American Journal of Biomedical Science & Research

www.biomedgrid.com

ISSN: 2642-1747

Review Article

Very Small Embryonic Like Stem Cells: A Review of Basic Science, Applications, and Potential Use in Orthopedics

Pierdanilo Sanna, Loubna Abdel Hadi, Rene Antonio Rivero Jimenez, Antonio Alfonso Bencomo Hernandez, Aya Zakaria, Samira Sheikh, Yendry Ventura Carmenate and John Phillips*

Abu Dhabi Stem Cells Center, UAE.

***Corresponding author:** John Phillips, Abu Dhabi Stem Cells Center, 25 Al Misaha St, Abu Dhabi, UAE.

To Cite This Article: *Pierdanilo Sanna, Loubna Abdel Hadi, Rene Antonio Rivero Jimenez, Antonio Alfonso Bencomo Hernandez, Aya Z, et al. Very Small Embryonic Like Stem Cells: A Review of Basic Science, Applications, and Potential Use in Orthopedics. Am J Biomed Sci & Res. 2022 17(4) AJBSR.MS.ID.002377,* DOI: 10.34297/AJBSR.2022.17.002377

Received: December 07, 2022; **Published:** December 12, 2022

Abstract

Research studies designed to show the clinical efficacy of the Pluripotent/Multipotent Stem Cells to differentiate into three germ layers have been conducted in the context of Regenerative Medicine (RM). This review describes recent clinical applications and the potential use of Very Small Embryonic-like Stem Cells (VSELs) in the context of orthopedic problems such as degenerative joint disease. VSELs are non-hematopoietic (Lin-/CD45-), rare, and quiescent cells; they were reported to be "dormant" cells in the Bone Marrow (BM). However, they are now found in cord blood, Peripheral Blood (PB), and other organs. Their positive expression of pluripotency markers, such as Oct-4+/Nanog+/SSEA-1/4+/CXCR4+, has been used to hypothesize that these cells could be deposited early during embryonic development as descendants of epiblast-derived stem cells, and perhaps from a more primordial germ cell. VSELs can be released/mobilized from the BM to the PB during tissue injury and stress, facilitating the regeneration of damaged tissues and stimulation of mesenchymal stem cells to divide/differentiate. Nowadays, VSELs can be expanded ex vivo. Their pluripotency could be suitable for applications in RM, solving several problems regarding the use of both controversial embryonic stem cells and induced pluripotent stem cells. Additional studies on VSELs and their characterization will provide a better understanding of their biological and regenerative potential for future clinical applications in orthopedics.

Keywords : Very Small Embryonic Like Stem Cells; Pluripotent Stem Cells; Regenerative Medicine; Orthopedics

Abbreviations: RM: Regenerative Medicine; Vsels: Very Small Embryonic-Like Stem Cells; BM: Bone Marrow; PB: Peripheral Blood; MSC: Mesenchymal Stem Cells; CB: Cord Blood; HESCS: Human Embryonic Stem Cells; IPSCS: Induced Pluripotent Stem Cells; HSCS: Hematopoietic Stem Cells; NHSCS: Non-Hematopoietic Stem Cells; EPSCS: Epiblast-Derived Stem Cells; PGCS: Primordial Germ Cells

Introduction

Many cell types have been studied and applied in cell therapy and RM. In orthopedics, Mesenchymal Stem Cells (MSC) are used to regenerate and repair bone, cartilage, and tendons. Identified in 1955, [1,2]. MSCs were first isolated from rat BM by Frieden stein in 1970 and characterized as non-hematopoietic, multipotent, plastic adherent, and fibroblastic-like stem cells [3]. Subsequently, these cells were isolated from several sources, in small numbers, and grown in a culture where they could differentiate into bone, cartilage, adipose tissue, tendon, muscle, and fibrous tissue. Since their discovery, numerous names have been suggested, and Caplan in 1991 [4] proposed to call them "Mesenchymal Stem Cells," [5,6] which is the most common name in the international nomenclature [7]. After 25 years of studies and debates, Caplan in 2017 suggested a new definition: "Medicinal Signaling Cells". Other proposed names include "Maintenance Stromal Stem Cells" [8]. Both definitions are more accurate in describing the functional characteristics of these cells since they possess a homing activity, towards injured or degenerated tissues, caused by their ability to secrete bioactive factors, including growth factors, cytokines, lipids, and nucleic acids present in their extracellular vesicles. These factors work in concert to activate the site-specific and tissue-specific resident

stem cells responsible for structuring and regenerating new tissue. In this context, MSCs have been described as cells with regenerative potential based on their immunomodulatory ability rather than an attributed stemness profile. The extensive use of MSCs in RM is related to a combination of the following: 1) ease of harvesting from fat tissue, Bone Marrow (BM), Cord Blood (CB), and other sources; and 2) their capability to be expanded and the standard laboratory culture conditions used for propagation. Many groups consider MSCs as "cell-drug" delivered in situ with the ability to drive, regulate, and maintain the tissue healing process. MSCs induce healing by activating resident progenitor stem cells, which are responsible for differentiating and regenerating the damaged tissue. Many studies report that only a small percentage of implanted MSC survive and undergo limited self-renewal and proliferation, but the rest undergo apoptosis after activation and release of bioactive factors. Indeed, most of them get trapped in the capillary network and disappear from the injection site in a short time [9,10]. Given the perceived lack of persistence, treatment protocols in RM suggest multiple administration doses of MSCs (2-4 weeks apart). Recent studies are ongoing to optimize their survival and engraftment to increase their performance [11].

