33

Derivation of Mesenchymal Stem Cells from Human Embryonic Stem Cells

Shelley E. Brown[1,2] and Paul H. Krebsbach[1,2]
[1]*Biologic and Materials Sciences Department, University of Michigan School of Dentistry*
[2]*Biomedical Engineering Department, University of Michigan College of Engineering*
USA

1. Introduction

In the early to mid 1970's, hallmark studies were published demonstrating the ability to isolate and derive adult fibroblast cell colonies from the bone marrow stroma and the spleen (Friedenstein *et al.*, 1970; Friedenstein and Kuralesova, 1971). These fibroblast-like cells, later termed bone marrow stromal cells (BMSCs) or bone marrow-derived mesenchymal stem cells (MSCs), were shown to proliferate in culture, to continually grow upon passaging while maintaining stable karyotypic characteristics, and were comprised of cells that had multipotent potential to differentiate along multiple mesenchymal cell lineages such as bone, cartilage, fat and could support hematopoietic stem cell (HSC) differentiation (Bab *et al.*, 1984; Bab *et al.*, 1986; Friedenstein *et al.*, 1970; Friedenstein *et al.*, 1974b). Numerous studies spurred from these findings, which also led researchers in this area to explore the functions of these cells in vitro and in their normal microenvironment. Bone marrow stromal cells were transplanted *in vivo* to determine if they had the ability to re-establish the marrow microenvironment, and it was reported that the *ex vivo* expanded stromal cells did indeed restore the hematopoietic niche within the bone marrow (Friedenstein *et al.*, 1974a). These experiments further developed the hypothesis that within the bone marrow stroma resided a heterogeneous mixture of cells that function as a repository of progenitors, known as MSCs, that may migrate out of their stem cell niche in response to disease, injury, and aging. Therefore, extensive investigation into the identification of MSCs and their utility for cell-replacement therapies were the basis for a new emerging field known as tissue engineering (Ashton *et al.*, 1980; Bab *et al.*, 1986; Owen and Friedenstein, 1988; Beresford, 1989; Jaiswal *et al.*, 1997; Krebsbach *et al.*, 1999).

In the 1980's and 1990's, many groups further demonstrated that culture-adherent MSCs present in the marrow stroma were capable of differentiation into bone, cartilage, muscle, tendon, and fat for multiple species such as canine, chicken, rabbit, rat, and mouse (Jaiswal *et al.*, 1997). Using the expertise gained from these culture systems, MSCs were then isolated and propagated from human adult bone marrow (hMSCs) (Bab *et al.*, 1988); (Krebsbach *et al.*, 1997). Human MSCs were then used with site-specific delivery vehicles to repair bone, cartilage, and other connective tissues (Haynesworth *et al.*, 1992a; Haynesworth *et al.*, 1992b). Additionally, a series of monoclonal antibodies were developed to identify characteristic surface markers on hMSCs, which would prove to be beneficial to researchers interested in not only identifying MSCs, but also subpopulations of osteoprogenitor cells

(Haynesworth *et al.*, 1992a; Haynesworth *et al.*, 1992b); (Gronthos *et al.*, 1999). Simultaneously, Caplan et al. used the embryonic chick limb bud mesechymal cell culture system as an assay for the purification of inductive factors in bone to further develop the technology for isolating, expanding, and preserving the stem cell capacity of adult human bone marrow-derived mesenchymal stem cells (Caplan, 2005). With this newly acquired knowledge and the emerging technologies in biomedical engineering, hMSCs became the principle cell source for cell-based pre-clinical bone tissue engineering studies.

