modality for male stress urinary incontinence
4	Carr et al. [132] (2013)	Autologous MDSCs	38 women	Intrasphincteric (transurethral injection) Low doses $1\text{--}16 \times 10^6$ High doses $32\text{--}128 \times 10^6$ for one or two times	Improvement of SUI symptoms and quality of life during the 1-year follow-up Improvement is related to cell dose for a greater percentage of patients in the high dose group. No major adverse events were reported	Cell therapy reduces SUI severity and improves quality of life A potential dose-dependent treatment effect was observed with a trend toward greater efficacy in patients who received doses of 32×10^6 or greater per treatment

(continued)

Table 14.3 (continued)

	Author	Stem cell type	Patients number	Injection/cell number	Outcome	Conclusion
5	Gras et al. [128] (2014)	Autologous minced skeletal Muscle (not expanded)	35 women	Periurethral injection	Significant decrease in the mean number of stress leaks and the impact of SUI symptoms during the 1-year follow-up	Treatment is simple and appears to be safe and moderately effective in women with uncomplicated SUI. The treatment compares well to a similar but more complicated regenerative therapy using in vitro expanded muscle-derived cells
6	Stangel-Wojcikiewicz et al. [134] (2014)	Autologous MDSCs	16 Women	Intrasphincteric (transurethral injection) 0.6–25 × 10 ⁶ cells	Success rate 75 %, with 50 % of patients cured and 25 % with improvement during the 2-year follow-up No serious adverse side effects or complications were noted	Treatment is safe and effective. In contrast with other reports, a relatively small number of cells was needed for relieving SUI symptoms
7	Jankowski et al. [133] (2014)	Autologous MDSCs	126 Women* *(enrolled in 4 phase I/II clinical trials)	Intrasphincteric injection 1–200 × 10 ⁶ cells	More patients are responsive to doses of 100 and 200 million AMDC at 12-month follow-up than to lower doses No adverse events attributed to MDSCs	Treatment is safe. Efficacy data suggest that more patients are responsive to doses of 100 and 200 million cells than to lower doses

ADSCs adipose-derived mesenchymal stem cells, *MDSCs* muscle-derived mesenchymal stem cells, *SUI* stress urinary incontinence

with a hydrogel matrix scaffold combined with myogenic stem cells. Neurophysiology tests and histologic examination, performed after 4 and 12 weeks, highlighted how, compared to the control groups, the addition of a biogel scaffold to the myogenic stem cells increases the contractile force and the histological evidence of sphincter restoration with steady improvement over time.

In Table 14.4 the studies on animal model of fecal incontinence reported in literature are summarized.

14.2.3.2 Clinical Study

Until today, only two pilot studies have been produced using MSCs to treat fecal incontinence in humans.

In 2013, Frudinger and colleagues published, for the first time, a study on ten women with damaged anal sphincter by obstetric trauma which were followed up for 1 year after treatment [145]. All patients, with a preventive endosonographic diagnosis of anterior defect of the external anal sphincter, were treated with autologous myoblast cells harvested from a pectoral muscle