Researchers aim to find the "perfect" stem cell, able to differentiate and regenerate all kinds of human tissues as needed. The use of Human Embryonic Stem Cells (hESCs) might be a candidate for therapeutic cells in RM, but several ethical and political dilemmas limit research on hESCs. However, substantial roadblocks still exist for their difficult isolation, genomic instability, inability of in vitro differentiation into distinct cell types, carcinogenic risk, and immune rejection [12]. Establishing core parameters essential for optimizing clinical efficacy and facilitating safety profiling of hESCs-derived products is an urgent need for future therapeutic applications.

Induced Pluripotent Stem Cells (iPSCs) are now being developed as a model to study genetic diseases and to provide a platform for personalized gene therapy and organ development. The development of iPSCs is still early. Many fundamental scientific questions remain regarding their inefficient derivation, tendency to differentiate into fetal counterparts, harboring mitochondrial mutations, genomic instability, and epigenetic state of somatic cells. Additionally, iPSCs are being developed and planned to be used in an autologous manner. This raises the concerns of considerable expenses and time required to isolate, expand, and differentiate, making iPSCs less useful for large-scale clinical trials [13].

Hematopoietic Stem Cells (HSCs) represent another potential source of stem cells in RM applications. Under physiological conditions, HSCs harboring multipotent capacity can fully reconstitute all the components of the blood system. Additionally, HSCs can regenerate multipotent progenitor cells that can maintain all lineages of blood cells for decades. There are several sources of HSCs, including BM, CB, peripheral blood (PB), and mobilized PB [14], the frequency of progenitors varies between sources. Interestingly, the ability of HSCs to trans-differentiate into different cell types not belonging to the hematopoietic system has been reported. Indeed, this concept of plasticity or trans-differentiation of HSCs has been published by several groups, showing that HSCs from BM are capable of giving rise to hepatic cells [15,16], skeletal muscle [17,18], cartilage [19], brain microglia as well as macroglia [20,21], endothelial precursors [22], and cardiac muscle cells [23,24]. Clinical Research programs are now focused on applying HSCs as a regenerative therapy in various non-hematological conditions such as neurological disorders (Parkinson's Disease, Autism), ischemic conditions (stroke, myocardial ischemia), and orthopedic conditions like cartilage degeneration or defects. However, significant additional research is needed for the thorough characterization and the trans-differentiation potential of HSCs.

Almost 20 years ago, a population of stem cells called Very Small Embryonic Like Stem Cells (VSELs) expressing pluripotency markers and possessing the ability to differentiate into the three germ layers was reported by Ratajczak, et al. [25]. Despite their multipotency, VSELs still need to be widely acknowledged by the scientific community. Additional studies must be completed to refine and optimize their isolation, characterization, and identification of their biological potential [13].

The scope of this review is to introduce VSELs, by providing an overview about their pluripotency, their application in RM, and, more specifically, their potential in the orthopedic field. Here, we address new studies that hopefully open up new frontiers to understand better VSEL's potential for future clinical applications. For this purpose, literature research of online databases (PubMed, EMBASE, and GOOGLE SCHOLAR) was performed by including the following terms from each of the following two groups. Group 1 consisted of "Very Small Embryonic-like Stem Cells," "VSEL" or "VSELs," or "pluripotent stem cells," or "pluripotency." Group 2 contained "stem cells," "regeneration," "Tissue regeneration," "musculoskeletal," "osteoporosis," "bone defect," "bone," "osteoarthritis," "chondral lesion," "degenerative arthritis," "Tendon" " ligament," "muscle," "fracture," "orthopedic." Abstracts and any articles not written in English were excluded.

Discussion

Very Small Embryonic-Like Stem Cells: Hidden Gems

Bone Marrow is a well-known source of stem cells containing HSC and Non-Hematopoietic Stem Cells (NHSC) populations. Both types have been shown to be critical players for tissue regeneration and repair. The ability of HSCs, endowed with "plasticity" features, to regenerate non-hematopoietic organs has been reported by several groups [26,27]. While the HSC plasticity concept may remain questionable, several studies have reported that in pathological conditions, HSCs can trans-differentiate in non-hematopoietic lineage with the ability to regenerate damaged tissues [13-15,18,20,23,28]. A survey by Kucia, et al. [27] indicated the presence of a heterogeneous population of NHSCs in BM [29-31]. A subpopulation of these NHSCs was identified and called