Currently, substantial advances have been made to address clinical needs for regeneration of damaged or diseased tissues. The three main approaches of cell-based clinical therapies that employ the use of hMSCs are: 1) from a tissue engineering standpoint where cells are incorporated into 3D biomaterial scaffolds for the replacement of tissue *in vivo*, 2) from a cell replacement therapy standpoint where allogeneic donor cells are used to replace ablated tumors and diseased cells; and 3) from a inductive standpoint where cells provide cytokine and growth factor cues that stimulate host reparative events and inhibit degenerative events (Caplan, 2005). Thus, clinical protocols were developed to establish that autologous hMSCs could be safely implanted back in order to reconstitute the marrow microenvironment for breast cancer and osteogenesis imperfecta (OI) patients following chemotherapy treatment (Koc *et al.*, 2000; Horwitz *et al.*, 2002). Additionally, hMSCs have been shown to have immunomodulatory effects and could induce immune suppression in patients (Le Blanc and Pittenger, 2005); (Aggarwal and Pittenger, 2005). Although the use of hMSCs has been successfully used in some cases, there are challenges that scientists and clinicians must overcome before the transplantation of these cells is incorporated into routine clinical practice. Specifically, the classic method to isolate MSCs from bone marrow relies on their capacity to adhere to plastic, their resistance to trypsinization during passaging, and proliferation in growth medium containing serum (Olivier *et al.*, 2006). However, cell availability is greatly limited with this method because MSCs are present at low concentrations in the marrow, occurring at less than 1 in 100,000-500,000 nucleated cells (Caplan, 2005). Also, the availability of tissues for their isolation remains limiting and requires invasive procedures that may cause severe donor site morbidity.

Therefore, an alternative source for generating MSCs can be found in human embryonic stem cells (hESCs) (Thomson *et al.*, 1998). Human ESCs are an alternative source for generating MSCs due to the fact that they can theoretically be expanded infinitely and also because using these cells would eliminate the need for invasive cell harvesting techniques. Host immune rejection could also be circumvented by the use of autologous hESCs generated from nuclear transfer or from immune compatible allogeneic hESCs. Derivation of mesenchymal stem cells from human ES cells will further the understanding of the differentiation pathways and important cellular events that occur during early human development and could also have useful clinical applications. Because of the therapeutic potential, particularly in the areas of cell therapy and regenerative medicine, derivation of MSCs from hESCs (hESC-MSCs) has specific advantages over the current "gold standard" use of autologous and allogeneic adult hMSCs for bone tissue engineering. (Olivier *et al.*, 2006).

2. Human embryonic stem cells

The major advancements in the area of stem cell culture, derivation, propagation, and differentiation paved the way for a pivotal discovery that was reported in a 1998 study from the University of Wisconsin. Thomson et. al. described the first successful isolation and long

term sustained culture of a small cluster cells from the inner cell mass of four-day old embryos (Thomson *et al.*, 1998). These cells, known as human embryonic stem cells (hESCs), represent a robust biologic tool and model system through which the scientific and medical communities will better understand human development, disease pathophysiology, organogenesis, and mechanisms for cellular differentiation; all of which will help develop and improve the field of regenerative medicine. These embryonic stem cells are derived by the selection and expansion of individual colonies rather than clonal expansion of a single cell. Human ESCs are pluripotent cells that are presumed to have virtually unlimited proliferation capacity in vitro, maintain normal karyotypic characteristics, sustain high levels of telomerase activity, and retain uniform undifferentiated morphology in prolonged culture (Thomson *et al.*, 1998). In addition, hESCs have the ability to differentiate along the three embryonic germ layers *in vivo* as evidenced by teratoma formation after injection into severe combined immunodeficient (SCID) mice. The teratomas can contain gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). They have also been shown to express certain cell surface markers that are widely used to confirm pluripotency, such as stage-specific embryonic antigen SSEA-4, TRA-1-60, TRA-1-81, and alkaline phosphatase. Oct-4, a transcription factor, has been identified as another key indicator of the undifferentiated state.

To maintain their self renewal capacity, hESCs were originally cultured on mouse embryonic fibroblast (MEF) feeder layers and grown under serum-free conditions using serum replacement (SR) with supplements of basic fibroblast growth factor (bFGF). Under these culture conditions, hESCs have been passaged continuously and maintained pluripotency as well as a normal karyotype. However, it has been reported that hESCs have been successfully cultured with feeder cells of human origin, such as human bone marrow stromal cells (hBMSCs), human placental fibroblasts, human foreskin fibrobalsts (hFFs), feeders derived from hESCs, and on polymeric substrates in feeder free conditions (Cheng *et al.*, 2003; Genbacev *et al.*, 2005; Wang *et al.*, 2005; Stojkovic *et al.*, 2005; Hovatta *et al.*, 2003; Villa-Diaz *et al.*, 2010). In order to safely use hESCs in a clinical setting, it is imperative that that feeder-free and animal product-free culture conditions are explored further to overcome the risks of cross-transfer of pathogens from xenogeneic sources.