Table 14.4 Stem cell studies in animal model of fecal incontinence

	Author	Stem cell type	Animal	Injury/injection	Outcome	Conclusion
1	Lorenzi et al. [135] (2008)	Rat BMSCs	24 male rat	Sphincterotomy and surgical repair of both internal and external anal sphincters 0.75×10^6 cells into each cut end of both sphincters at the same time	The area at the site of injury appeared rich in irregularly disposed muscle cells of different sizes Improved response of anal sphincters to electrical field stimulation compared to control group	BMSCs increases contractile function and improves muscle regeneration
2	Kang et al. [136] (2008)	Rat MDSCs	15 female rat	Anal cryoinjury. Intrasphincteric injection 5 min after injury 3×10^6 cells	Regenerating smooth and skeletal fibers in the area of MDSCs injection Contraction amplitude higher than in the control group but not significantly In BMSCs group decreased muscle fibrosis	Autologous MDSCs grafts may be used as a tool for improving anal sphincter contractility
3	Aghaee-Afshar et al. [138] (2009)	hUCM and rabbit BMSCs	35 male rabbits	External anal sphincterotomy Intrasphincteric injection 2 weeks after injury 10^6 cells	Improvement in the electrical activity at 2 weeks after transplantation compared to pretreatment values and controls	BMSCs may provide an effective tool for treating anal sphincter injuries in humans
4	Kajbafzadeh et al. [139] (2010)	Rabbit myoblast	21 male rabbits	Subtotal external sphincterotomy Intrasphincteric injection 3 weeks after injury 7×10^7 cells	Regenerated myotubes in the injection area. Significant decrease in interstitial fibrosis. Improvement in the mean resting pressure of anal canal at 4 weeks after cell injection	Autografting of muscle progenitor cells showed the potential for a myogenic procedure
5	White et al. [137] (2010)	Rat myoblast	120 female rat	External anal sphincter transection with or without surgical repair Intrasphincteric injection at the same time of the injury 3.2×10^6 cells	Myogenic stem cells enhanced contractile function at both 7 days and 90 day in sphincter repair group	Myogenic stem cells may serve as positive adjunctive therapy in the treatment of anal sphincter injury

(continued)

Table 14.4 (continued)

	Author	Stem cell type	Animal	Injury/injection	Outcome	Conclusion
6	Pathi et al. [140] (2012)	Rat heterologous BMSCs	204 female rat	External anal sphincter transection and surgical repair Intrasphincteric or intravenous injection At the same time of the injury 4×10^6	Direct, not intravenous, resulted in full functional recovery of compromised sphincter at 21 days after cell injection Increased level of lysyl oxidase and TFG-Beta1	MSC may play an important role in increased collagen deposition in the injured site Directly injected MSCs, but not intravenously injected, not only may provide progenitor cells for new myoblasts but also may stimulate matrix production to orient and guide migration of the cells into the injured muscle
7	Salcedo et al. [141] (2013)	Rat BMSCs	70 female rat	Sphincterotomy or pudendal nerve crush Intrasphincteric or intravenous injection 24 h after injury	In sphincterotomy group, not in pudendal nerve crush group, significant increase in anal pressure and sphincter EMG frequency at 9 days after cell injection	MSC treatment either IM or IV resulted in improved anal sphincter pressures after a direct injury to the anal sphincter complex
8	Salcedo et al. [142] (2014)	Rat BMSCs	50 female rat	Excision of 25% of the external and internal anal sphincter Intrasphincteric or intravenous injection 24 h after injury 5×10^5 cells	Significant improvement in anal pressure Marked decrease in fibrosis and scar tissue in both intramuscular and intravenous BMSCs transplanted groups, at 10 and 35 days after cell injection	The process of increased pressures after i.v. infusions can be explained by MSCs homing to the site of injury in response to the cytokine There is a possibility of a bulking effect of an i.m. injection that needs to be explored

	Author	Stem cell type	Animal	Injury/injection	Outcome	Conclusion
9	Fitzwater et al. [143] (2015)	Heterologous rat myoblast	40 female rat	External anal sphincter transection and surgical repair Intrasphincteric injection at the same time of the injury 3.2×10^6	Substantial improvement in contractile force; no difference in anal sphincter volume, compared to the control, at 7 and 90 days after cell injection	The study suggests that the myogenic stem cells can improve the contractile ability of the anal sphincter through more complex cellular mechanisms besides merely the volumetric or morphological findings
10	Montoya et al. [144] (2015)	Heterologous rat myoblast	80 female rats	External and internal anal sphincter transection Intrasphincteric injection of stem cells combined with <i>hydrogel matrix scaffold 2</i> weeks after injury 3.2×10^6 cells	Increasing of contractile force and histological evidence of sphincter restoration at 4 and 12 weeks after injection	A biologically compatible matrix may facilitate stem cell survival, differentiation, or functions leading to recovery of tissue function