VSELs in 2006 [32]. They are similar in size to the cells in the inner cell mass of the blastocyst, found to be ~ 2–4 μm in mice and ~5-7 μm in humans, and they are larger than peripheral blood platelets and smaller than erythrocytes. VSELs present a simple morphology, characterized by a thin rim of cytoplasm around a large round nucleus containing unorganized and lightly packed euchromatin [33,34]. In addition, VSELs are reported as novel primitive stem cells negative for both hematopoietic lineage markers (Lin) and the pan leukocyte marker CD45 and positive for CD34+, CD133+, CXCR4+, C-met, and LIF-R [33,34]. VSELs highly express pluripotency core Transcription Factors (TFs), such as Oct4, Nanog, Sox2, SSEA-1/4, Klf4 and the telomerase protein Rif1. These TFs form an interconnected autoregulatory network in maintaining stem cells pluripotency and self-renewal [34,35]. Their high nuclear-to-cytoplasmic ratio as well as the low number of mitochondria make them developmentally primitive with very low metabolic activity, earning VSELs the title of "dormant cell". Accordingly, their existence in BM, CB, PB, and adult organs is maintained under a strict condition of quiescence. Based on their pluripotent nature, it has been hypothesized that VSELs are direct descendants of epiblast-derived stem cells (EPSCs) and likely primordial germ cells (PGCs) [25,36] (Figure. 1).

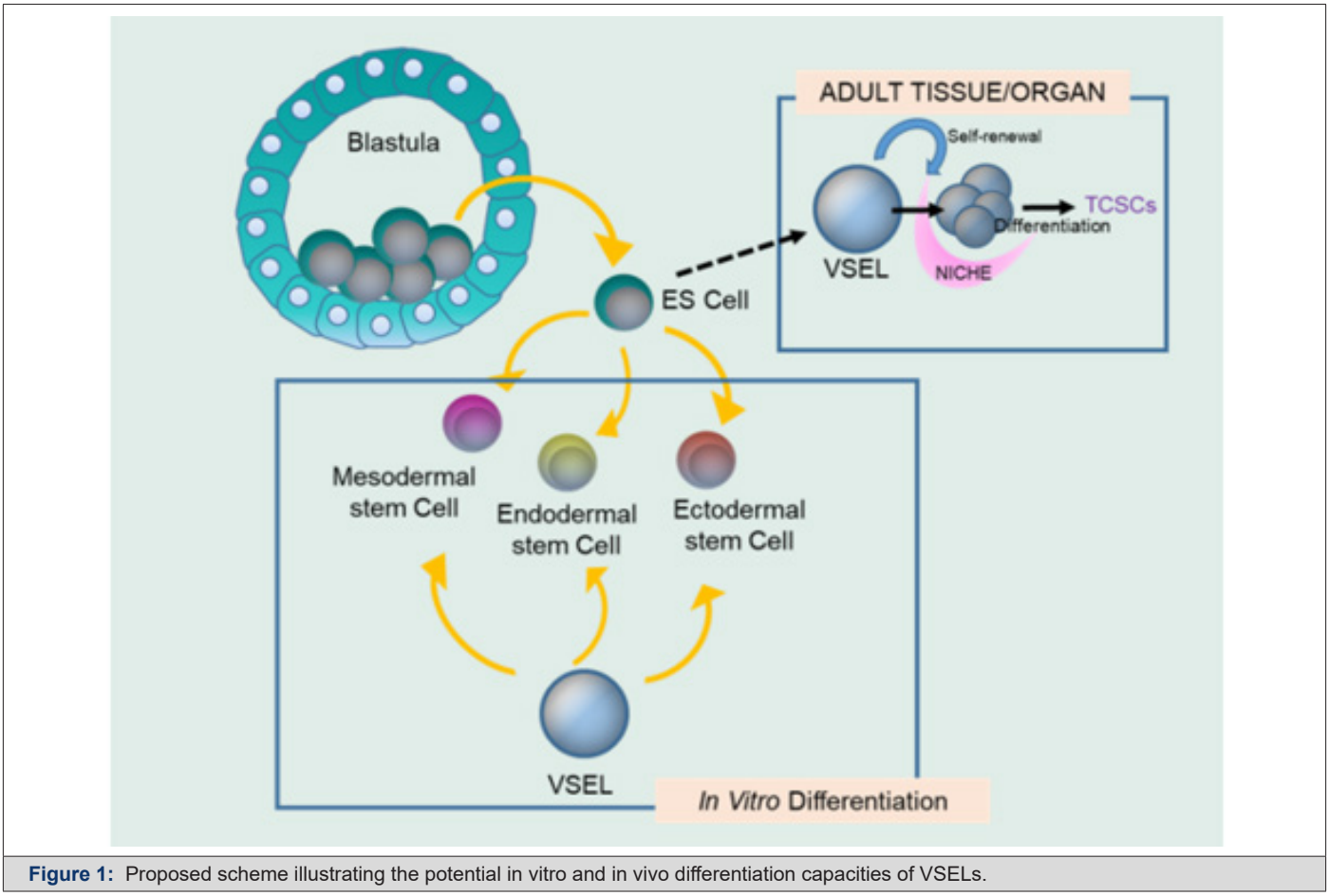

Figure 1: Proposed scheme illustrating the potential in vitro and in vivo differentiation capacities of VSELs.

The origin of the VSELs is likely from cells of the germline lineage and are deposited as reservoirs to augment adult stem cells in developing organs during embryogenesis. *In Vivo*, VSELs reside as a population of Pluripotent Stem Cells (PSCs) able to self-renew and differentiate by interacting with their niche to monopotent Tissue Committed Stem Cells (TCSCs). In Vitro, VSELs demonstrate their multilineage cellular differentiation ability by mimicking the Embryonic Stem Cell (ES cells) properties.