The ability of hESCs to maintain an undifferentiated state indefinitely in culture and to differentiate into all cell types and tissues within the human body has created a high demand for research. Although the cells are of great scientific interest, progression of this type of research has been met with great controversy and resistance due to the ethical concern of destroying early human embryos for derivation of hESC lines (Knowles, 2004; Baschetti, 2005; Gruen and Grabel, 2006). Nevertheless, once the ethical concerns are abated through placement of the appropriate guidelines and policies on research, the hESC field will not only evolve, but will continue to rapidly progress toward monumental medical and scientific breakthroughs.

3. Human embryonic stem cell derived mesenchymal stem cells

The current major goal for hESC research in regenerative medicine is the controlled differentiation into specific progenitor cells for the purpose of replacing or regenerating damaged tissue. Therefore, the ability to obtain large quantities of multipotent cells from hESCs represents a challenge for cell based therapy and tissue engineering strategies that currently rely on human bone marrow stromal cells (hMSCs). Within the diverse population

of hMSCs, there exist early progenitor mesenchymal stem cells capable of self-renewal and multi-lineage differentiation into cell types such as osteoblasts, chondrocytes, and adipocytes (Bianco *et al.*, 2003; Wagers and Weissman, 2004). While hMSCs make a useful source of osteoprogenitor cells for tissue engineering strategies, they have limited proliferation and differentiation capacity. In contrast, hESCs which are able to proliferate indefinitely in vitro, represent a potentially unlimited source of mesenchymal stem cells.

Recent studies demonstrate that the derivation of hESC-MSCS, mesenchymal precursors derived from hESCs, has been achieved via various isolation methods, and the generation of osteoblasts has been achieved in co-culture with primary bone derived cells (PBDs), in the presence of known osteogenic supplements, and in transwell co-culture with hBMSCs (Ahn *et al.*, 2006; Cao *et al.*, 2005; Duplomb, 2007; Karner *et al.*, 2007; Karp *et al.*, 2006; Sotille *et al.*, 2003; Tong *et al.*, 2007). Although the identification and characterization of hESC-MSCs has been reported, the data are quite vast and varied in terms of the derivation method, cell culture conditions, the mechanism of differentiation (epithelial-mesenchymal transition vs neural crest stem cell-mesenchymal differentiation), multilineage differentiation potential, and surface markers used to select for a pure mesenchymal stem cell subpopulation. As the field continues to evolve, careful attention should be placed on standardizing these parameters along clinical-grade good manufacturing practice (GMP guidelines). Through the isolation and identification of hESC-MSCs and the ability to produce a large supply of progenitor cells that can be genetically modified, the field hESC-MSC based tissue engineering and regenerative medicine strategies holds great promise.

3.1 Derivation methods

Thorough and extensive investigation into the definition, differentiation, and identification of mesenchymal stem cells has occurred over the last three decades. However, there are fundamental mechanistic and developmental concepts that remain poorly understood. The foundation laid by pioneers in the MSC field has provided current researchers with a breadth of knowledge to draw upon because the same fundamental questions are being investigated to identify the true ''MSC" from differentiating hESCs. Many investigators state that although MSCs isolated from the adult bone marrow have been shown to differentiate *in vitro* and *in vivo*, as well as have been successfully used in a clinical setting to repopulate the marrow environment in cancer patients, harvesting and utilizing adult hMSCs has disadvantages such as tissue availability, donor site morbidity, and host immune rejection (Caplan, 2005; Horwitz *et al.*, 2002; Karp *et al.*, 2006). Therefore, hESCs have been the topic of great discussion and interest as a potential repository of cells that can provide an unlimited number of specialized mesenchymal stem cells known as hESC-MSCs.

