**Title: The “Ins” and “Outs” of Mesenchymal Stem Cell Osteogenesis in Regenerative Medicine**

**Running Title:** Mesenchymal Stem Cells in Bone Regeneration

**Authors:** Dean T Yamaguchi

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Dean T Yamaguchi,** Research Service,Veteran Administration Greater Los Angeles Healthcare System and David Geffen School of Medicine at University of California at Los Angeles, Los Angeles, CA 90073, United States

**Author Contribution:** Yamaguchi, DT solely contributed to this review

**Supported by** Veterans Administration Merit Review Award 2 I01 BX000170-05

**Correspondence to:** Dean T Yamaguchi, MD, PhD, Research Service 151, VA Greater Los Angeles Healthcare System, 11301 Wilshire Blvd, Bldg 114, Rm 330, Los Angeles, CA., 90073-1003, United States. [Dean.yamaguchi@va.gov](mailto:Dean.yamaguchi@va.gov)

Telephone +1-310-268-3459 Fax +1-310-268-4856

**Abstract**

Repair and regeneration of bone requires mesenchymal stem cells that by self-renewal, are able to generate a critical mass of cells with the ability to differentiate into osteoblasts that can produce bone protein matrix (osteoid) and enable its mineralization. The number of human mesenchymal stem cells (hMSCs) diminishes with age and *ex vivo* replication of hMSCs has limited potential. While propagating hMSCs under hypoxic conditions may maintain their ability to self-renew, the strategy of using human telomerase reverse transcriptase (hTERT) to allow for hMSCs to prolong their replicative lifespan is an attractive means of ensuring a critical mass of cells with the potential to differentiate into various mesodermal structural tissues including bone. However, this strategy must be tempered by the oncogenic potential of TERT-transformed cells, the unknown differentiating potential of high population doubling hMSCs and the source of hMSCs (e.g. bone marrow, adipose-derived, muscle-derived, umbilical cord blood, etc.) that may provide peculiarities to self-renewal, differentiation, and physiologic function that may differ from non-transformed native cells. Tissue engineering approaches to use hMSCs to repair bone defects utilize the growth of hMSCs on three-dimensional scaffolds that can either be a base on which hMSCs can attach and grow or as a means of sequestering growth factors to assist in the chemoattraction and differentiation of native hMSCs. The use of whole native extracellular matrix (ECM) produced by hMSCs, rather than individual ECM components, appear to be advantageous in not only being utilized as a three-dimensional attachment base but also in appropriate orientation of cells and their differentiation through the growth factors that native ECM harbor or in simulating growth factor motifs. The origin of native ECM, whether from hMSCs from young or old individuals is a critical factor in “rejuvenating” hMSCs from older individuals grown on ECM from younger individuals.

**Key Words:** mesenchymal stem cell; TERT; extracellular matrix; osteogenesis; regenerative medicine; tissue engineering; proliferation; differentiation

**Core Tip:** When hTERT transformed hMSCs are used to prolong replicative potential and osteogenic differentiation, consideration should be given to using lower population doubling hTERT-transformed hMSCs to avoid potential oncogenesis. An inducible hTERT system may also avoid oncogenic transformation. Demonstration of *in vivo* bone forming capacity of hTERT-transformed cells should be used as standard in determining osteogenic differentiation of such cells rather than *in vitro* culture mineralization; the CD146 marker may be a suggested surface marker for hTERT-transformed hMSCs that may have the capacity to form bone *in vivo.* Native ECM from early population doubling hMSCs or hMSCs from a younger source may be best when seeking to extend the proliferative and differentiating potential of hMSCs from either young or older sources.

**INTRODUCTION**

The regeneration of mesodermal and neural crest-derived structural or connective tissues such as bone, cartilage, muscle and tendon continues to be a widely pursued for the reason that such structural tissues are generally homogeneous with either a predominantly single cell type or limited number of cells that contribute to the make-up of the tissue and that precursors to the mature cell types can be found in adult tissues. These precursor cells are generally multipotent, in that they can differentiate into a variety of connective tissue phenotypes. These precursor cells are generally referred to as adult mesenchymal stem cells (MSCs) or bone marrow stromal cells and can be found in the bone marrow but also as similar multipotent cells in specific tissues as well as circulating cells in blood.

Tissue engineering seeks to replace tissues that are either lost by traumatic events or by disease through the use of specific cell types that can recapitulate the lost or diseased tissue, and generally used in combination with a three-dimensional structural scaffold, and in many instances in combination with various growth factors, cytokines, and hormones or other biological molecules to assist in either the creation of a critical mass of needed cells or to assist in differentiating these cells to the required tissue type.

Because generating a critical mass of cells used in the regenerative process is a key to successful tissue engineering followed by differentiating those cells into the specific cell type comprising the tissue, stem cells have been the preferred starting cell type in many tissue engineering trials. This minireview will focus only on human adult bone marrow MSCs (herein assumed to be synonymous with bone marrow stromal cells) as much as possible and the telomerase strategy of inducing self-renewal of these cells to create a critical cell mass. Secondly, the minireview will examine the strategy of using extracellular matrix as a native scaffold upon which mesenchymal stem cells can self-renew and differentiate into bone.

**Mesenchymal Stem Cell Self-Renewal**

The ability to self-renew is a hallmark of any stem cell[1]. Self-renewal is simply defined as the ability of the resulting daughter cells, after mitotic division of the original mother cell, to retain the ability to generate a variety of differentiated cell types identical to that of the ability of the mother cell to differentiate in to those same cell types, and for a daughter cell to be able to generate daughter cells that also maintain the ability to differentiate into the same variety of cell types as the original “grandmother” and mother cells[2]. The maintenance of self-renewal and pluripotency of stem cells occurs in the stem cell niche, where stem cells are able to receive cues from the stroma and other cell types either by direct contact or by secreted soluble factors within this microenvironmental niche[3,4].

Adult MSCs also share the ability to self-renew. This potential to self-replicate and to differentiate into connective tissue phenotypes has led to the exploration to utilize MSCs in the repair of injured tissues[5,6]. While the bone marrow has been a common site to harvest MSCs, other cell types similar to bone marrow-derived MSCs can also be found in other sites. Adipose-derived stem cells, satellite cells in muscle, and pericytes around blood vessels and umbilical cord blood cells also may share multipotent characteristics for differentiation into connective tissue phenotypes under specific conditions which include selective differentiation media and growth factors[7-10]. However, whether MSC types from non-bone marrow sites have the same potential for self-replication as do bone marrow-derived MSCs is currently unknown. Additionally, whether non- bone marrow-derived MSCs favor differentiation into specific connective tissue types or even non-mesodermal cell types as in the case of umbilical cord blood MSCs in an *in vivo* environment is still a ripe area of investigation.

Age of the organism is a determinant of the number of bone marrow MSCs present as well as *in vitro* tissue culture conditions that are critical for MSCs to retain their ability to self-renew yet demonstrate plasticity in their ability to differentiate into various mesodermal tissues[11]. The number of cells from human bone marrow that are MSCs as determined by colony forming unit-fibroblastic (CFU-f) assay are less than 0.1% of total bone marrow mononuclear cells, thus demonstrating a minimal number of hMSCs that can be used in bone regeneration[12]. The numbers of CFU-f and the capacity of CFU-fs that can differentiate into osteoblasts further decrease as a function of age of the bone marrow donor up to age 40; after age 40, there does not appear to be any further diminishing of CFU-fs that can differentiate into osteoblasts[13]. It was suggested that hMSCs have decreased proliferative capacity as a function of age[14]. Thus hMSCs from young individuals ages 18-29 years achieved an average population doubling level of 41 whereas hMSCs from older individuals ages 66-81 years achieved an average population doubling level of 24 with about a 55% lower population doubling rate than in hMSCs from the younger individuals. However, no difference in *in vivo* bone formation was noted as a function of donor age with early passage cells from either age group. Thus, once placed in primary culture, hMSCs have a limited lifespan (average 20 to 40 population doublings, but the number of population doublings may differ depending on growth medium or any added growth factors)[14-16] under environmental conditions normally used for *in vitro* cell culture (humidified 5% CO2 and 95% air (21% O2) and when grown on tissue culture plastic. hMSCs grown in such conditions attain the Hayflick limit where cell division ceases, and the usual hMSC size becomes larger and the usual spindle shape of normal hMSCs becomes more polygonal or with a variety of shapes and sizes, at times with multinucleation, and overall with less cell density per culture than cells undergoing cell division[17]. As the number of population doublings for such cells is limited practically in primary culture, slower cell division and finally lack of cell division ensues and the above morphological changes are noted, and the expression of senescence-associated β-galactosidase, and p16, markers of cellular senescence, are increased[18]. However, it has been shown that if environmental conditions simulate the MSC niche in the bone marrow, specifically low oxygen tension, that self-renewal of hMSCs can be prolonged. D’Ippolito, et. al. (2004)[19] developed a multilineage inducible MSC model from human cadaveric vertebral body marrow (MIAMI cells) and propagated them in 3% O2/5% CO2/ 92% N2. They reported that more than 50 cell doublings beyond the Hayflick limit for primary cells could be achieved from hMSCs from at least 3 of 12 donors and at least 30 population doublings could be achieved from all of their donors. In a follow-up communication, they reported that MIAMI cells grown in 3% O2 doubled more quickly than those grown at 21% O2 and maintained the embryonic transcription factors OCT-4, REX-1, and hTERT and had suppressed osteoblastic differentiation when exposed to osteogenic differentiation medium. At higher O2 concentrations of 21%, these embryonic transcription factors were lost and osteogenic differentiation was enhanced[20]. The mechanism by which hypoxia regulates stem cell self-renewal appears to be via hypoxia inducible factor-1α (HIF-1α). Low oxygen concentrations stabilize HIF-1α by inhibiting its degradation by the proteasome. Mazumdar, et. al. (2010)[21]  reported that hypoxia induced canonical Wnt/β-catenin signaling and increased transcription of Lef/Tcf genes which have hypoxia response elements in their promoter regions that bind HIF-1α. Canonical Wnt/β-catenin signaling thus can induce increased cell proliferation.