VSELs have the capacity of self-renewal and can be considered a backup pool of stem cells that actively contribute to the turnover of other tissue specific mono-potent stem cells located in peripheral niches. Once activated, they can contribute to tissue/organ regeneration. Indeed, VSELs are mobilized to various organs under stressful conditions, injury, or diseases like myocardial infarction [37], stroke [26,38], leg ischemia [39], pulmonary diseases [40], or cytotoxic treatments [26]. It has been demonstrated that they can give rise to tissue-committed progenitors that maintain lifelong homeostasis [25]. Kucia, et al. [27] postulated that because VSELs show tropism to stromal cells and undergo emperipolesis in cocultures with BM-derived fibroblasts, they could be co-isolated with other cells fractions of BM cells, including MSC [41], Multipotent Adult Progenitor Cells (MAPCs) [42], Marrow-Isolated Adult Multilineage Inducible (MIAMI) Cells [43], and Multilineage Differentiating Stress Enduring Cell (MUSE) [44,45]. However, there is still a lack of consensus on the phenotypic markers used

to describe this cell type due to the overlapping nature of the pluripotent cells and the lack of consistency used in the markers followed in the isolation protocols of pure VSELs. Further studies are needed to solidify a platform for the isolation and characterization of VSELs.

VSELs mostly remain in the G0 state of the cell cycle and undergo rare asymmetrical cell divisions to self-renew. They can also give rise to progenitors that divide rapidly by undergoing symmetrical cell divisions and clonal expansion. This is thought to be then followed by differentiation into tissue-specific cell types based on their location [46]. Unlike ESC, HSCs, or ES/iPSC, VSELs can spontaneously differentiate into adult cell types regenerating *in vivo* tissues and organs, like the pancreas and even gametes [36]. Therefore, VSELs may have the most significant potential, among other endogenous stem cell candidates, to regenerate adult tissues of many lineages (Table 1).

Table 1: Comparison of Stem Cells: Human Embryonic, induced Pluripotent, Hematopoietic, Mesenchymal, and Very Small Embryonic-Like.

Cells	Potency	Markers	Source	Immunological Issue	Origin	Differentiation/ Plasticity	Expansion	Stemness	Tumor Formation (Teratomas)
hEC	Pluripotent	OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81	Embryo/ Placenta	yes	Embryo/ Placenta	3 layers	+++	++++	++
iPS	Pluripotent	OCT4, SOX2, SSEA4, TRA-1-60 TRA-1-81	Skin/ Blood (reprogrammed)	yes	Somatic Cells	3 layers	+++	++++	+++
HSC	Multipotent	CD34+, CD59+, CD90/Thy1+ CD38low-c-Kit-/low, Lin-	BM PB UC	No	Red BM	MESODERM Hematopoietic Plasticity +		+++ Committee Progenitors	-
MSC	Multipotent	CD73+, CD90+, CD105+ CD34-, CD45-CD11b-, CD14-, CD19-, CD79a, HLA-DR	BM Adipose Tissue Dental Pulp Derma Myocardium	No	Pericyte/ stroma	MESODERM-bone, cartilage, adipose tissue, tendon, muscle, and fibrous tissue.	+++	+ Committee Progenitors	-
VSELs	Pluripotent	CD90+ CD133+ CD44+ Sca1+Oct-4+ NANOG+ SSEA 1/4+ Rex1+ CXCR4	BM PB UC Adipose Tissue Ovarian Testis Myocardial	No	Epiblast/ Germ Cells	3 layers	+	++++	-

Note: hEC: Human embryonic stem cells; iPS: Induced pluripotent stem cells; HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells; VSELs: Very small embryonic-like stem cells; OCT4: Octamer binding transcription factor 4; SOX2: Sex determining region Y-box 2; SSEA4: Stage-Specific Embryonic Antigen-4; TRA-1-60/ TRA-1-81: Surface Antigen Podocalyxin; HLA-DR: Human leukocyte antigen DR isotype; Sca1: Stem cells antigen 1; Rex1: Reduced expression 1; CXCR4: Chemokine receptor type 4; BM: Bone marrow; PB: Peripheral blood; UC: Umbilical cord.

One of the main problems in using VSELs in RM is the limited frequency and difficulty of isolation. A common question arises: Will the isolated population have sufficient capacity to expand to achieve the desired regeneration? Despite sorting and other technical difficulties, VSELs can be grown in vitro, maintaining their pluripotency and ability to differentiate without manipulation [47]. Several methods have been tested to improve VSEL's culture conditions by using cytokine combinations, feeder cell co-cultures, recombinant proteins, small molecules, and cold temperature [48-50]. Lahlil et al. have demonstrated that VSELs expanded in a pyrimidoindole derivative (UM171) medium to remain mononuclear by maintaining undifferentiated morphological features even after 12 days of culture [48]. To date, the knowledge of how and for how long native VSELs maintain their pluripotent and differentiation potential remains unknown. Kucia, et al. [27] group has demonstrated that highly purified mouse BM-derived VSELs express a low level of mitotic genes and have a similar but not identical transcriptome to ESCs, which proliferate and may differentiate with the correct stimulation [51]. Co-culturing of VSELs with a C2C12 supportive cell line produced a unique pattern of differentiation where the imprinted gene methylation is reverted, which may explain, in part, VSELs quiescent status [52]. Owing to their limited number, quiescence, and poor ability to expand in vitro, these challenges are viewed by many groups as a limitation for VSELs use in RM [53].