Numerous isolation protocols have been reported describing successful derivation and differentiation of hESC-MSCs (Arpornmaeklong *et al.*, 2009; Barberi *et al.*, 2005; Brown *et al.*, 2009; de Peppo *et al.*, 2010a; de Peppo *et al.*, 2010b; Evseenko *et al.*, 2010; Karlsson *et al.*, 2009; Karp *et al.*, 2006; Kopher *et al.*, 2010; Kuznetsov *et al.*, ; Lian *et al.*, 2007; Olivier *et al.*, 2006; Smith *et al.*, 2009; Trivedi and Hematti, 2007; Xu *et al.*, 2004). One of the first reports of the derivation of a MSC-like progenitor population was in 2004, where fibroblast-like hESC derivatives were infected with a human telomerase reverse transcriptase (hTERT) retrovirus, as a result showed extended proliferative capacity, supported undifferentiated growth of hESCs as a feeder layer, and differentiated into osteoblasts (Xu *et al.*, 2004). Following that study, another group reported the successful production of hESC-MSCs when cultured on murine OP9 stromal cells in the presence of heat-inactivated FBS, and

indicated that the hESC-MSCs had a similar immunophenotype to hMSCs after flow cytometry was performed to purify the hESC-MSC population from the stromal cell feeder (Barberi *et al.*, 2005). Another method for hESC-MSC production involved the use of spontaneously differentiated hESC colonies. The cells obtained became morphologically fibroblastic and homogenous after multiple passages, possessed a characteristic MSC immunophenotype, and supported hESC and hematopoietic progenitor cell growth (Olivier *et al.*, 2006). Of particular importance, two reports showed the ability to reproducibly derive clinically compliant hESC-MSCs in a xeno-free environment where all contaminating animal-derived components were replaced with human-derived or recombinant components. Thus, they cultivated a hESC-MSC line suitable for clinical use ((Karlsson *et al.*, 2009; Lian *et al.*, 2007). Other groups described similar findings, demonstrating that hESCs had the ability to reproducibly proliferate, differentiate, and commit to the mesodermal lineage in various cell culture conditions (both in monolayer and 3D) while retaining their multilineage differentiation potential and self renewal capacity, further demonstrating their high potential for tissue engineering applications (Arpornmaeklong *et al.*, 2009; Brown *et al.*, 2009; de Peppo *et al.*, 2010a; de Peppo *et al.*, 2010b; Evseenko *et al.*, 2010; Kopher *et al.*, 2010; Lian *et al.*, 2007; Smith *et al.*, 2009; Trivedi and Hematti, 2007).

In summary, multiple approaches have attempted to achieve the most direct and efficient derivation of hESC-MSCs. A variety of studies have compared using the embryoid body (EB) step versus omitting this step, using multiple media formulations with and without serum, and using feeder-free cultures versus co-culture. These reports greatly contributed to the field, however, a concensus on the most appropriate method of isolation and culture is absolutely necessary to make hESC-MSC based therapies in a clinical setting a reality.

3.2 Osteoprogenitor cell differentiation from hESCs

Currently, there are major gaps in the knowledge about the growth factors and three-dimensional milieu that influence and direct osteoblast differentiation. The generation of osteoprogenitors from hESC-MSCs has been shown to be successful as evidenced by osteogenic gene expression of runt-related transcription factor 2 (Runx2), collagen type 1A (Col1A1), bone-specific alkaline phosphatase (ALP), and osteocalcin (OCN); mineralized matrix confirmed by von Kossa and Alizarin Red staining; bone nodule formation *in vitro;* and bone formation *in vivo* in diffusion chambers and transplants to orthotopic sites (Duplomb, 2007). One of the first differentiation studies used cultured hESCs in the presence of defined osteogenic supplements for 21 days, and was able to demonstrate mineralization and induction of osteoblastic marker expression (Sotille *et al.*, 2003). Human ESCs have been co-cultured with primary bone derived cells (PBDs) to induce osteoblast differentiation without the addition of exogenous factors, and cultured *in vitro* in the presence of known osteogenic factors without the embryoid body (EB) formation step – both studies confirming that hESCs have the capacity to differentiate into osteoblasts (Ahn *et al.*, 2006; Karp *et al.*, 2006). Whereas, other findings suggest that 12 day EB-derived hESC-MSCs are equally capable of undergoing multilineage differentiation *in vitro* (Cao *et al.*, 2005). It has also been shown that hESC-MSCs can not only differentiate into functional osteoblasts and adipocytes and express markers characteristic of hMSCs, but they can also be successfullly transduced with an osteogenic lineage specific Col2.3-GFP lentivirus in order to track and isolate cells as they underwent differentiation. The transgene construct used has been shown to be a useful tool for studying hBMSC differentiation (Brown *et al.*, 2009). When the hESC-MSCs began as pre-osteoblasts there was low GFP expression, however, increased GFP expression was