**hTERT Transformation of hMSCs – the “In’s” for Self-Renewal**

In lieu of special resources needed to grow hMSCs in a hypoxic environment to maintain a proliferative state, a self-renewal strategy, engineering of hMSCs to over express telomerase has been an alternative means to maintain a longer proliferative lifespan of such cells. Telomerase, which is a multi-subunit ribonucleoprotein found in the cell nucleus and perhaps closely associated with nucleoli, allows for the addition of non-coding telomere DNA at the 3’ end of linear chromosomes[22-24]. Maintenance of telomere length by the addition of TTAGGG repeats onto the ends of telomeres allows for cells to continue to divide[25]. Telomerase is expressed in human embryonic cells and in fetal, newborn, and adult testes and ovaries but not in mature spermatozoa or oocytes. Moreover, expression of telomerase disappears in human somatic cells in the neonatal period and later in life[26]. Thus lacking telomerase, telomeres shorten with each cell division leading to replicative senescence once cells reach a critical shortened telomere length. Specifically, with respect to MSCs, a number of laboratories have reported that hMSCs from bone marrow do not express telomerase activity or have activity below detectable levels by telomeric repeat amplification protocol (TRAP) assay when hMSCs are asynchronously dividing[15,27-29]. However, human telomerase reverse transcriptase (hTERT) expression and telomerase activity could be detected when cells were synchronized to S-phase[29]. Others have found that telomere length in hMSCs is short upon initial isolation and tend to further shorten with cell passage *in vitro* and appear to correlate with low to undetectable levels of hTERT[30]. Thus theoretically, maintaining telomerase expression should prevent replicative senescence. Additionally, the decrease in telomere length correlates with colony forming unit – fibroblastic (CFU-f) numbers suggesting that telomere length and telomerase activity could also be related to the ability of hMSCs to differentiate along various cell lineages including the osteogenic lineage[30]. Gronthos, et. al. (2003)[31] reported that expression of hTERT in human bone marrow-derived MSCs not only increased proliferative capacity by up-regulating G1 to S phase transition cell cycle genes but also increased the expression of osteogenic genes for cbfa-1, osterix, and osteocalcin and induced bone formation earlier and to a much larger degree in an *in vivo* ectopic bone formation assay of hTERT-transformed hMSCs. Saeed, et. al. (2011)[32] demonstrated that in telomerase-deficient mice (*Terc-/-),* there was delayed ossification in occipital bone, sternum, vertebrae, and metatarsals. Overall bone volume was decreased compared to wild type controls, and trabecular bone parameters showed decreased trabecular thickness and increased trabecular spacing[32]. Additionally, bone formation rate was decreased which correlated with decreased osteoblast surface per bone surface, and osteoclast surface per bone surface was increased. The proliferative ability of bone marrow-derived MSCs from *Terc-/-* mice was diminished compared to wild type mice, and there was increased β-galactosidase staining of *Terc-/-* cells suggesting a more senescent phenotype of MSCs. There was up-regulation of pro-inflammatory genes (e.g. IL-1 receptor type 2, toll-like receptor 6, leukotriene B4 receptor 1, tumor necrosis factor, etc) indicative of osteoclastic activity as well as a decrease of osteoblast-specific bone markers. Thus both decreased bone formation and increased bone resorption as a result of an inflammatory microenvironment were found in this telomerase deficient model.

The critical components of human telomerase include the hTERT catalytic subunit and the RNA subunit, telomerase RNA ( hTR), that provides a template for the synthesis of the DNA repeats at the ends of chromosomes. However, generally only hTERT is sufficient to maintain telomere length when transfected into various cell types although integration of ectopic hTERT alone to extend cell replicative ability may be dependent on integration site, availability of other proteins associated with telomeres, or cell specificity[27]. Thus a number of studies have demonstrated the feasibility of using hTERT in hMSCs to allow for prolonged replicative lifespan as well as capability of differentiating hTERT-transformed hMSCs towards the osteogenic lineage[33-37]. The strategy used to transform hMSCs to over express the hTERT gene is generally a retroviral vector approach that uses green fluorescent protein expression as a positive selection marker to enable sorting of positively transformed cells by fluorescence activated cell sorting[36]. An alternative approach to select transformed cells is an antibiotic resistance strategy[37]. A technique to control hTERT expression in transfected hMSCs on demand utilizes the tetracycline inducible approach (Tet-On) so that proliferative and differentiation ability can be assessed at selected population doublings although “leakiness” of hTERT even in the Tet-off state could be a limitation[35]. hTERT-transformed hMSCs have been reported to undergo at least 70 population doubling levels[37] but upwards of 120 to 400 population doubling levels have been reported depending on the length of time in culture, plating density of cells, and subcultured clonal populations[27,34-36]. The interesting aspect of hTERT-transformed hMSCs is that they are able to maintain their proliferative ability while being induced to differentiate along osteogenic, but also adipogenic, and chondrogenic lineages. Thus hTERT-transformed cells are different from non-transformed hMSCs and mesenchymal (stromal) cells from other species that are able to differentiate into osteoblasts where it is observed that as osteogenic differentiation proceeds, the proliferative ability of the cells diminishes[38,39].

Three important criteria must be met when hMSCs are transformed by hTERT expression to achieve a critical mass of cells via self-renewal that would be necessary to populate fabricated scaffolds for tissue engineering. Firstly, markers of hMSCs should be maintained after hTERT transformation that would suggest maintenance of multipotency of the cells to undergo differentiation into various mesenchymal cell lineages. Secondly, it is important that hTERT transformation of hMSCs does not lead to malignant transformation either in the pluripotent state or in differentiated cell types. Thirdly, it is critical that hTERT expressing hMSCs will be able to specifically differentiate along the osteogenic lineage and to form bone which is the tissue of interest in this minireivew.

Surface markers have been traditionally used to identify hMSCs. The International Society for Cellular Therapy set minimal criteria for positive markers to define hMSCs which are >95% expression of CD105 (endoglin), CD73 (ecto-5’-nucleotidase), CD90 (Thy-1) and <2% expression of hematopoietic stem cell markers, CD45 (protein tyrosine phosphatase, receptor type, C), CD34 (sialomucin family adhesion factor), CD14 (monocyte differentiation antigen/lipoglycan receptor) or CD11b (integrin alpha M), CD79α (immunoglobulin associated alpha) or CD19 (B-lymphocyte antigen), and HLA-DR[40]. Other markers used to identify hMSCs include STRO-1, CD146 (melanoma cell adhesion molecule/MUC18), CD49a (integrin alpha subunit), CD271 (low-affinity nerve growth factor receptor), CD63 (lysosome-associated membrane protein-3), found on only on marrow-derived hMSCs and CD166 (activated leucocyte cell adhesion molecule)[41-46]. Interestingly, stage-specific embryonic antigen-4 (SSEA-4), found on human embryonic stem cells, was identified as a marker for both mouse and human bone marrow-derived MSCs that had the ability to differentiate into both adipogenic and osteogenic lineages[47]. Most recently CD44 was identified as a negative marker in freshly isolated although acquisition of the CD44 marker may be a function of *in vitro* cell culture of hMSCs[48].

Telomerase expression and activity has been found in a majority of human tumors thus suggesting that hTERT expression in human cells could potentially lead to uncontrolled cell proliferation[49]. However, it has also been suggested that the immortalization induced by hTERT may only in part be due to maintaining telomere length and stabilization and that non-canonical functions of hTERT such as the up-regulation of NF-κB transcription by TERT binding to the p65 subunit of NF-κB as well as activating the Wnt/β-catenin pathway and its target genes, MYC and CCND1 (Cyclin D1), which are regulators of oncogenic targets, and the ability of NF-κB to inhibit apoptosis, may be more important in promoting tumorigenesis[50]. The loss of expression of p16INK4a, the protein transcript of the CDKN2A gene, in addition to loss of p53 tumor suppressor function, and resistance to growth inhibition by transforming growth factor-β (TGF-β), are among other observations found in the acquisition of oncogenic potential in TERT transformed cells[51].

Specifically in hMSCs that are transformed with hTERT, there is still the potential of such cells to express tumorigenic properties. Yamaoka, et. al. (2011)[52], constructed hTERT transformed bone marrow hMSCs and found that teratocarcinoma formation could occur when such transformed cells were implanted in immune deficient mice. However, the cells that these investigators transformed with hTERT had first been selected due to their ability to be maintain a proliferative state in the presence of fibroblast growth factor-2 (FGF-2) (>100 population doubling levels) compared to hMSCs not cultured with FGF-2 that could proliferate to only 20 population doubling levels. As telomerase activity was absent in these FGF-2 maintained clones but had maintained long telomere length, an alternative lengthening of telomeres (ALT) pathway induced by FGF-2 in combination with TERT immortalization could have accounted for the malignant transformation. Serakinci, et. al. (2004)[53] also reported that hMSCs transformed with hTERT could exhibit neoplastic characteristics as shown by loss of contact inhibition and development of mesenchymal tumors after implantation of cells in immunodeficient mice. Loss of p16INK4a and hypermethylation of DBCCR1 (deleted in bladder cancer chromosomal region candidate 1), a cell-cycle associated gene, were observed. Interestingly, tumors were generated only in high population doubling level hTERT-transformed hMSCs and not in relatively lower population doubling level hTERT-transformed hMSCs. Similarly, Abdallah, et. al., (2005)[34] reported that mesodermal type tumors formed from hTERT transformed hMSCs that had a short population doubling time and accelerated growth, but no tumors developed in hTERT transformed hMSC clones with longer population doubling times that were slower growing. Thus the potential for neoplastic change may be associated with loss of proliferative control as evidenced by cell cycle gene alterations with continued proliferation.