BMSCs bone marrow-derived mesenchymal stem cells, *MDSCs* muscle-derived mesenchymal stem cells

biopsy and cultured for 39 days. Muscle-derived stem cells were injected under ultrasonography guidance into external anal sphincter ends and also in the interposed scar tissue. Twelve to 14 individual injections of 0.5 ml for a total of 6–7 ml (the number of myoblasts is not specified) were administered, making sure not to treat directly the internal anal sphincter. No adverse events were observed, and the procedure was well tolerated. At 12 months, all patients stated that their symptoms had improved and the Rockwood Fecal Incontinence Quality of Life Scale [146] resulted significantly improved. The Wexner Incontinence Score [147] had a significant decrease from a mean value of 15.3–1.6. However, the initial significant increase in mean and maximal anal squeeze pressures seen at 1 and 6 months was transient and not sustained at 12 months. As matter of fact, between the 6 and 12 months assessment, also a significant decrease in mean and maximal anal resting pressure occurred. The author reported in an additional article published in 2015 the updates of the 5-year follow-up for the ten patients reporting significant improved symptoms of anal incontinence.

The second study on regenerative treatment in patients affected by fecal incontinence was produced by the group of Giori and coinvestigators. In this pilot study, performed according to the Declaration of Helsinki and approved by the ethics committee, they treated 15 patients (14 females and 1 male) with incontinence due to obstetric injury and anorectal-pelvic surgery. Preliminary results of this study were published in 2015 [148]. Actual experience of the authors in the use, for the first time, of Lipogems® technique to treat patients with fecal incontinence is described below.

14.2.4 Personal Experience with Technique Lipogems®

Lipogems® is a regenerative product by autologous lipoaspirated fat rich in mesenchymal stem cells, obtained in a completely closed system by a disposable device without using enzymes, additives, and other manipulations. Mild mechanical

processes of microfracturing, washing, and filtration progressively reduce the size of the fragments of adipose tissue and remove oily substances and blood residues [149] (Fig. 14.5). Differently, from the mesenchymal cells expanded in laboratory, Lipogems micro-tissue is not a “pool” of individual stem cells, but it is composed of small adipose spherical clusters (400/900 µm) with micro-fragments of intact connective structure maintained viable by a stromal vascular fraction (SVF), particularly rich in pericytes and MSCs incorporated in their “natural niche tissue” [150, 151] and exposed on the surface of the vascular stroma. The micro-fragmented tissue enclosing stromal vascular fraction (SVF) can be ideally assimilated to a biologic matrix scaffold that facilitates the engraftment and the biological activity of MSCs, as evident in previous experience described by Montoya [144]. These properties contribute to make Lipogems® able to survive in a suffering tissue, facilitating the engraftment and the paracrine activity of the embedded cells when autologously inoculated in target tissues [152–154].

However, studies have shown that, when the Lipogems® product was cultured in vitro, it yielded a virtually pure population of hMSCs exhibiting the typical characteristics of surface markers isolated from other sources, including CD90, CD73, CD105, and CD44 [32, 155].

In clinical practice, Lipogems procedure is conducted in a single surgical session that includes three different steps: (1) harvesting of subcutaneous adipose tissue from abdomen or thigh of the patient, (2) processing of adipose tissue with Lipogems® device, and (3) reinjecting the product in the same patient under ultrasound guidance.