detected after 28 days culture in osteogenic medium, suggesting that hES-MSCs differentiated into mature osteoblasts. The ability to track differentiation allowed the isolation of osteoprogenitor cells from the derived hESC-MSC population. These studies suggest that in particular, the osteoprogenitor populations derived from hESCs have tremendous potential, and can serve as a tool through which we can characterize early bone development and cellular behavior on bone-related biomaterials.

3.3 Gene transcription and proteomic array analyses

The therapeutic capacity of hESC-MSCs to treat a variety of diseases lies within their capability to different into numerous cell phenotypes to repair or regenerate tissues and organs. However, it remains to be determined if transplanted MSCs, whether of hESC or adult stem cell origin, contribute to and integrate within the majority of newly formed tissue, or perhaps via paracrine action mediate and stimulate host repair and regeneration. To that end, investigation into the therapeutic potential of the hESC-MSC paracrine proteome has been conducted. Within the study, defined serum-free culture medium was conditioned by hESC-MSCs and subsequently analyzed via multidimensional protein identification and cytokine antibody array analysis (Sze *et al.*, 2007). The array data revealed over 200 unique gene products that play a role in biological processes such as metabolism, defense, response, and tissue differentiation including vascularization, hematopoiesis, and skeletal development. These processes and pathways are associated with numerous cellular processes that are activated to participate in injury, repair, and regeneration, as well as to facilitate immune cell migration to the site of injury, ECM remodeling, and increases in cellular metabolism (Sze *et al.*, 2007). The identification of a large number of MSC secretory products that can act as paracrine modulators provides insight into the potential mechanism of action by which hESC-MSCs may participate in tissue repair and disease treatment.

Another study investigated the gene expression profile of differentiating hESC-MSCs and reported that during derivation major transcriptional changes occurred, resulting in an expression profile very similar to that of hMSCs (de Peppo *et al.*, 2010b). The major questions addressed were how the transcriptome may be affected by the hESC-MSC derivation process and whether hESCs and their MSC derivatives were distinct or equivalent to one another. The findings in the hESC-MSC population revealed a down-regulation in pluripotency genes such as the *OCT* family of genes, *NANOG*, *TDGF1, LIN28, GDF3,* and *ZIC3,* down regulation in tumor development *p53*-associated genes *LTBP2* and *TFAP2A*, up-regulation of mesodermal lineage commitment genes such as *RUNX2, TGBR2, BMPR2,* and *TFAP2A,* and up-regulation of genes supportive of craniofacial development and osteogenesis such as *DLX1, DLX2,* and *MSX1.* Lastly, and importantly, the immunological profile of hESC-MSCs displayed lower expression than hMSCs of HLA-ABC and HLA-DR, two markers characteristic of the inflammatory immune response. These findings suggest that the hESC-MSCs may be more immuno-privileged than hMSCs, thus another piece of evidence supporting the notion that hESC-MSCs represent a suitable alternative for cell transplantation therapies (Romieu-Mourez *et al.*, 2007; de Peppo *et al.*, 2010a).

3.4 Epithelial-mesenchymal transition

Cells within the body are derived from a single cell, with variations of cell phenotypes resulting from expression of a specific and defined transcriptome, thus further imparting diversity in cellular signaling and function. Epithelia are considered to be highly plastic during embryogenesis and have the ability to shuttle back and forth between mesenchyme

and epithelia through the process know as epithelial-mesenchymal transition (EMT). It is one mechanism that gives rise to mesenchymal-like behavior to cells in numerous different settings (Kalluri, 2009). Historically, it has been proposed that epithelial cells have to be terminally differentiated to in order to perform defined functions involved in organ development. However, experimental evidence has suggested that epithelial cells can alter their phenotype based on the influence of microenvironment (Boyer *et al.*, 2000). Therefore, EMT has been accepted as a mechanism by which fibroblasts and mesenchymal cells are formed in injured tissues. In the adult, the process of EMT occurs during tissue regeneration and wound healing by facilitating mesenchymal cell migration to invade surrounding tissues. This was described as one of the three EMT subtypes that occurs, and is also suggested to be an underlying mechanism for derivation of hESC-MSCs (Zeisberg and Neilson, 2009; Ullmann *et al.*, 2007).