Nevertheless, others have reported that hTERT-transformed hMSCs did not exhibit changes associated with neoplasia even at higher population doubling levels (up to 275)[27,36,54]. However, whether or not potential oncogenic development occurs in hTERT-transformed hMSCs, functional changes in hMSC parameters need to be considered. Baumer, et. al. (2011)[55] reported that hTERT-transformed human coronary artery endothelial cells demonstrated changes in an *in vitro* co-culture angiogenesis assay where TERT-transformed human coronary artery endothelial cells co-cultured with human fibroblasts and treated with vascular endothelial growth factor (VEGF) did not form tubular networks indicative of angiogenesis; non-TERT-transformed endothelial cells in co-culture with fibroblasts and treated with VEGF were able to form tubular networks. Moreover, hTERT-transformed endothelial cells responded differently to exogenous tumor necrosis factor-α (TNF-α) compared to non-hTERT transformed cells where vascular cell adhesion molecule-1 (VCAM-1) expression was lower, and endothelial barrier function as measured by transepithelial resistance was lost in hTERT-transformed cells. Since hMSCs are immunomodulatory cells that can affect the function of immune hematopoietically derived cells (lymphocytes, monocytes, etc) in an inflammatory environment, there needs to be further investigation if hTERT transformation of hMSCs do not affect these immunomodulating properties of normal hMSCs or have altered function in differentiation or on angiogenesis when interacting with other cell types in a microenvironmental setting.

Perhaps the most prudent approach to ensure that hTERT transformed hMSCs would be useful for bone repair after induction of osteogenic differentiation would be to use inducible vectors for hTERT expression that can then be regulated both temporally and spatially to avert problems with continuous cell proliferation that could result in oncogenic transformation of hTERT-transformed hMSCs[35].

**Osteogenic Differentiation of hTERT-transformed hMSCs**

Differentiation of hMSCs along the osteogenic lineage has been demonstrated using both *in vitro* and *in* *vivo* techniques. Induction of *in vitro* osteogenic differentiation in hMSCs include addition of dexamethasone, ascorbate, and a source of phosphate, mainly β-glycerophosphate to a culture medium base (generally Dulbecco’s modified Eagle’s medium) containing 10% bovine serum. However, recently it was reported that hMSCs from bone marrow may not require the addition of dexamethasone and ascorbate to form bone *in vivo* although bone marrow-derived hMSCs respond to dexamethasone and ascorbate with increased proliferation *in vitro*[56]. Osteogenic marker expression by mRNA and protein is usually assessed over the course of *in vitro* cell culture. Early markers of osteogenesis include core binding factor 1 (cbfa1 or runx2 (Runt-related transcription factor 2)) which is found in chondro-osseous precursor cells, osterix which appears in committed osteogenic cells, and collagen type I. Intermediate markers of osteogenesis include alkaline phosphatase and osteopontin and bone sialoprotein and osteocalcin (usually induced in hMSCs by 1,25 dihydroxyvitamin D3) are generally used as later markers of terminally differentiated osteoblasts. Determination of mineralization of culture *in vitro* is also critical in assessing terminal differentiation along the osteogenic lineage. This is usually accomplished by staining cell cultures using alizarin red or von Kossa stains which bind to calcium and/or eluting these stains for semi-quantitation of calcium spectrophotometrially. It is also suggested that to distinguish amorphous calcium-phosphate precipitation in cultures from hydroxyapatite (Ca10P8(OH)2), x-ray diffraction, nuclear magnetic resonance, or other technique be used to compare the calcium-phosphate complexes in *in vitro* cell cultures with standard hydroxyapatite patterns by these techniques. Additionally, negative markers for other mesodermal cell types that can be differentiated from hMSCs should be assessed. These are usually markers for the adipogenic lineage (adipsin, peroxisome proliferator-activated receptor gamma (PPAR-γ), adiponectin), the chondrogenic lineage (sox9, collagen type II, collagen type X, aggrecan), tenogenic lineage (scleraxis)[57], and myogenic lineage (Pax3, Pax7 (myogenic precursors), MyoD and myogenin (skeletal muscle), α-smooth muscle actin, vascular endothelial (VE) cadherin (smooth muscle). Essentially, similar techniques to demonstrate osteogenic differentiation have been used for hTERT-transformed hMSCs.

*In vivo* osteogenesis of hMSCs, whether or not transformed with hTERT, is usually accomplished by ectopic bone ossicle formation assay. In this assay, hMSCs are usually mixed with hydroxyapatite and/or treated with various bone morphogenetic proteins (BMPs) and are implanted into subcutaneous pockets in either immunocompromised rodents (e.g.nude mice; NOD/ SCID mice)[27,34,58,59] or into immune competent rodents[36]. Assessment for bone formation is done by microCT and/or histology to identify trabecular bone formation and the expression of the above bone marker genes and proteins in tissue sections. hMSCs have been shown to create a locally immunosuppressive microenvironment and are able to avoid allo-recognition[60] perhaps in rodent species although it is unknown if the same holds true for transplantation of hMSCs into human recipients or if there are any consequences of immunogenicity of hMSCs once they are differentiated into specific lineages in a human recipient[61].

It is highly important that the both *in vitro* and *in vivo* confirmation of hydroxyapatite or bone formation be done especially in hTERT-transformed hMSCs. It is possible that not all hTERT-transformed hMSCs will be able to form bone *in vivo*. Larsen, et. al., (2010)[62] established subclones from hTERT transformed hMSCs at a relatively early population doubling level (PDL 77) and from a later PDL 233. They found that both subclones retained surface markers for hMSCs (CD63, CD73, CD105, and CD166) as well as expressed osteoblast markers, alkaline phosphatase, collagen type I, and osteocalcin upon induction with osteogenic medium. Both clones also formed mineralized matrix *in vitro* as assessed by alizarin red staining. However, the PDL 77 clone was able to form bone in an *in vivo* ectopic bone formation assay while the PDL 233 clone did not form bone. Interestingly, these investigators reported that CD146 was highly expressed in the hTERT-transformed hMSC clone that could form bone *in vivo* while CD146 was minimally expressed in the hTERT-transformed clone that did not form bone *in vivo*. Thus the criteria for *in vivo* bone formation and expression of CD146 should be helpful in assessing hTERT-transformed hMSCs that may be useful for potential bone repair or regenerative therapy, and sole dependence on osteogenic markers and *in vitro*, two-dimensional cell culture mineralization assays may be insufficient. Also observed in additional hTERT-transformed hMSC clones that formed bone *in vivo* was the increased number of extracellular matrix genes expressed as well as the increased number of Sp3 binding sites in the promoter regions of these expressed genes compared to that of hTERT-transformed hMSC clones that did not form bone *in vivo*. Sp3 is a transcription factor necessary for bone development and ossification.

In attempts to seed hTERT-transformed hMSCs in areas requiring their presence for tissue repair, strategies such as intracardiac or intravenous injection of hMSCs expressing a fluorescent marker (e.g. green fluorescent protein) have been used to identify sites where such injected hMSCs populate as well as to assess the longevity of transplanted hMSCs in the desired regions. Bentzon, et. al. (2005)[63] reported that hTERT-transformed hMSCs injected intracardiac or intravenously into NOD/SCID mice were trapped mainly in microvasculature of the lungs, kidneys and heart. It was also found that only a small fraction of the injected telomerized hMSCs survived or were retained possibly due to protracted trans-endothelial migration. Thus direct engraftment of hTERT-transformed hMSCs may be a better approach to healing bone.

In addition to cells, such as MSCs, that have the potential to self-replicate and differentiate into the cell type of choice, tissue engineering in regenerative medicine strategies generally combine the cellular component with various growth and differentiation factors that can promote differentiation of undifferentiated precursor cells and with the employment of a structural framework on which either such cells and/or growth and differentiation factors can be assembled. The use of three-dimensional culture platforms may simulate the natural three-dimensional *in vivo* tissue architecture and provide advantages over that of assessing hMSC growth and differentiation on tissue culture plastic in a two-dimensional format[64,65]. Two dimensional cultures may only yield woven type bone (random orientation of collagen fibrils) and not allow for the formation of lamellar bone, the final desired bone product, and microenvironments that may develop in a three-dimensional framework that could affect cell-cell and cell-matrix interactions cannot fully develop in a two dimensional culture system.