Notwithstanding these in vitro challenges, VSELs are potent cells able to differentiate in vitro into all three germ-layer lineages (Ectoderm-Mesoderm-Endoderm) without forming teratomas [54]. Lack of VSELs teratoma development can be explained by modified methylation of specific genes residing in their Differentially Methylated Regions (DMRs): a deletion of paternally imprinted genes within the Igf2-H19 and Rasgrf1 loci and a hyper-methylation of the Igf2 receptor (Igf2R), Kcnq1-p57KIP2, and Peg1 loci were reported. Because paternally expressed imprinted genes (Igf2 and Rasgrf1) enhance embryonic growth and maternally expressed genes (H19, p57KIP2, and Igf2R) inhibit cell proliferation, the unique genomic imprinting pattern observed in VSELs demonstrates the growth-repressive influence of imprinted genes on these cells [51]. As a result, there is no uncontrolled proliferation like late migratory PGCs, which may also explain the quiescent state of VSELs in adult tissues [54,55].

Potential of VSELs

A better understanding of the phenotype of VSELs is still required for their biology and molecular mechanism governing their quiescence, activation, proliferation, targeting, and differentiation. Deep phenotyping will provide a precise stratification of VSELs into subsets and, accordingly, a better understanding of their biological mechanisms. There are reports of different sub-populations of VSELs that have different gene expression profiles and therefore different in their functional response, Lahlil, et al. [56] have demonstrated that VSELs Lin-CD34+CD45- expressing CD133 or NANOG have the same expansion, and differentiation capacities towards the mesodermal and endodermal pathways. The same study reported that VSELs expressing the CXCR4 marker have less ability to proliferate and differentiate [56]. This suggests that the VSELs phenotype regulates their response to pathophysiological needs; in fact, they are well-oriented to determine *in vivo* which of their subpopulation is needed to repopulate the tissue where they reside. By achieving a thorough characterization, VSELs can represent a valid alternative candidate for stem cells in clinical applications since their proliferation and differentiation *in vivo* are well monitored and controlled.

Additionally, studies will be needed to characterize the VSELs transcriptome to determine which genes govern VSELs quiescence [54]. It is also necessary to demonstrate that VSELs can regenerate damaged tissue in animal models and humans on administration. During the pandemic of COVID-19 in Abu Dhabi, UAE, researchers developed a patented procedure to harvest an autologous peripheral blood non-hematopoietic enriched stem cells cocktail (PB-NHESC-C) called UAECell-19® [57] in which VSELs constituted a significant component. This cocktail was administered by jet nebulization to improve the clinical conditions of COVID-19 patients in the SENTAD-COVID Study (NCT04473170), an adaptive, prospective, and multicentric phase I/II clinical trial involving hospitalized adult patients with confirmed COVID-19 infection. The primary objective was to assess the safety and efficacy of the UAECell-19® for therapy as an add-on treatment to standard care for COVID-19 patients. The study included a total of 139 randomized COVID-19 patients, with 69 in the experimental group and 70 in the control group (standard care). The study conclusion was the first report of an autologous treatment with minimally manipulated stem cells. The main component of the cocktail is non-hematopoietic cells, which were obtained using a simplified autologous cell isolation procedure that can be implemented in blood banks or transfusion center facilities. The UAECell-19® was safe and improved the clinical and laboratory outcomes in most treated patients with the potential to reduce hospitalization and mortality [58]. Several clinical studies were designed using VSELs as a cell drug in facial skin antiaging (NCT03976206), Erectile dysfunction (NCT03973021) and premature ovarian failure (NCT03985462).

Potential Application of VSELs in Orthopedics

The ability of VSELs to differentiate and become committed to the regeneration of some tissues and organs has been demonstrated; however, their use in orthopedics is limited thus far. VSELs research is needed in this area to better understand how they can dovetail into potentially novel applications within orthopedics and RM.

The first study that hypothesizes that VSELs could play a pivotal role in the normal rejuvenation of adult tissues and involvement in the regeneration of damaged organs was published in 2008, soon after discovering VSELs by the Ratajczak, et al. [25] and Kucia, et al. [27,57]. These authors envisioned that the potential of these cells for tissue and organ regeneration could also be harnessed to decelerate the aging processes. Since then, several studies and considerable research has been conducted to better understand the possible clinical application of these cells in several medical fields.

In 2013 Havens et al. published one exciting article showing the capability of human VSELs (hVSELs) to generate skeletal structure *in vivo* [59]. VSELs isolated from blood by apheresis following granulocyte–colony-stimulating factor mobilization was fractionated and enriched by elutriation and fluorescence activated cell sorting. Sponge scaffolds made with collagen-containing 2,000–30,000 hVSELs were implanted into cranial defects in SCID mice. A microcomputed tomography analysis showed that a cell population, including VSEL, produced mineralized tissue within the cranial defects compared with controls at three months. Histological studies showed significant bone formation and cellular organization within the defects compared with cellular or scaffold controls alone. When hVSELs cells were implanted into a cranial wound defect, woven human bone was generated with marrow cavities and contained within the marrow spaces of the human bone, has been found osteoblasts, chondrocytes, and human neural adipocytes as well [60]. These studies also pointed out that both human (hVSELs) and murine (MuVSELs) cells could be induced to express markers that are consistent with the acquisition of osteoblastic (Runx2, osteocalcin), adipocytic (PPAR-g) and endothelial phenotype (CD31, Factor VIII) cells that are mesenchymal derivatives.