It has been reported that hESCs grown in monolayer in feeder-free conditions, without MEFs or other supporting cells, form uniform sheets of epithelial cells after removal from standard feeder culture systems (Boyd *et al.*, 2009; Ullmann *et al.*, 2007). The uniform epithelial sheets exhibit characteristic mesodermal gene expression patterns that appear to undergo EMT that results in a highly proliferative population of cells that over time become uniformly homogenous with a mesenchymal stem cell morphology. It is in fact these homogenous cells that many researchers identify as hESC-MSCs, which have the ability to differentiate along multiple mesenchymal cell lineages *in vitro.* More specifically, these studies find that the hESCs that underwent mesenchymal differentation in monolayer culture were over 80% positive for E-cadherin, a characteristic epithelium marker, and maintained expression while cell morphology changed. Additionally, the cells that were undergoing apparent EMT were positive for the characteristic markers such as CD73, CD90, CD 105 and CD166, and negative for CD31, CD34, CD45, CD133 and CD146, further confirming the formation of a mesenchymal progenitor cell population (Boyd *et al.*, 2009). The key significance of these studies is the finding that hESCs are behaving in culture in a manner similar to that of normal embryogenesis, thus underscoring the importance of using hESCs as a tool for better understanding overall human development.

3.5 Tissue engineering strategies for human clinical applications

A major challenge for using stem cells in a clinical setting is the need to identify an ideal stem cell candidate that is multipotent while retaining its self-renewal capacity. Although hMSCs make a useful source of progenitor cells for tissue engineering strategies, as evidenced by their multipotent potential and immunosuppressive characteristics, their limited proliferative and differentiation capacity represent an obstacle for therapeutic application. In contrast, hESCs with their ability to proliferate indefinitely *in vitro* and multi lineage differentiation capacity represent an unlimited source of progenitor cells, specifically, mesenchymal progenitor cells. Therefore, it is necessary to establish clinical-grade GMP protocols for the derivation, identification, and isolation of hES-MSCs, to produce large quantities of genotypically homogenous progenitor cells that can be modified, and to fully characterize these cells for tissue regeneration strategies. Tissue engineering is an emerging field of research aimed at regenerating functional tissues by combining cells with a supporting substrate or biomaterial that possesses design characteristics that deliver progenitor cells and important signalling molecules in a spatially and temporally controlled manner, while promoting vascularization and tissue invastion into the interior of the scaffold. Ideally, biomimetic scaffolds designed for hESC-MSC based tissue engineering

strategies would contain inductive signaling cues for proliferation and differentiation, possess composite material properties that conferred the ability to generate multi-layered hybrid tissues, and have tunable three-dimensional geometrical architecture that appropriately restores form and function to anatomical defects or diseased tissues.