For *in vivo* uses, three-dimensional platforms or scaffolds need to be biocompatible, potentially biodegradable, have sufficient porosity to allow great surface area for cell attachment, and in general be non-immunogenic. The more rigid platforms or scaffolds composed of material such as hydroxyapatite or other calcium-phosphate bases which are osteoinductive and can induce ectopic bone formation. Titanium has been used to grow hMSCs that can then be differentiated along the osteogenic lineage with or without BMP stimulation prior to direct surgical implantation into bone defects in translational models of bone repair[66-68]. Biological scaffolds that are composed of polymer blends such as poly(l-lactide-co-glycolide) (PLGA) are biocompatible and can be degraded by the body have also been used as a base on which hMSCs can be grown and differentiated[69]. Polymer blends have also been used in combination with inorganic hydroxyapatite crystals or naturally occurring proteins such as collagen to construct composite scaffolds that improve mechanical and osteoinductive properties of the scaffolds have also been designed[70]. Hydrogels have also been used as scaffold material due to biocompatibility; natural hydrogels are derived from collagen or gelatin, while synthetic hydrogels can be made from poly(ethylene glycol). While natural hydrogels are excellent for cell adhesion and biodegradation, immunogenic reactions may be a concern if the hydrogels are derived from animal-derived extracellular matrix (ECM) protein. Synthetic hydrogels have the advantage of creating scaffolds *in situ* using photopolymerization and also are non-immunogenic[71]. Hydrogels as well as polymer blends with or without ceramic material (e.g. hydroxyapatite )have also been useful in serving as reservoirs for bioactive molecules such as growth factors[70-72]. Thus scaffolds impregnated with various growth factors or composed in part of ECM-derived short peptides, modified heparin, chondroitin sulfate or hyaluronic acid to tether growth factors such as the BMPs, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), TGF-β, FGF-2 have been useful in the differentiation of transplanted hMSCs and/or the chemotaxis of native MSCs useful in bone repair[68,70-75]. Stromal-derived factor 1 (SDF-1), a chemokine, has also been impregnated in scaffolds to serve as a chemotactic factor for bone marrow-derived MSCs[76-78]. VEGF has been used to stimulate angiogenesis that would allow for improved blood supply to repairing tissues; use of VEGF incorporated into natural hydrogels or injected directly into scaffolds and in combination with BMPs and MSCs attached to scaffolds have been tested to improve bone healing[74,79]. In the absence of seeding MSCs onto scaffolds, delivery of SDF-1 via implantable infusion pump to poly-€-caprolactone scaffolds preceded by delivery of VEGF to the scaffolds and followed by BMP-6 to induce osteogenic differentiation was able to induce mature mineralized bone formation[80]. Tasso, et. al., (2009)[81] also reported that in a mouse model of ectopic bone formation, donor murine bone marrow MSCs loaded onto hydroxyapatite scaffolds were needed in the early development of ectopic bone (up to one week after implantation) to recruit host osteoprogenitor cells, but native (host) osteoprogenitor cells actually contributed the most to the bone formation via endochondral ossification. Thus native MSCs can be induced to populate scaffolds using SDF-1 and osteogenically differentiate to form vascularized bone. Finally, MSCs harboring viral vectors (adeno-associated virus or lentivirus) to over express growth factors and chemoattractants and attached to various types of scaffolds have been used as an alternative strategy to increase local concentrations of bioactive molecules such as BMP-2, BMP-7, VEGF, and CXCR4, the transmembrane G-protein coupled receptor for SDF-1-induced chemoattraction to enhance osteogenic marker expression[82-87].

Three dimensional spheroid cultures consisting of high density cell aggregates in agarose or alginate have also been used to traditionally differentiate chondrocytes from hMSCs[88,89]. Burns, et. al, (2010)[90] used a variation of this method by using caroxymethylcellulose in their high density cell preparation to form spheroids of hTERT transformed hMSC cells. When combined with hydroxyapatite / β-tricalcium phosphate scaffolds, induced with osteogenic medium, and implanted into immunodeficient mice in an *in vivo* ectopic bone formation assay, lamellar bone formation was observed in scaffold concavities in addition to the expression of usual osteoblastic markers of cbfa1, alkaline phosphatase, osteonectin, osteopontin, collagen type I and osteocalcin. CD146 expression which had been high in hMSCs was lost as osteogenic differentiation proceeded. Interestingly, transcriptional co-activator with PDZ binding motif (TAZ)[91], a cbfa1 binding transcription co-activator that allows for commitment to the osteogenic lineage while inhibiting adipogenic differentiation of hMSCs was also induced in the hTERT transformed hMSC spheroids. Stimulated expression of other extracellular matrix proteins such as biglycan, lumican, elastin, periostin,, microfibrillar-associated proteins (MFAP2 and MFAP5), tetranectin and decorin also occurred suggesting correlation between these extracellular matrix protein and osteogenesis.

**Extracellular Matrix (ECM) and hMSCs – the “Outs” for Osteogenesis**

The use of ECM components to enhance either rigid type scaffolds or hydrogel scaffolds or to serve as scaffolds themselves has become more popular in tissue engineering. For instance, collagen type I in the form of gels or sponges or as a protein coating of hydroxyapatite platforms has been useful in providing an attachment for cells in addition to being able to deliver growth factors such as TGF-β, BMPs, or VEGF[92]. ECM contains proteoglycans which are comprised in part of heparin sulfate that can bind many types of growth factors such as FGFs and VEGF and degradation of ECM by matrix metalloproteases can release these growth factors to subsequently bind to their receptors on specific cells[93]. Other ECM proteins such as laminin and tenascin have epidermal growth factor (EGF)-like motifs that could potentially bind to EGF receptors on cells and then initiate an EGF signaling cascade through tyrosine kinase activation resulting in cell proliferation and/or differentiation[94]. The binding of cells to naturally occurring proteins such as collagen occur via integrins, comprised of α and β subunits and binding cell membranes to ECM proteins with the arginine-glycine-aspartic acid (RGD) or leucine-valine-aspartic acid (LVD) (consensus sequence L/I (isoleucine)-D/E (glutamic acid)-V (valine)/S(serine)/T(threonine)-P(proline)/S) domains[95]. The short cytoplasmic domains of integrins interact with various cytoskeletal elements such as talin and kindlin to initiate inside-out signaling through integrin-linked kinase that is involved in activating integrins to bind to ECM components[96-98]. Outside-in signaling occurs with the interaction of specific sequences of ECM proteins and activated integrins to activate focal adhesion kinase to allow in part for functions such as cell spreading and migration but also activating other signaling pathways enabling cell proliferation, and survival[99].

Thus scaffolds composed of native ECM proteins such as collagen have been applied as one strategy to expand hMSCs *ex vivo* and to promote osteogenic differentiation and to enhance bone repair[12,100,101]. Bone marrow-derived hMSCs, express various integrins such as α1β1, α2β1, α5β1, α6β1, αvβ3, and αvβ5; however, the β1 integrin subunit was found to be most responsible for hMSCs to adhere to collagen, laminin and fibronectin and be involved in proliferation of hMSCs and for their differentiation into osteoblasts[102]. However, pre-coating scaffolds with a specific protein such as collagen type 1 or MatrigelTM (BD Biosciences) composed of collagen type IV, entactin, and laminin, may not yield the natural three dimensional environment, nor account for all appropriate ECM proteins that interact with hMSCs *in vivo*, nor retain the natural elasticity or stiffness required for proper self-renewal or tissue-specific differentiation. Degree of stiffness or elasticity of support structures or ECM has been shown to be important in part to be a determinant of stem cell differentiation. In reference to MSCs, softer substrates favor adipocyte or chondrogenic differentiation while stiffer substrates direct osteogenic differentiation. Intermediate stiffness can assist in directing myogenesis from MSCs[103-106]. ECM or bioengineered support structure stiffness or elasticity can be sensed by cells through the organization of stress fibers composed of actin microfilaments and myosin. Specifically, non-muscle myosin II isoforms, IIA, IIB, and IIC appear to be involved in the MSC’s ability to sense matrix stiffness through their interaction with cortical actin that is linked to focal adhesions. Increased matrix stiffness is associated with increased activity of non-muscle myosin II. The increased non-muscle myosin II also correlates with specific lineage determination of MSCs[107]. Interestingly, ECM stiffness that can set the stage for specific lineage differentiation via expression or repression of specific genes is transduced to nuclear chromatin via lamin-A[108]. Cytoskeletal stresses and tension increase with increasing ECM stiffness and the degree of lamin-A expression and phosphorylation is inversely related to ECM stiffness. Thus osteogenic differentiation of MSCs is correlated with increased lamin-A levels and decreased lamin-A phosphorylation when MSCs are grown on a stiff ECM. It would follow that lamin-A would act in a manner to maintain nuclear rigidity or stiffness which could translate into epigenetic regulation of chromatin thus enabling transcription of osteogenic genes and repression of genes specific with other mesenchymal lineages through lamina-associated domains which contain repressive heterochromatin.

Thus the use of cell-free preparations of secreted ECM proteins produced by MSCs or cells of the desired differentiated type (i.e. osteoblasts) may perhaps allow for better osteogenic differentiation of MSCs in a native three dimensional microenvironment similar to the MSC niche found in bone marrow. Chen, et. al., (2007)[109] prepared ECM from mouse MSCs that supported self-renewal of mouse MSCs when cultured on this native ECM and the proliferative ability of the MSCs grown on native ECM was greater than MSCs grown on fibronectin or collagen type I individually. Differentiation of mouse MSCs into both adipogenic (in response to rosiglitazone) and osteogenic lineages (in the presence of exogenous BMP-2) was also enhanced in cells cultured on native ECM compared to tissue culture plastic or culture plastic coated with fibronectin alone or with collagen type I alone. However, mouse MSCs had a delay in osteogenic differentiation when grown on native ECM in the absence of exogenous BMP-2, and it was suggested that the native ECM components such as collagen and biglycan bind BMP-2, making it less available to MSCs to allow for earlier osteogenic differentiation.

hMSCs can also be used to generate native ECM that supports self-replication of hMSCs, and the degree of enhanced proliferation of hMSCs was found to be greater than that of hMSCs grown on tissue culture plastic, or fibronectin or collagen type I independently[110]. It was also found that SSEA-4, a marker for bone marrow-derived hMSCs, was maintained at a high level throughout the culture period on native ECM and interestingly, telomerase activity was stable and reactive oxygen species was low on ECM-grown hMSCs compared to hMSCs grown on plastic, fibronectin, or collagen type I. *In vivo* bone formation was also significantly higher in hMSCs grown on native ECM compared to those grown on plastic. Thus native ECM from hMSCs can better support self-renewal and osteogenic differentiation compared to single ECM components or a two dimensional culture platform (plastic).