Unlike to Frudinger, who had injected MSC only in the area of the sphincter lesion [145], in our study, we injected Lipogems not only in the area of muscle defects but also in the intersphincteric space and around the external anal sphincter [148]. Moreover, because a lesion of the sphincter muscle that affects from outside, such as in childbirth, is always associated to a lesion of the peripheral nerve endings, we have found it useful to also stimulate a neural-regenerative effect injecting Lipogems® also along the course of

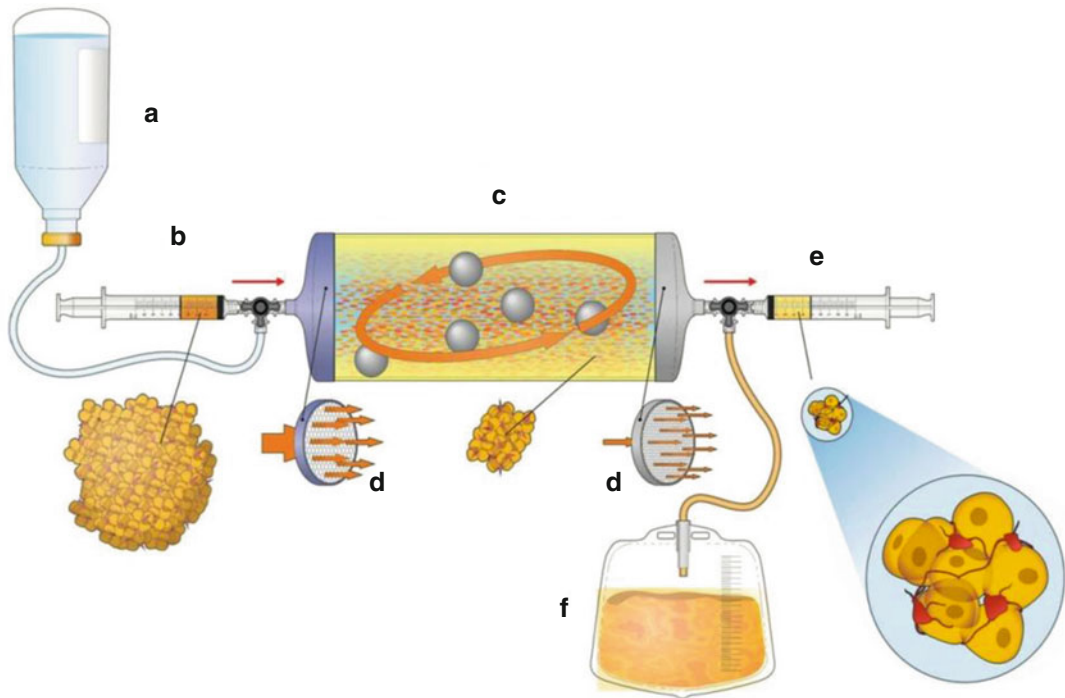


Fig. 14.5 Lipogems device is a disposable closed system filled with physiologic solution. It reduces the size of lipoaspirate clusters after washing of oil, blood, and cellular debris. (A) sac with saline solution; (B) syringe with lipoaspirate; (C) washing chamber containing marbles for

the emulsion of fluid and elimination of oil and blood; (D) mechanical filters, (E) syringe with clusters of microfractured adipose tissue rich in MSCs; and (F) sac with waste oil and blood

peripheral pudendal nerve. An average of 340 cc of lipoaspirate was collected from subcutaneous fat of each patient. As a result of processing with the technique Lipogems®, an average of 87 cc of product was obtained, ready for injection in every patient. After treatment, the 15 patients of this series were followed up for 2 years. Wexner Incontinence Score, Rockwood Fecal Incontinence Quality of Life Scale, digital exploration, proctoscopy, endoanal ultrasound, and anorectal manometry were used before treatment and after 3, 6, 12, 18, and 24 months from the injection of ADSCs, to assess fecal incontinence. The procedure has proven to be safe and well tolerated. In all patients, there were no adverse events related to ADSCs injection. Only in one patient occurred a hematoma at the site of harvesting in the subcutaneous adipose tissue, which resolved spontaneously. Improvement both short and long term was observed in all patients.

Patients' satisfaction for the treatment was very good, and the Fecal Incontinence Quality of Life Scale increased from a mean preoperative overall value of 53–102 after 3 months from treatment, essentially unchanged for the 2 years of follow up.