Lepik et al. in a study published in 2020, made significant bone defects in the femurs of 38 Sprague Dawley female rats and treated them with β-TCP scaffold granules seeded with male

VSELs. This work demonstrated that VSELs isolated from rat BM-Derived Mononuclear Cells (BM-MNC) contribute to bone healing. In a separate study, BM-MNC, VSEL-depleted BM-MNC or scaffold alone, and bone healing were evaluated after eight weeks post-surgery. Bone healing was remarkably increased in defects treated with VSEL and BM-MNC compared to defects treated with VSEL-depleted BM-MNC. Donor cells were detected in new bone tissue, all the defects treated with cells, and fibrous tissue only in defects treated with VSEL-depleted BM-MNC [58].

As we know, osteogenesis and bone remodeling are complex processes that involve several mechanisms and interactions between different cell populations, not only the osteoblastic and osteoclastic cell lineages. A synergy between progenitor stem cells that maintain the bone cell repertoire and hematopoietic and immune tissue cells also occurs, thanks to the secretion of local cytokines and growth factors [59] and the activation of transcription factors. Osteoporosis is a metabolic disorder related to the osteoblast and osteoclast imbalance and other functions, like bone-vessel coupling and bone-adipocyte coupling [60]. VSELs seem to be promising for use in this kind of skeletal disorder and decelerate the aging processes in bone metabolism that lead to osteoporosis [61]. In 2015, Young Hun et al. induced osteoporosis by ovariectomy in mice and demonstrated, using DEXA and μCT examination, that following a tibial injection of hVSELs, significant differences in the trabecular number (11.24±0.67 vs. 6.78±0.35, P<0.001), bone volume (0.301±0.05 vs. 0.25±0.01, P=0.025), and bone mineral density (1108.56±115.6 vs. 913.02±17.11, P=0.006) of the tibias were noted in animals treated with hVSELs and estrogen vs. those animals not receiving estrogen and hVSELs alone. The authors declared that this study represents the first demonstration in SCID mice that hVSELs can induce bone regeneration in osteoporotic animals [62].

Conclusion

Based on the encouraging results from several studies, it can be concluded that VSELs are an excellent source of regenerative stem cells for Orthopedics and Sports Medicine applications, both for acute and degenerative processes. Considering their propensity to form bones, they could be considered for treating diseases like Osteogenesis Imperfecta, other types of bone defects, pseudarthrosis, and healing delay, as well as avascular necrosis and osteoporosis. Due to their pluripotency, VSELs also represent a valid option to treat several chronic degenerative conditions, such as cartilage erosion and osteochondral defects, joint arthritis, ligaments or tendon injury and degeneration, but more preclinical and clinical research needs to be done regarding these pluripotent cells that and the potential use in clinical regenerative therapies including skeletal and connective tissue disorders.

Acknowledgements

In loving memory of Dr. Pierdanilo Sanna

Conflict of Interest

None of the contributing authors have any conflict of interest.

References

1. Berman L, Stulberg CS, Ruddle FH (1955) Long-term tissue culture of human bone marrow I Report of isolation of a strain of cells resembling epithelial cells from bone marrow of a patient with carcinoma of the lung. Blood 10(9): 896-911.
2. McCulloch EA, Parker RC (1957) Continuous cultivation of cells of hemic origin. Proc Can Cancer Conf 2:152-167.
3. Friedenstein AJ, Chailakhjan RK, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 3(4): 393-403.
4. Caplan AI (1991) Mesenchymal stem cells. J Orthop Res 9(5): 641-650.
5. Caplan AI (2015) Adult Mesenchymal Stem Cells: When, Where, and How. Stem Cells Int 2015: 628767.
6. Caplan AI (2017) Mesenchymal Stem Cells: Time to Change the Name. Stem Cells Transl Med 6(6): 1445-1451.
7. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper Cortenbach I, et al. (2005) Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 7(5): 393-395.
8. Kumar A, Ghosh Kadamb A, Ghosh Kadamb K (2020) Mesenchymal or Maintenance Stem Cell & Understanding Their Role in Osteoarthritis of the Knee Joint: A Review Article. Arch Bone Jt Surg 8(5): 560-569.
9. Liu XB, Chen H, Chen HQ, Zhu MF, Hu XY, et al. (2012) Angiopoietin-1 preconditioning enhances survival and functional recovery of mesenchymal stem cell transplantation. J Zhejiang Univ Sci B 13(8): 616-623.
10. Satue M, Schuler C, Ginner N, Erben RG (2019) Intra-articularly injected mesenchymal stem cells promote cartilage regeneration, but do not permanently engraft in distant organs. Sci Rep 9(1): 10153.
11. Garcia Sanchez D, Fernandez D, Rodriguez Rey JC, Perez Campo FM (2019) Enhancing survival, engraftment, and osteogenic potential of mesenchymal stem cells. World J Stem Cells 11(10): 748-763.
12. Pappas JJ, Yang PC (2008) Human ESC vs iPSC-pros and cons. J Cardiovasc Transl Res 1(2): 96-99.
13. Bhartiya D (2017) Pluripotent Stem Cells in Adult Tissues: Struggling to be Acknowledged Over Two Decades. Stem Cell Rev Rep 13(6): 713-724.
14. Kanji S, Pompili VJ, Das H (2011) Plasticity and maintenance of hematopoietic stem cells during development. Recent Pat Biotechnol 5(1): 40-53.
15. Alison MR, Poulsom R, Jeffery R, Dhillon AP, Quaglia A, et al. (2000) Hepatocytes from non-hepatic adult stem cells. Nature 406(6793): 257
16. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, et al. (1999) Bone marrow as a potential source of hepatic oval cells. Science 284(5417): 1168-1170.
17. Ferrari G, Cusella De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, et al. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279(5356): 1528-1530.
18. Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, et al. (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401(6751): 390-394.
19. Saw KY, Anz A, Siew Yoke Jee C, Merican S, Ching Soong Ng R, et al. (2013) Articular cartilage regeneration with autologous peripheral blood stem cells versus hyaluronic acid: a randomized controlled trial. Arthroscopy 29(4): 684-694.
20. Brazelton TR, Rossi FM, Keshet GI, Blau HM (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290(5497): 1775-1779.

21. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR, et al. (2000) Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290(5497): 1779-1782.

22. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, et al. (1999) Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 85(3): 221-228.

23. Bittner RE, Schofer C, Weipoltshammer K, Ivanova S, Streubel B, et al. (1999) Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Anat Embryol (Berl) 199(5): 391-396.

24. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, et al. (2001) Transplanted adult bone marrow cells repair myocardial infarcts in mice. Ann N Y Acad Sci 938: 221-229

25. Ratajczak MZ, Machalinski B, Wojakowski W, Ratajczak J, Kucia M. et al (2007) A hypothesis for an embryonic origin of pluripotent Oct-4(+) stem cells in adult bone marrow and other tissues. Leukemia 21(5): 860-867.

26. Grymula K, Tarnowski M, Piotrowska K, Suszynska M, Mierzejewska K, et al. (2014) Evidence that the population of quiescent bone marrow-residing very small embryonic/epiblast-like stem cells (VSELs) expands in response to neurotoxic treatment. J Cell Mol Med 18(9): 1797-1806.

27. Kucia M, Reca R, Jala VR, Dawn B, Ratajczak J, et al. (2005) Bone marrow as a home of heterogenous populations of nonhematopoietic stem cells. Leukemia 19(7): 1118-1127.

28. Eglitis MA, Mezey E (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci U S A 94(8): 4080-4085.

29. Kmiecik TE, Keller JR, Rosen E, Vande Woude GF (1992) Hepatocyte growth factor is a synergistic factor for the growth of hematopoietic progenitor cells. Blood 80(10): 2454-2457.

30. Kucia M, Wu W, Ratajczak MZ (2007) Bone marrow-derived very small embryonic-like stem cells: their developmental origin and biological significance. Dev Dyn 236(12): 3309-3320.

31. Nagasawa T (2000) A chemokine SDF-1/PBSF, and its receptor, CXC chemokine receptor 4, as mediators of hematopoiesis. Int J Hematol 72(4): 408-411.

32. Kucia M, Reca R, Campbell FR, Zuba Surma E, Majka M, et al. (2006) A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia 20(5): 857-869.

33. Kucia M, Halasa M, Wysoczynski M, Baskiewicz Masiuk M, Moldenhawer S, et al. (2007) Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood: preliminary report. Leukemia 21(2): 297-303.

34. Shin DM, Suszynska M, Mierzejewska K, Ratajczak J, Ratajczak MZ, et al. (2013) Very small embryonic-like stem-cell optimization of isolation protocols: an update of molecular signatures and a review of current in vivo applications. Exp Mol Med 45(11): e56.

35. Bhartiya D, Shaikh A, Anand S, Patel H, Kapoor S, et al. (2016) Endogenous, very small embryonic-like stem cells: critical review, therapeutic potential and a look ahead. Hum Reprod Update 23(1): 41-76.

36. Kucia M, Machalinski B, Ratajczak MZ (2006) The developmental deposition of epiblast/germ cell-line derived cells in various organs as a hypothetical explanation of stem cell plasticity? Acta Neurobiol Exp (Wars) 66(4): 331-341.

37. Abdel Latif A, Zuba Surma EK, Ziada KM, Kucia M, Cohen DA, et al. (2010) Evidence of mobilization of pluripotent stem cells into peripheral blood of patients with myocardial ischemia. Exp Hematol 38(12): 1131-1142.

38. Borlongan CV, Glover LE, Tajiri N, Kaneko Y, Freeman TB, et al. (2011) The great migration of bone marrow-derived stem cells toward the ischemic brain: therapeutic implications for stroke and other neurological disorders. Prog Neurobiol 95(2): 213-228.