It has been shown that ECM from human foreskin young fibroblasts (<20-30 population doublings) supported the proliferation of old fibroblasts ( >68 population doublings) so that the proliferative rate of the old fibroblasts approached that of young cells grown on ECM from young cells[111]. Additionally, telomere length was restored in old fibroblasts grown on ECM from young cells by a telomerase independent mechanism and reduced reactive oxygen species similar to young cells was also found. Interestingly, SIRT 1, a gene for the NAD-dependent histone deacetylase, sirtuin 1, which was downregulated during senescence was increased when old fibroblasts were grown on ECM from young cells. This suggests that epigenetic mechanism(s) may play a role the mechanism of how young ECM can restore the proliferative ability of old fibroblasts. SIRT 1 can be directly activated by lamin A[112] , which is critically involved in the process of information flow from ECM to the nucleus to perhaps determine chromatin configuration and thus confer epigenetic regulation on gene expression or repression. Thus the potential role of epigenetics in ECM rejuvenation of old fibroblast cells is an area of interesting investigation.

With regards to MSCs, the composition of ECM from young (low passage) adipose-derived MSCs compared to that of old (higher passage) MSCs is different. For instance, while collagen type I is increased in young MSCs, laminin, fibronectin, vimentin, keratin, and lamin A/C are decreased in old MSCs. When old MSCs are seeded onto ECM from young MSCs, the pluripotency markers of Oct4, Sox2, and Nanog are increased and growth factors such as TGFβ are also upregulated[113]. The ECM component, biglycan, has been shown to increase canonical Wnt/β-catenin signaling. Wnt signaling is a critical morphogen in osteoprogenitor development . Bone marrow MSCs from mice deficient in biglycan were less proficient in Wnt-induced mineral deposition in culture, did not respond to exogenous Wnt3a, and made significantly less trabecular bone when used in an *in vivo* ectopic bone formation assay[114]. Thus one could speculate that ECM from young MSCs may have more biglycan than ECM from old MSCs and thus young ECM would be able to enhance Wnt signaling to enhance both proliferation of osteoprogenitors and potentially more bone formation. However, the exact mechanism of how biglycan can regulate either canonical or non-canonical Wnt signaling is unclear.

In another interesting study, Sun, et. al. (2011)[115] reported the differential effect of ECM from mouse bone marrow stromal cells derived from young (3 months) versus old mice (18 months). Replicative ability was restored in MSCs from old mice cultured on ECM from young mice, similar to that of the replicative ability of young mice grown on ECM from young mice. However, the replicative ability of MSCs from either young or old mice was significantly less when cultured on ECM from old mice. Telomerase levels were also increased in MSCs from young and old mice cultured on ECM from young mice compared to that of MSCs cultured on tissue culture plastic or on ECM from old animals. Examination of bone forming ability using an *in vivo* assay where MSCs from young or old mice pre-cultured on ECM from young or old mice demonstrated that MSCs from old mice pre-cultured on ECM from young mice had increased cancellous bone formation compared to MSCs from young or old mice pre-cultured on tissue culture plastic. Culture of MSCs from either young or old mice on ECM from old mice demonstrated less bone formation. In trying to dissect the differential effect of ECM from old versus young mice, these investigators founds that ECM from old mice contained more mineral phosphate and less collagen although the total amount of ECM produced by young or old cells were the same. Furthermore, reactive oxygen species levels were higher in MSCs grown on ECM from old mice but were reduced in MSCs grown on ECM from young mice; there was also an inverse correlation of the number of colony forming units-osteoblast and the level of reactive oxygen species. How ECM from old mice is incapable of handling reactive oxygen species and how this may relate to changes in ECM composition (lower collagen and proteoglycans) remains unknown.

In a recent communication, Prewitz, et. al., (2013)[116] used early passage bone marrow-derived hMSCs to generate native ECM but used either osteogenic medium to allow the hMSCs to differentiate towards the osteogenic lineage or ascorbic acid alone in the growth medium to allow the hMSCs under these conditions to generate an “enriched” ECM. These generated ECMs were then tethered to tissue culture plastic using poly(octadecene-*alt*-maleic anhydride). These investigators reported that ascorbic acid-stimulated native ECM contained twice as much collagen and sulfated glycosaminoglycans compared to native ECM generated using osteogenic medium although the spectrum of ECM protein were the same. Release of hepatocyte growth factor, FGF, VEGF, and interleukin-8 was also higher from ascorbic acid-stimulated ECM. Nevertheless, both types of ECM supported higher population doublings of hMSCs grown on these surfaces compared to hMSCs grown on either plasma-treated tissue culture plastic, fibronectin or Matrigel. Both ascorbic acid and osteogenic-induced ECM also stimulated more osteogenic differentiation as well as adipogenic differentiation although the ascorbic acid-induced ECM yielded better osteogenic and adipogenic differentiation than osteogenic-induced ECM. Finally, both ascorbic acid-induced and osteogenic-induced ECM were able to support the engraftment of hematopoietic stem and progenitor cells, similar to a hematopoietic stem cell niche. Hence, bolstering native ECM by stimulation its production from hMSCs with either ascorbic acid or osteogenic medium could potentially be a useful strategy in rejuvenating old hMSCs.

Thus whether the total or individual amounts of native ECM, the breadth of composition of native ECM, the geometry of ECM organization, or the ability of ECM to sequester growth factors, retain growth factor-like motifs (e.g. similar to the EGF-like repeats found on laminin and tenascin), or regulate other morphogens such as Wnt signaling that can potentially regulate MSC proliferation and differentiation are important factors in explaining the mechanism(s) of how young ECM can rejuvenate old MSCs are salient areas for future investigation.