In the 15 patients, a significant improvement in the average values of the overall Wexner Incontinence Score was observed which decreased by a mean preoperative of 14.1 units to 3.4 units at 3 months and remained quite stable over the time of the study with a value of 4.1 at 24 months (Fig. 14.6). After treatment, the overall mean values of the anal pressure at rest and in squeeze improved in all the patients as reported in Fig. 14.7. In the graph, a remarkable increasing of the anal squeeze pressure during the entire second year of follow-up is evident. Ultrasound examinations showed a progressive reabsorption of the hyperechogenic Lipogems tissue from 3 to 12 months and increasing of muscle

Fig. 14.6 Average values of the Wexner Incontinence Score (0–20) before and after treatment

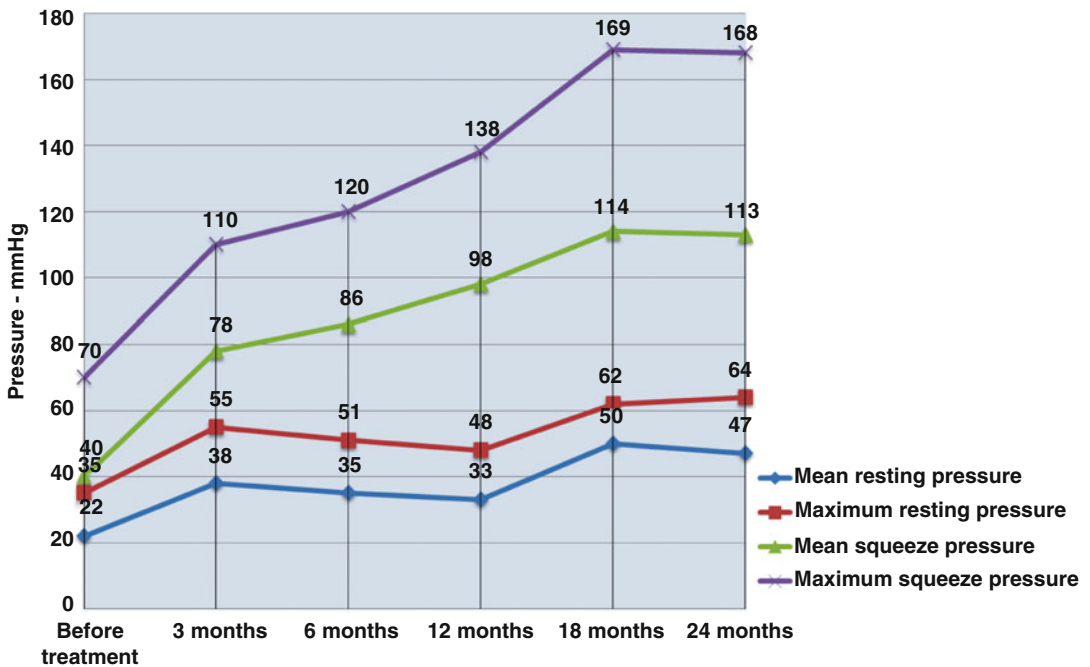
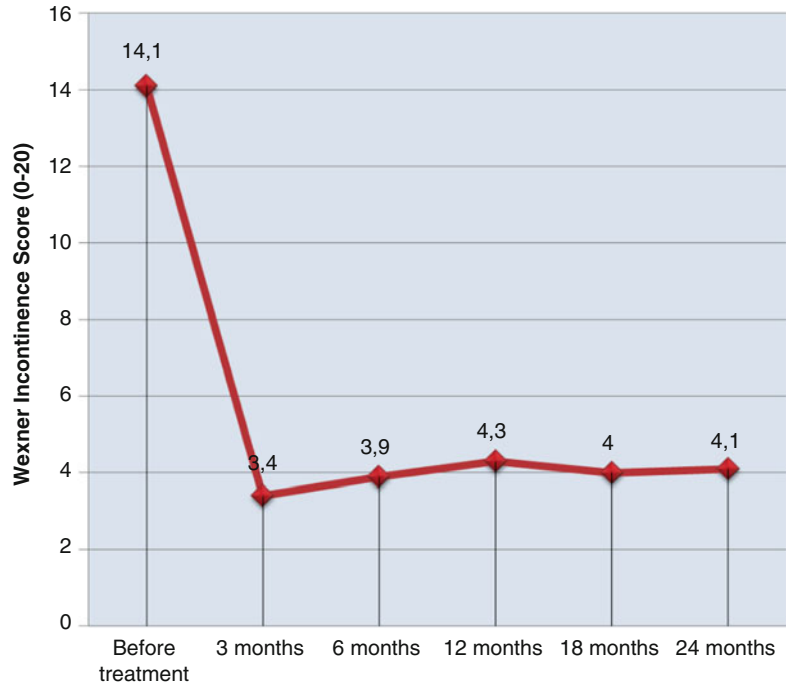