39. de Lima M, McNiece I, Robinson SN, Munsell M, Eapen M, et al. (2012) Cord-blood engraftment with ex vivo mesenchymal-cell coculture. N Engl J Med 367(24): 2305-2315.

40. Guerin CL, Loyer X, Vilar J, Cras A, Mirault T, et al. (2015) Bone-marrow-derived very small embryonic-like stem cells in patients with critical leg ischaemia: evidence of vasculogenic potential. Thromb Haemost 113(5): 1084-1094.

41. Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276(5309): 71-74.

42. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, et al. (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418(6893): 41-49.

43. D Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, et al. (2004) Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. J Cell Sci 117(Pt 14):2971-2981.

44. Heneidi S, Simerman AA, Keller E, Singh P, Li X, et al. (2013) Awakened by cellular stress: isolation and characterization of a novel population of pluripotent stem cells derived from human adipose tissue. PLoS One 8(6): e64752.

45. Kuroda Y, Kitada M, Wakao S, Nishikawa K, Tanimura Y, et al. (2010) Unique multipotent cells in adult human mesenchymal cell populations. Proc Natl Acad Sci U S A 107(19): 8639-8643.

46. Bhartiya D, Patel H, Ganguly R, Shaikh A, Shukla Y, et al. (2018) Novel Insights into Adult and Cancer Stem Cell Biology. Stem Cells Dev 27(22): 1527-1539.

47. Ratajczak MZ, Zuba Surma EK, Shin DM, Ratajczak J, Kucia M, et al.(2008) Very small embryonic-like (VSEL) stem cells in adult organs and their potential role in rejuvenation of tissues and longevity. Exp Gerontol 43(11): 1009-1017.

48. Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, et al. (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science 329(5997):1345-1348.

49. Weiss JN (2021) Very Small Embryonic-Like Stem Cells for Knee Osteoarthritis. Orthopedic Stem Cell Surgery Springer: 145-147.

50. Shin DM, Liu R, Wu W, Waigel SJ, Zacharias W, et al. (2012) Global gene expression analysis of very small embryonic-like stem cells reveals that the Ezh2-dependent bivalent domain mechanism contributes to their pluripotent state. Stem Cells Dev 21(10): 1639-1652.

51. Shin DM, Zuba Surma EK, Wu W, Ratajczak J, Wysoczynski M, et al. (2009) Novel epigenetic mechanisms that control pluripotency and quiescence of adult bone marrow-derived Oct4(+) very small embryonic-like stem cells. Leukemia 23(11): 2042-2051.

52. Alvarez Gonzalez C, Duggleby R, Vagaska B, Querol S, Gomez SG, et al. (2013) Cord blood Lin(-)CD45(-) embryonic-like stem cells are a heterogeneous population that lack self-renewal capacity. PLoS One 8(6): e67968.

53. Ratajczak J, Wysoczynski M, Zuba Surma E, Wan W, Kucia M, et al. (2011) Adult murine bone marrow-derived very small embryonic-like stem cells differentiate into the hematopoietic lineage after coculture over OP9 stromal cells. Exp Hematol 39(2): 225-237.

54. Mierzejewska K, Heo J, Kang JW, Kang H, Ratajczak J, et al. (2013) Genome-wide analysis of murine bone marrow-derived very small embryonic-like stem cells reveals that mitogenic growth factor signaling pathways play a crucial role in the quiescence and ageing of these cells. Int J Mol Med 32(2): 281-290.

55. Shin DM, Liu R, Klich I, Ratajczak J, Kucia M, et al. (2010) Molecular characterization of isolated from murine adult tissues very small embryonic/epiblast like stem cells (VSELs). Mol Cells 29(6): 533-538.

56. Lahlil R, Scrofani M, Barbet R, Tancredi C, Aries A, et al. (2018) VSELs Maintain their Pluripotency and Competence to Differentiate after Enhanced Ex Vivo Expansion. Stem Cell Rev Rep 14(4): 510-524.

57. Ventura Carmenate Y, Bencomo Hernandez A, Alkaabi F (2020) inventor; Abu Dhabi Stem Cells Center, assignee. Simplified methods for harvesting non-hematopoietic enriched stem cells cocktail (PBNHESCC) from adult peripheral blood. United Arab Emirate patent 3263. 2020–04-302007.

58. Havens AM, Shiozawa Y, Jung Y, Sun H, Wang J, et al. (2013) Human very small embryonic-like cells generate skeletal structures, in vivo. Stem Cells Dev 22(4): 622-630.

59. Teitelbaum SL (2010) Stem cells and osteoporosis therapy. Cell Stem Cell 7(5): 553-554.

60. Bionaz M, Monaco E, Wheeler MB (2015) Transcription Adaptation during In Vitro Adipogenesis and Osteogenesis of Porcine Mesenchymal Stem Cells: Dynamics of Pathways, Biological Processes, Up-Stream Regulators, and Gene Networks. PLoS One 10(9): e0137644.

61. Paspaliaris V, Kolios G (2019) Stem cells in Osteoporosis: From Biology to New Therapeutic Approaches. Stem Cells Int 2019: 1730978.

62. Younghun J (2015) Efficacy of Human VSELs to Reverse Bone Loss in Osteoporosis.