**REFERENCES**

1. Fuchs E, Chen T. A matter of life and death: self-renewal in stem cells. EMBO Reports 14:39-48, 2013. PMID: 23229591 doi: 10.1038/embor.2012.197
2. Rando TA. Stem cells, ageing and the quest for immortality. Nature 441:1080-1086, 2006. PMID: 16810243 doi: 10.1038/nature04958
3. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. Nature Rev Mol Cell Biol 9:11-21, 2008. PMID: 18097443 doi: 10.1038/nrm2319
4. Bianco P. Minireview: The stem cell next door: skeletal and hematopoietic stem cell “niches” in bone. Endocrinology 152:2957-2962, 2011. PMID: 21610157 doi: 10.1210/en.2011-0217
5. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nature Rev Immunol 8:726-736, 2008. PMID: 19172693 doi: 10.1038/nri2395
6. Bianco P, Cao X, Frenette PS, Mao JJ, Robey PG, Simmons PJ, Wang C-Y. The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. Nature Med 19:35-42, 2013. PMID: 23296015 doi: 10.1038/nm.3028
7. Tapp H, Hanley Jr, EN, Patt JC, Gruber HE. Adipose-derived stem cells: characterization and current application in orthopaedic tissue repair. Exp Biol Med 234:1-9, 2009. PMID: 19109553 doi: 10.3181/0805/MR-170
8. Fukada S, Uezumi A, Ikemoto M, Masuda S, Segawa M, Tanimura N, Yamamoto H, Miyagoe-Suzuki Y, Takeda S. Molecular signature of quiescent satellite cells in adult skeletal muscle. Stem Cells 25:2448-2459, 2007. PMID:17600112 doi: 10.1634/stemcells.2007-0019
9. Drisan M, Yap S, Casteilla L, Chen C-W, Corselli M, Part TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng, P-N, Traas J, Schugar R, Deasy BM, Badylak S, Buhring H-J, Giacobino J-P, Lazzari L, Huard J, Peault B. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3:301-313, 2008. PMID: 18786417 doi: 10.1016/j.stem.2008.07.003
10. Ali H, Al-Mulla F. Defining umbilical cord blood stem cells. Stem Cell Discovery 2:15-23, 2012. doi: 10.4236/scd.2012.21003
11. Delorme B, Chateauvieux S, Charbord P. The concept of mesenchymal stem cells. Regen Med 1:497-509, 2006. PMID: 17465844 doi: 10.2217/17460751.1.4.497
12. Mauney JR, Kirker-Head, Abrahamson L, Gronowicz G, Volloch V, Kaplan DL. Matrix-mediated retention of *in vitro* osteogenic differentiation potential and *in viv*o bone-forming capacity by human adult bone marrow-derived mesenchymal stem cells during *ex vivo* expansion. J Biomed Mater Res 79A:464-475, 2006. PMID: 16752403 doi: 10.1002/jbm.a.30876
13. D’Ippolito G, Schiller PC, Ricordi C, Roos BA, Howard GA. Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. J Bone Miner Res 14:1115-1122, 1999. PMID: 10404011 doi: 10.1359/jbmr.1999.147.1115
14. Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 33:919-926, 2003. PMID: 14678851 doi: 10.1016/j.bone.2003.07.005
15. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F. Human bone marrow-derived mesenchymal stem cells do not undergo transformation after long-term *in vitro* culture and do not exhibit telomere maintenance mechanisms. Cancer Res 67:9142-9149, 2007. PMID: 17909019 doi: 10.1158/0008-5472.CAN-06-4690
16. Yu K-R, Kang K-S. Aging-related genes in mesenchymal stem cells: a mini-review. Gerontology Published online August 17, 2013. doi: 10.1159/000353857.
17. Shay JW, Wright WE. Hayflick, his limit, and cellular aging, Nature Reviews Molecular and Cell Biology 1: 72-76, 2000. PMID: 11413492 doi: 10.1038/35036093
18. Campisi J, d’Adda diFagagna F. Cellular senescence: when bad things happen to good cells. Nature Reviews Mol Cell Biol 8: 729-740, 2007. PMID: 17667954 doi:10.1038/nrm2233
19. D’Ippolito, G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC. 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 Science 117: 2971-2981, 2004. PMID: 15173316 doi: 10.1242/jcs.01103
20. D’Ippolito G, Diabira S, Howard GA, Roos BA, Schiller PC. Low oxygen tension inhibits osteogenic differentiation and enhances stemness of human MIAMI cells. Bone 39: 513-522, 2006. PMID: 16616713 doi: 10.1016/j.bone.2006..02.06
21. Mazumdar J, O’Brien WT, Johnson RS, LaManna JC, Chavez JC, Klein PS, Simon MC. O2 regulates stem cells through Wnt/β-catenin signaling. Nature Cell Biol 12:1007-1013, 2010. PMID: 20852629 doi: 10.1038/ncb2102
22. Bachand F, Auxtexier C. Functional regions of human telomerase reverse transcriptase and human telomerase RNA required for telomerase activity and RNA-protein interactions. Mol Cell Biol 21:1888-1897, 2001. PMID: 11238925. doi: 10.1128/MCB.21.5.1888-1897.2001
23. Nguyen BN, Elmore LW, Holt SE. Mechanisms of dominant-negative telomerase function. Cell Cycle 8:3227-3233, 2009. PMID: 19738429. doi: 10.4161/cc.8.19.9788
24. Zhang Q, Kim N-K, Feigon J. Architecture of human telomerase RNA. Proc Nat Acad Sci (USA) 108:20325-20332, 2011. PMID: 21844345. doi:10.1073/pnas.1100279108.
25. Shay JW, Wright WE. Hallmarks of telomeres in ageing research. J Path 211:114-123, 2007. PMID: 17200948. doi: 10.1002/path.2090
26. Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. Dev Genet 18:173-179, 1996. PMID:8934879
27. Simonsen JL, Rosada C, Serakinci N, Justesen J, Stenerup K, Rattan SIS, Jensen TG, Kassem M. Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat Biotechnol 20:592-596, 2002. PMID: 12042863 doi: 10.1038/nbt0602-592
28. Zimmermann S, Voss M, Kaiser S, Kapp U, Waller CF, Martens UM. Lack of telomerase activity in human mesenchymal stem cells. Leukemia 17:1146-1149, 2003. PMID: 12764382. doi: 10.1038./sj.leu.2402962
29. Zhao Y-M, Li J-Y, Lan J-P, Lai X-Y, Luo Y, Sun J, Yu J, Zhu Y-Y, Zeng F-F, Zhou Q, Huang H. Cell cycle dependent telomere regulation by telomerase in human bone marrow mesenchymal stem cells. Biochem Biophys Res Commun 369:1114-1119, 2008. PMID: 18339310. doi: 10.1016/j.bbrc.2008.03.011
30. Samsonraj RM, Raghunath M, Hui JH, Ling L, Nurcombe V, Cool SM. Telomere length analysis of human mesenchymal stem cells by quantitative PCR. Gene 519:348-355, 2013. PMID:23380569. doi: 10.1016/j.gene.2013.01.039
31. Gronthos S, Chen S, Wang C-Y, Robey PG, Shi S. Telomerase accelerates osteogenesis of bone marrow stromal stem cells by upregulation of CBFA1, osterix, and osteocalcin. J Bone Miner Res 18:716-722, 2003. PMID: 12674332 doi: 10.1359/jbmr.2003.18.4.716
32. Saeed H, Abdallah BM, Ditzel N, Catala-Lehnen P, Qiu W, Amling M, Kassem M. Telomerase-deficient mice exhibit bone loss owing to defects in osteoblasts and increased osteoclastogenesis by inflammatory microenvironment. J Bone Miner Res 26:1494-1505, 2011. PMID: 21308778. PMID: 21308778 doi: 10.1002/jbmr.349
33. Kassem M, Abdallah BM, Yu Z, Ditzel N, Burnes JS. The use of hTERT-immortalized cells in tissue engineering. Cytotechnology 45:39-46, 2004. PMID: 19003242 doi: 10.1007/s10616-004-5124-2
34. Abdallah BM, Haack-Sorensen M, Burnes JS, Elsnab B, Jakob F, Hokland P, Kassem M. Maintenance of differentiation potential of human bone marrow mesenchymal stem cells immortalized by human telomerase reverse transcriptase gene in despite of extensive proliferation. Biochem Biophys Res Commun 326:527-538, 2005. PMID: 15596132 doi: 10.1016/j.bbrc.2204.11.059
35. Piper SL, Wang M, Yamamoto A, Malek F, Luu A, Kuo AC, Kim HT. Inducible immortality in hTERT-human mesenchymal stem cells. J Orthop Res 30:1879-1885, 2012. PMID: 22674533 doi: 10.1002/jor.22162
36. Nakahara H, Misawa H, Hayashi T, Kondo E, Yuasa T, Kubota Y, Seita M, Kawamoto H, Hassan WARA, Hassan RARA, Javed SM, Tanaka M, Endo H, Noguchi H, Matsumoto S, Takata K, Tashiro Y, Nakaji S, Ozaki T, Kobayashi N. Bone repair by transplantation of hTERT-immortalized human mesenchymal stem cells in mice. Transplantation 88:346-353, 2009. PMID: 19667936 doi: 10.1097/TP.0b013e3181ae5ba2
37. Bischoff DS, Makhijani NS, Yamaguchi DT Constitutive expression of human telomerase enhances the proliferation potential of human mesenchymal stem cells. BioResearch Open Access 1:273-279. PMID: 23515239 doi: 10.1089/biores.2012.0252
38. Bellows CG, Heersche JN, Aubin JE. Determination of the capacity for proliferation and differentiation of osteoprogenitor cells in the presence and absence of dexamethasone. Dev Biol 140:132-138, 1990. PMID: 2358113
39. Malaval L, Liu F, Roche P Aubin JE. Kinetics of osteoprogenitor proliferation and osteoblast differentiation *in vitro*. J Cell Biochem 74:616-627, 1999. PMID: 10440931
40. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy :315-317, 2006. PMID: 16923606 doi: 10.1080/14653240600855905
41. Bianco P, Cao X, Frenette PS, Mao JJ, Robey PG, Simmons PJ, Wang C-Y. The meaning, the sense, and the significance: translating the science of mesenchymal stem cells. Nature Medicine 19:35-42. doi: 10.1038/nm.3028