Within the hESC field, the use of 3D scaffolds has been employed in only a few reports (Arpornmaeklong *et al.,* 2009; Ferreira *et al.,* 2007; Kaufman *et al.,* 2010; Levenberg *et al.*, 2003; Kim *et al.*, 2007; Kuznetsov *et al.,* 2010; Smith *et al.,* 2009). Investigators have used collagen scaffolds for hepatocye differentiation, and porous polylactic/polyglycolic biomaterial sponges to direct neural, chondrogenic, or hepatocytic lineages (Levenberg et al., 2003). While other studies have shown that 3D porous alginate scaffolds to provide a conducive environment for generation of well-vascularized embryoid body derived hESCs (Ferreira et al., 2007). Within the bone tissue engineering field, the use of architecturally designed scaffolds with hESC-MSCs is seen even less frequently. It has been reported that hESC-MSCs were capable of forming bone tissue *in vivo* when implanted subcutaneously after 8 weeks in the presence of BMP-2 (Kim *et al.,* 2007). In 2009, Arpornmaeklong et al. reported the influence of composite collagen scaffolds on the osteogenic differentiation of hESC-MSCs *in vitro* as indicated by osteogenic gene induction, increased ALP activity, and the presence of mature bone ECM proteins; all of which are characteristic of the osteoblast phenotype. From an *in vivo* standpoint, enriched osteoprogenitor cells were encaspulated in fibrin gels mixed with ceramic particles and implanted in a rat calvarial defect model. After six weeks, the identification of transplanted hESC-MSCs in newly formed bone verified the role that MSCs derived from hESCs played in the bone regeneration process (Arpornmaeklong *et al.*, 2009). Another study demonstrated that hESC-MSCs can form mineralized tissue *in vitro* when cultured on 3D nanofibrous polylactic acid (PLLA) in the presence of BMP-7, illustrating the capability of hESC-MSCs to differentiate in 3D culture for bone regeneration purposes (Smith *et al.,* 2009). Most recently, a comprehensive study investigated multiple media formulations and cell culture conditions for efficient derivation of a homogenous hESC-MSC population. To determine their *in vivo* osteogenic potential, cells were implanted up to 16 wks with biphasic ceramic particles and histology revealed cells of human origin were embedded with the bone, including broad areas of multiple intertwining trabeculae (Kuznetsov *et al.,* 2010).

It is hypothesized that the hESC-MSCs not only require a 3D biomaterial, but also inductive cues. This suggests that for tissue formation, hESCs may require additional biological cues such as pro-osteogenic factors for attachment, proliferation, and directed differentiation on biomaterials. For bone formation specifically, hESCs may require an osteoconductive biomaterial with not only the appropriate scaffold architecture, but one that also can associate cellular and molecular elements to increase cellular response to the biomaterial.

4. Conclusion

Human ESC research is a rapidly developing field, and has the potential to impact the medical and scientific community immensely. It is vitally important that we continue to explore hESC biology in order to realize the potential of hESCs to cure diseases. The derivation of mesenchymal stem cells from human embryonic stem cells is an area of active investigation in that hESC-MSCs potentially offer insight into embryonic mesodermal development events, as well as provide information about underlying differentiation mechanisms and signaling pathways that have been unclear heretofor. In addition to elucidating the mechanisms by which hESC-MSCs differentiate, it is equally important to

better understand how the 3D biomaterial microenvironment can be manipulated to direct and control this process. In general, stem cell research advances the knowledge and understanding of how an organism develops and how progenitor cells migrate from the stem cell niche to the site of damaged or diseased tissue. To improve upon the overall quality of human health, scientists must continue to work collaboratively with clinicians to drive translational "bench-to-bedside" research. To this end, extensive investigation into the xeno-free derivation, robustness, and non-tumorigenic safety of hESC-MSCs will be absolutely necessary as the field progresses toward the realization of clinical tissue engineering and regenerative medicine therapies.

5. References

Aggarwal S, Pittenger MF (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105(4):1815-22.

Ahn S, Kim S, Park K, Moon S, Lee H, Kim G, Lee Y, Park K, Cha K, Chung H (2006). Primary bone-derived cells induce osteogenic differentiation without exogenous factors in human embryonic stem cells. *Biochem Biophys Res Commun* 340(2):403-8.

Arpornmaeklong P, Brown SE, Wang Z, Krebsbach PH (2009). Phenotypic characterization, osteoblastic differentiation, and bone regeneration capacity of human embryonic stem cell-derived mesenchymal stem cells. *Stem Cells Dev* 18(7):955-68.

Ashton BA, Allen TD, Howlett CR, Eaglesom CC, Hattori A, Owen M (1980). Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. *Clin Orthop Relat Res* (151):294-307.

Bab I, Howlett CR, Ashton BA, Owen ME (1984). Ultrastructure of bone and cartilage formed in vivo in diffusion chambers. *Clin Orthop Relat Res* (187):243-54.

Bab I, Ashton BA, Gazit D, Marx G, Williamson MC, Owen ME (1986). Kinetics and differentiation of marrow stromal cells in diffusion chambers in vivo. *J Cell Sci* 84:139-51.