Fig. 14.7 Average values of anorectal manometry (mmHg) before and after treatment

fibers with images of sphincter restoration at 12 and 24 months in several patients (Fig. 14.8). After treatment, physical examination and proctoscopy

did not show any new pathological findings of the anorectal complex. At palpation the enhancement in the contractile activity of the anal sphincters and

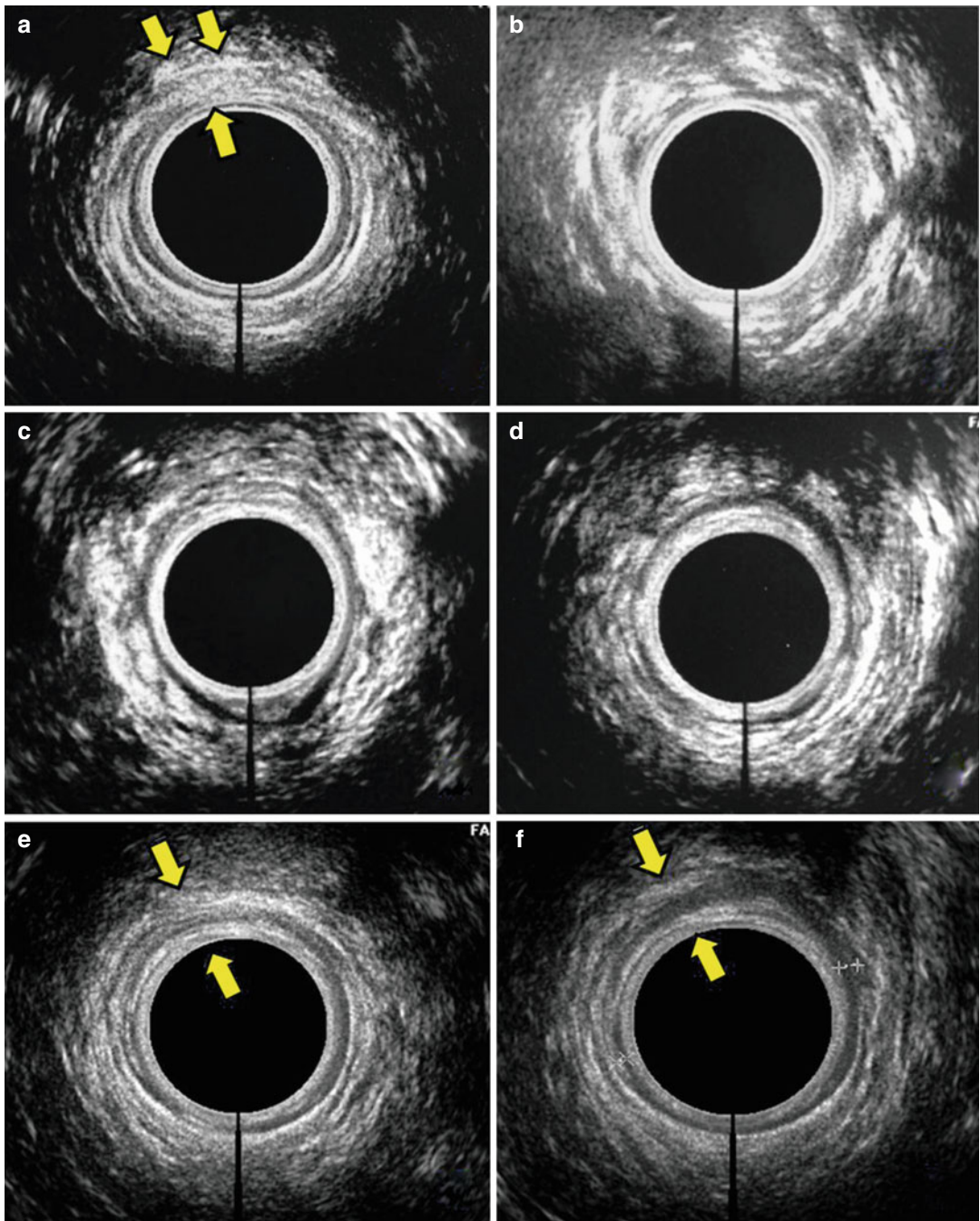


Fig. 14.8 Endoanal ultrasonography images of a representative patient at preoperative, intraoperative stage and at 3, 6, 12, 24 months after treatment. (a) Preoperative stage. Lesion of the external and internal anal sphincter localized in the anterior area, evidenced by arrows (b) Intraoperative stage. Diffuse hyperechoic spots at the sites of inoculation of Lipogems. (c) 3rd month. Partial resorption of hyperechoic spots of

Lipogems. (d) 6th month. Aspect of the early development of new muscle tissue at the site of the lesion. Are still evident few hyperechoic spots of Lipogems. (e, f) 12th and 24th month respectively. The image shows muscle restoration in the area of previous lesion of the anal sphincters evidenced by arrows. No more evidence of hyperechoic depots of Lipogems

improvement of anocutaneous reflex, where it was lacking before treatment, was clearly observable.

From a detailed analysis of patients' data, between the 3rd and the 12th month, we have observed a slight decline of the resting pressure associated with mild worsening of the Wexner index. This feature is, in our opinion, attributed to a temporary bulking effect of the "not stem cells" component of Lipogems, which is reabsorbed in a period between 3 and 12 months from treatment, as it