42. Gronthos S, Graves SE, Ohta S, Simmons PJ. The STRO-1+ fraction of adult human bone marrow contains the osteogenic precursors. Blood 84:4164-4173, 1994. PMID: 7994030
43. Delorme B, Chateauvieux S, Charbord P. The concept of mesenchymal stem cells. Regenerative Med 1:497-509, 2006. PMID: 17465844 doi: 10.2217/17460751.1.4.497
44. Pilz GA, Braun J, Ulrich C, Felka T, Warstat K, Ruh M, Schewe B, Abele H, Larbi A, Aicher WK. Human mesenchymal stromal cells express CD14cross-reactive epitopes. Cytometry Part A 79:635-645, 2011. PMID: 21735544 doi: 10.1002/cyto.a.21073
45. Niehage C, Steenblock C, Pursche T, Bornhauser M, Corbeil D, Hoflack B. The cell surface proteome of human mesencymal stromal cells. PLoS One 6:e20399, 2011. PMID: 21637820 doi: 10.1371/journal.pone.0020399
46. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 28:875-884, 2000. PMID: 10989188 doi: 10.1016/S0301-472X(00)00482-3
47. Gang EJ, Bosnakovski D, Figueiredo CA, Visser JW, Perlingeiro RCR. SSEA-4 identifies mesenchymal stem cells from bone marrow. Blood 109:1743-1751, 2007. PMID: 17062733 doi: 10.1182/blood-2005-11-010504
48. Qian H, Le Blanc K, Sigvardsson M. Primary mesenchymal stem cell and progenitor cells from bone marrow lack expression of CD44 protein. J Biol Chem 287: 25795-25807, 2012. PMID: 22654106 doi: 10.1074/jbc.M112.339622
49. Mu J, Wei LX. Telomere and telomerase in oncology. Cell Res 12:1-7, 2002. PMID: 11942406 doi: 10.1038/sj.cr.7290104
50. Low KC, Tergaonkar V. Telomerase: central regulator of all of the hallmarks of cancer. Trends Biochem Sci 38:426-434, 2013. PMID: 23932019 doi: 10.1016/j.tibs.2013.07.001
51. Belgiovine C, Chiodi I, Mondello C. Telomerase: cellular immortalization and neoplastic transformation. Multiple functions of a multifaceted complex. Cytogenet Genome Res 122:255-262, 2008.PMID: 19188694 doi: 10.1159/000167811
52. Yamaoka E, Hiyama E, Sotomaru Y, Onitake Y, Fukuba I, Sudo T, Sueda T, Hiyama K. Neoplastic transformation by TERT in FGF-2-expanded human mesenchymal stem cells. Int J Oncol 39:5-11, 2011. PMID: 21573488 doi: 10.3892/ijo.2011.1029
53. Serakinci N, Guldberg P, Burns JS, Abdallah B, Schrodder H, Jensen, T, Kassem, M. Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 23:5095-5098, 2004. PMID: 15107831 doi: 10.1038/sj.onc.1207651
54. Huang G, Zheng Q, Sun J, Guo C, Yang J, Chen R, Xu Y, Wang G, Shen D, Pan Z, Jin J, Wang J. Stabilization of cellular properties and differentiation multipotential of human mesenchymal stem cells transduced with hTERT gene in a long-term culture. J Cell Biochem 103:1256-1269, 2008. PMID: 18027880 doi: 10.1002/jcb.21502
55. Baumer Y, Scholz B, Ivanov S, Schlosshauer B. Telomerase-based immortalization modifies the angiogenic/inflammatory responses of human coronary artery endothelial cells. Exp Biol Med 236:692-700, 2011. PMID: 21558092 doi: 10.1258/ebm.2011.010300
56. Kuznetsov SA, Mankani MH, Robey PG. *In vivo* formation of bone and haematopoietic territories by transplanted human bone marrow stromal cells generated in medium with and without osteogenic supplements. J Tissue Eng and Regen Med 7:226-235, 2013. PMID: 22052864 doi:10.1002/term.515
57. Liu PP, Rui YE, Ni M, Chan KM. Tenogenic differentiation of stem cells for tendon repair-what is the current evidence. J Tissue Eng Regen Med 5:144-163, 2011. PMID: 21548133 doi: 10.1002/term.424
58. Dayoub H, Dumont RJ, Li JZ, Dumont AS, Hankins GR, Kallmes DF, Helm GA. Human mesenchymal stem cells transduced with recombinant bone morphogenetic protein-9 adenovirus promote osteogenesis in rodents. Tissue Eng 9:347-356, 2003. PMID: 12740097 doi: 10.1089/107632703764664819
59. Janicki P, Boeuf S, Steck E, Egermann M, Kasten P, Richter W. Prediction of *in vivo* bone forming potency of bone marrow-derived human mesenchymal stem cells. Eur Cell Mater 21:488-507, 2011. PMID: 21710441
60. Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflammation 2:8, 2005. PMID: 16045800 doi: 10.1186/1476-9255-2-8
61. Griffin MD, Ryan AE, Alagesan S, Lohan P, Treacy, O, Ritter T. Anti-donor immune responses elicited by allogeneic mesenchymal stem cells: what have we learned so far? Immunol and Cell Biol 91:40-51, 2013. PMID: 23207278 doi: 10.1038/icb.2012.67
62. Larsen KH, Frederiksen CM, Burns JS, Abdallah BM, Kassem M. Identifying a molecular phenotype for bone marrow stromal cells with *in vivo* bone-forming capacity. J Bone Miner Res 25:796-808, 2010. PMID: 19821776 doi: 10.1359/jbmr.091018
63. Bentzon JF, Stenderup K, Hansen FD, Schroder HD, Abdallah BM, Jensen TG, Kassem M. Tissue distribution and engraftment of human mesenchymal stem cells immortalized by human telomerase reverse transcriptase. Biochem Biophys Res Commun 330:633-640, 2005. PMID: 15809044 doi: 10.1016/j.bbrc.2005.03.072.
64. Kale S, Biermann S, Edwards C, Tarnowski C, Morris M, Long MW. Three-dimensional cellular development is essential for *ex vivo* formation of human bone. Nature Biotechnol 18:954-958, 2000. PMID: 10973215 doi: 10.1038/79439
65. Fisher MB, Mauck RL. Tissue engineering and regenerative medicine: recent innovations and the transition to translation. Tissue Eng Part B 19:1-13, 2013. PMID: 23253031 doi: 10.1089/ten.teb.2012.0723
66. Billstrom GH, Blom AW, Larsson S, Beswick AD. Application of scaffolds for bone regeneration strategies: current trends and future directions. Injury 44 (Supplement 1): S28-S33, 2013. PMID: 23351866 doi: 10.1016/S0020-1383(13)70007-X
67. Mankani MH, Kuznetsov SA, Marshall GW, Robey PG. Creation of new bone by the percutaneous injection of human bone marrow stromal cell and HA/TCP suspensions. Tissue Eng Part A 14:1949-1958, 2008. PMID: 18800877 doi: 10.1089/ten.tea.2007.0348
68. Van der Stok J, Wang H, Yavari SA, Siebelt M, Sandker M, Waarsing JH, Verhaar JAN, Jahr H, Zadpoor AA, Leeuwenburgh CG, Weinans H. Enhanced bone regeneration of cortical segmental bone defects using porous titanium scaffolds incorporated with colloidal gelatin gels for time- and dose-controlled delivery of dual growth factors. Tissue Eng Part A 2013 PMID: 23822814 doi: 10.1089/ten.tea.2013.0181
69. Kruger EA, Im DD, Bischoff DS, Pereira CT, Huang W, Rudkin GH, Yamaguchi DT, Miller TA. *In vitro* mineralization of human mesenchymal stem cells on three-dimensional type I collagen versus PLGA scaffolds: a comparative analysis. Plast ReconstrSurg 127:2301-2311, 2011. PMID: 21617464 doi: 10.1097/PRS.0b013e318213a004
70. Amini AR, Laurencin CT, Nukavarapu SP. Bone Tissue Engineering: recent advances and challenges. Crit Rev Biomed Eng 40:363-408, 2012. PMID: 23339648
71. Zhu J. Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. Biomaterials 31:4639-4656, 2010. PMID: 20303169 doi: 10.1016/j.biomaterials.2010.02.044
72. Ohba S, Hojo H, Chung U-I. Bioactive factors for tissue regeneration: state of the art. Muscles, Ligaments and Tendons Journal 2:193-203, 2012. PMID: 23738297
73. Lee J, Yoo JJ, Atala A, Lee SJ. The effect of controlled release of PDGF-BB from heparin-conjugated electrospun PCL/gelatin scaffolds on cellular bioactivity and infliltration. Biomaterials 33:6709-6720, 2012. PMID: 22770570 doi: 10.1016/j.biomaterials.2012.06.017
74. Patel ZS, Young S, Tabata Y, Jansen JA, Wong MEK, Mikos AG. Dual delivery of an angiogenic and an osteogenic growth factor for bone regeneration in a critical size defect model. Bone 43:931-940, 2008. PMID: 18675385 doi: 10.1016/j.bone.2008.06.019
75. Duan B and Wang M. Customized Ca-P/PHBV nanocomposite scaffolds for bone tissue engineering: design, fabrication, surface modification and sustained release of growth factor. J R Soc Interface 7: S615-S629, 2010. PMID: 20504805 doi: 10.1098/rsif.2010.0127.focus
76. Kucia M, Wojakowski W, Reca R, Machalinski B, Gozdizik J, Majka M, Baran J, Ratajczak, Ratajczak MZ. The migration of bone marrow-derived non-hematopoietic tissue-committed stem cells is regulated in an SDF-1-, HGF-, and LIF-dependent manner. Arch Immunol Ther Exp 54:121-135, 2006. PMID: 16648972 doi: 10.1007/s0005-006-0015-1
77. He X, Ma J, Jabbari E. Migration of marrow stromal cells in response to sustained release of stromal-derived factor-1α from poly(lactide ethylene oxide fumarate) hydrogels. Int J Pharmaceutics 390:107-116, 2010. PMID: 20219655 doi: 10.1016/j.ipharm.2009.12.063
78. Lau TT and Wang D-A. Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine. Expert Opin Biol Ther 11:189-197, 2011. PMID: 21219236 doi: 10.1517/14712598.2011.546338
79. Young, S, Patel ZS, Kretlow JD, Murphy MB, Mountziaris PM, Baggett LS, Ueda H, Tabata Y, Jansen JA, Wong M, Mikos AG. Dose effect of dual delivery of vascular endothelia growth factor and bone morphogenetic protein-2 on bone regeneration in a rat critical-sized defect model. Tissue Eng Part A 15:2347-2362, 2009. PMID: 19249918 doi: 10.1089/ten/tea.2008.0510
80. Schantz J-T, Chim H, Whiteman M. Cell guidance in tissue engineering: SDF-1 mediates site-directed homing of mesenchymal stem cells within three-dimensional polycaprolactone scaffolds. Tissue Eng 13:2615-2624, 2007. PMID: 17961003 doi: 10.1089/ten.2006.0438
81. Tasso R, Augello A, Boccardo S, Salvi S, Carida M, Postglione F, Fais F, Truini M, Cancedda R, Pennesi G. Recruitment of a host’s osteoprogenitor cells using exogenous mesenchymal stem cells seeded on porous ceramic. Tissue Eng Part A 15:2203-2212, 2009. PMID: 19265473 doi: 10.1089/ten.tea.2008.0269
82. Kumar S, Chanda D, Ponnazhagan. Therapeutic potential of genetically modified mesenchymal stem cells. Gene Ther 15:711-715, 2008. PMID: 18356815 doi: 10.1038/gt.2008.35
83. Evans C. Gene therapy for the regeneration of bone. Injury 42:599-604, 2011. PMID: 21489526 doi: 10.1016/j.injury.2011.03.032
84. Evans, CH. Gene delivery to bone. Adv Drug Deliv Rev 64:1331-1340, 2012. PMID: 22480730 doi: 10.1016/j.addr.2012.03.013
85. Huang Y-C, Kaigler D, Rice KG, Kresbach PH, Mooney DJ. Combined angiogenic and osteogenic factor delivery enhances bone marrow stromal cell-driven bone regeneration. J Bone Miner Res 20: 848-857, 2005. PMID: 15824858 doi: 10.1359/JBMR.041226
86. Meinel L, Hofmann S, Betz O, Fajardo R, Merkle HP, Langer R, Evans CH, Vunjak-Novakovic G, Kaplan DL. Osteogenesis by human mesenchymal stem cells cultured on silk biomaterials: comparison of adenovirus mediated gene transfer and protein delivery of BMP-2. Biomaterials 27:4993-5002, 2006. PMID: 16765437 doi: 10.1016/j.biomaterials.2006.05.021
87. Thieme S, Ryser M, Gentsch M, Navratiel K, Brenner S, Stiehler M, Rolfing J, Gelinsky M, Rosen-Wolff A. Stromal cell-derived factor-1α-directed chemoattraction of transiently CXCR4-overexpressing bone marrow stromal cells into functionalized three-dimensional biomimetic scaffolds. Tissue Eng Part C Methods 4:687-696, 2009. PMID: 19260802 doi: 10.1089/ten.tec.2008.0556
88. Arufe MC, De la Fuente A, Fuentes-Boquette I, De Toro FJ, Blanco FJ. Differentiation of synovia CD105+ human mesenchymal stem cells into chondrocyte-like cells through spheroid formation. J Cell Biochem108:145-155, 2009. PMID: 19544399 doi: 10.1002/jcb.22238
89. Pelttari K, Steck E, Richter W. The use of mesenchymal stem cells for chondrogenesis. Injury 39 (Suppl 1):S58-S65, 2008. PMID: 18313473 doi: 10.1016/j.injury.2008.01.038
90. Burns JS, Rasmussen PL, Larsen KH, Schroder HD, Kassem M. Parameters in three-dimensional osteospheroids of telomerized human mesenchymal (stromal) stem cells grown on osteoconductive scaffolds that predict *in vivo* bone-forming potential. Tissue Eng Part A 16:2331-2342, 2010. PMID: 20196644 doi: 10.1089/ten.tea.2009.0735
91. Hong JW, Hwang ES, McManus MT, Amsterdam A, Tian Y, Kalmukova R, Mueller E, Benjamin T, Spiegelman BM, Sharp PA, Hopkins N, Yaffe MB. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. Science 309:1074-1078, 2005. PMID: 16099986 doi: 10.1126/science.1110955
92. Pang Y, Greisler HP. Using a type 1 collagen-based system to understand cell-scaffold interactions and to deliver chimeric collagen-binding growth factors for vascular tissue engineering. J Invest Med 58:845-848, 2010. PMID: 20683346 doi: 10.231/JIM.0b013e318ee81f7
93. Hynes RO. Extracellular matrix: not just pretty fibrils. Science 326:1216-1219, 2009. PMID: 19965464 doi: 10.1126/science.1176009.
94. Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. Curr Opin Cell Biol 24:645-651, 2012. PMID: 22898530 doi: 10.1016/j.ceb.2012.07.001
95. Humphries JD, Byron A, Humphries MJ. Integrin ligands at a glance. J Cell Sci 119:3901-3903, 2006. PMID: 16988024 doi: 10.1242/jcs.03098
96. Shattil SJ, Kim C, Ginsberg MH. The final steps of integrin activation: the end game. Nature Rev Mol Cell Biol 11:288-300, 2010. PMID: 20308986 doi:10.1038/nrm2871
97. Kim C, Ye F, Ginsberg MH. Regulation of integrin activation. Ann Rev Cell Develop Biol 27:321-345, 2011. PMID: 21663444 doi: 10.1146/annurev-cellbio-100109-104104
98. Anthis NJ, Campbell ID. The tail of integrin activation. Trends Biochem Sci 36:191-198, 2011. PMID: 21216149 doi: 10.1016/j.tibs.2010.11.002
99. Shen B, Delaney MK, Du X. Inside-out, outside-in, and inside-outside-in: G-protein signaling in integrin-mediated cell adhesion, spreading, and retraction. Curr Opin Cell Biol 24:600-606, 2012. PMID: 22980731 doi: 10.1016/j.ceb.2012.08.011
100. Shih Y-R, Chen C-N, Tsai S-W, Wang YJ, Lee OK. Growth of mesenchymal stem cells on electrospun type I collagen nanofibers. Stem Cells 24:2391-2397, 2006. PMID: 17071856 doi: 10.1634/stemcells.2006-0253
101. Mauney JR, Kaplan DL, Volloch V. Matrix-mediated retention of osteogenic differentiation potential by human adult bone marrow stromal cells during *ex vivo* expansion. Biomaterials 25:3233-3243, 2004. PMID: 14980418 doi: 10.1016/j.biomaterials.2003.10.005
102. Gronthos S, Simmons PJ, Graves SE, Robey PG. Integrin-mediated interactions between human bone marrow stromal precursor cells and the extracellular matrix. Bone 28:174-181, 2001. PMID: 11182375 doi: 10.1016/S8756-3282(00)00424-5
103. Watt FM, Huck WT. Role of the extracellular matrix in regulating stem cell fate. Nature Rev Mol Cell Biol 14:467-473, 2013. PMID: 23839578 doi: 10.1038/nrm3620
104. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 6:483-495, 2004. PMID: 15068789 doi: 10.1016/S1534-5807(04)00075-9
105. Mathieu PS, Loboa EG. Cytoskeletal and focal adhesion influences on mesenchymal stem cell shape, mechanical properties, and differentiation down osteogenic, adipogenic, and chondrogenic pathways. Tissue Eng Part B Rev 18:436-444, 2012. PMID: 22741572 doi: 10.1089/ten.teb.2012.0014
106. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science 324:1673-1677, 2009. PMID: 19556500 doi: 10.1126/science.1171643
107. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 126:677-689, 2006. PMID: 16923388 doi: 10.1016/j.cell.2006.06.044
108. Swift J, Ivanovska IL, Buxboim A, Harada T, Dingal PCDP, Pinter J, Pajerowski JD, Spinler KR, Shin J-W, Tewari M, Rehfeldt F, Speicher DW, Discher DE. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science 341: 1240104, 2013. PMID: 23990565 doi: 10.1126/science.1240104
109. Chen, X-D, Dusevich V, Feng JQ, Manolagas SC, Jilka RL. Extracellular matrix made by bone marrow cells facilitates expansion of marrow-derived mesenchymal progenitor cells and prevents their differentiation into osteoblasts. J Bone Miner Res 22:1943-1956, 2007. PMID: 17680726 doi: 10.1359/JBMR.070725
110. Lai Y, Sun Y, Skinner CM, Son EL, Lu Z, Tuan RS, Jilka RL, Ling J, Chen X-D. Reconstitution of marrow-derived extracellular matrix *ex vivo*: a robust culture system for expanding large-scale highly functional human mesenchymal stem cells. Stem Cells Dev 19:1095-1107, 2010. PMID: 19737070 doi: 10.1089/scd.2009.0217
111. Choi HR, Cho KA, Kang HT, Lee JB, Kaeberlein M, Suh Y, Chung K, Part SC. Restoration of senescent human diploid fibroblasts by modulation of the extracellular matrix. Aging Cell 10:148-157, 2011. PMID: 21108727 doi: 10.1111/j..1474-9726.2010.00654.x
112. Liu B, Ghosh S, Yang X, Zheng H, Liu X, Wang Z, Jin G, Zheng B, Kennedy BK, Suh Y, Kaeberlein M, Tryggvason K, Zhou Z. Resveratrol rescues SIRT-1-dependent adult stem cell decline and alleviates progeroid features in laminopathy-based Progeria. Cell Metabolism 16:738-750, 2012. PMID: 23217256 doi: 10.1016/j.cmet.2012.11.007
113. Kurtz A, Oh SJ. Age related changes of the extracellular matrix and stem cell maintenance. Preventive Med 54:S50-S56, 2012. PMID: 22285957 doi: 10.1016/j.ypmed.2012.01.003
114. Berendsen AD, Fisher LW, Kilts TM, Owens RT, Robey PG, Gutkind JS, Young MF. Modulation of canonical Wnt signaling by the extracellular matrix component biglycan. Proc Natl Acad Sci USA 108:17022-17027, 2011. PMID: 21969569 doi: 10.1073/pnas.1110629108
115. Sun Y, Li W, Lu Z, Chen R, Ling J, Ran Q, Jilka RL, Chen X-D. Rescuing replication and osteogenesis of aged mesenchymal stem cells by exposure to a young extracellular matrix. FASB J 25:1474-1485, 2011. PMID: 21248241 doi: 10.1096/fj.10-161497
116. Prewitz MC, Seib FP, von Bonin M, Friedrichs J, Stiβel A, Niehage C, Muller K, Anastassoados K, Waskow C, Hoflack B, Bornhauser M, Werner C. Tightly anchored tissue-mimetic matrices as instructive stem cell microenvironments. Nature Methods 10:788-794, 2013. PMID: 23793238 doi: 10.1038/nmeth.2523