usually occurs with the traditional techniques of biological lipofilling [156]. Thus, the clear increase in the contractile ability of the anal sphincter recorded by anorectal manometry after 12 months (Fig. 14.8e, f), when deposits of material inoculated disappeared and ultrasound images showed muscle restoration, is attributable to the effect of mesenchymal cells.

This is also indirectly confirmed by the results of Frudinger, who obtained a temporary increase in anal squeeze pressures not sustained at 12 months as reported in her first article. However, the extension of the follow-up described in the second publication has proven a slow and gradual improvement of incontinence, which remained unchanged through all the 5 years of observation [145, 157].

14.2.5 Final Considerations

On the basis of preclinical studies and clinical trials conducted both in the field of urinary incontinence and fecal incontinence, the use of autologous MSCs revealed to be safe, minimally traumatic, well tolerated, and effective in improving the symptoms of incontinence. In particular, new techniques like Lipogems®, which uses autologous stem cells derived from adipose tissue easy to harvest and prepare the product using a device directly in the operating room in the course of a single surgical time, make the use of mesenchymal stem cells of simple execution and that are inexpensive and widely applicable in clinical practice. Further investigations are necessary to clarify the efficacy and the biological mechanism of this very simple regenerative procedure, but a future association together with surgical repair of damaged anal sphincters is also possible.

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