Bab I, Passi-Even L, Gazit D, Sekeles E, Ashton BA, Peylan-Ramu N, Ziv I, Ulmansky M (1988). Osteogenesis in in vivo diffusion chamber cultures of human marrow cells. *Bone Miner* 4(4):373-86.

Barberi T, Willis LM, Socci ND, Studer L (2005). Derivation of Multipotent Mesenchymal Precursors from Human Embryonic Stem Cells. *PLoS Medicine* 2(6):e161.

Baschetti R (2005). Evolutionary, biological origins of morality: implications for research with human embryonic stem cells. *Stem Cells Dev* 14(3):239-47.

Beresford JN (1989). Osteogenic stem cells and the stromal system of bone and marrow. *Clin Orthop Relat Res* 240:270-80.

Bianco P, Riminucci M, Gronthos S, Robey P (2003). Bone Marrow Stromal Stem Cells: Nature, Biology, and Potential Applications. *Stem Cells* 19(3):180-92.

Boyd NL, Robbins KR, Dhara SK, West FD, Stice SL (2009). Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells. *Tissue Eng Part A* 15(8):1897-907.

Boyer B, Valles AM, Edme N (2000). Induction and regulation of epithelial-mesenchymal transitions. *Biochem Pharmacol* 60(8):1091-9.

Brown SE, Tong W, Krebsbach PH (2009). The derivation of mesenchymal stem cells from human embryonic stem cells. *Cells Tissues Organs* 189(1-4):256-60.

Cao T, Heng B, Ye C, Liu H, Toh W, Robson P, Li P, Hong Y, Stanton L (2005). Osteogenic differentiation within intact human embryoid bodies result in a marked increase in

osteocalcin secretion after 12 days of in vitro culture, and formation of morphologically distinct nodule-like structures. *Tissue and Cell* 37(4):325-34.

Caplan AI (2005). Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. *Tissue Eng* 11(7-8):1198-211.

Cheng L, Hammond H, Ye Z, Zhan X, Dravid G (2003). Human adult marrow cells support prolonged expansion of human embryonic stem cells in culture. *Stem Cells* 21(2):131-42.

de Peppo GM, Sjovall P, Lenneras M, Strehl R, Hyllner J, Thomsen P, Karlsson C (2010a). Osteogenic Potential of Human Mesenchymal Stem Cells and Human Embryonic Stem Cell-Derived Mesodermal Progenitors: A Tissue Engineering Perspective. *Tissue Eng Part A*.

de Peppo GM, Svensson S, Lenneras M, Synnergren J, Stenberg J, Strehl R, Hyllner J, Thomsen P, Karlsson C (2010b). Human embryonic mesodermal progenitors highly resemble human mesenchymal stem cells and display high potential for tissue engineering applications. *Tissue Eng Part A* 16(7):2161-82.

Duplomb L, Dagouassat, M, Jourdon, P, Heymann, D (2007). Concise Review: Embryonic Stem Cells: A New Tool to Study Osteoblast and Osteoclast Differentiation. *Stem Cells* 25(3):544-52.

Evseenko D, Zhu Y, Schenke-Layland K, Kuo J, Latour B, Ge S, Scholes J, Dravid G, Li X, MacLellan WR, Crooks GM (2010). Mapping the first stages of mesoderm commitment during differentiation of human embryonic stem cells. *Proc Natl Acad Sci U S A* 107(31):13742-7.

Friedenstein A, Kuralesova AI (1971). Osteogenic precursor cells of bone marrow in radiation chimeras. *Transplantation* 12(2):99-108.

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.

Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV (1974a). Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* 17(4):331-40.

Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, Ruadkow IA (1974b). Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. *Exp Hematol* 2(2):83-92.

Genbacev O, Krtolica A, Zdravkovic T, Brunette E, Powell S, Nath A, Caceres E, McMaster M, McDonagh S, Li Y, Mandalam R, Lebkowski J, Fisher SJ (2005). Serum-free derivation of human embryonic stem cell lines on human placental fibroblast feeders. *Fertil Steril* 83(5):1517-29.

Gronthos S, Graves SE, Ohta S, Simmons PJ (1994). The STRO-1+ Fraction of Adult Human Bone Marrow Contains the Osteogenic Precursors. *Blood* 84(12):4164-73.

Gronthos S, Zannettino AC, Graves SE, Ohta S, Hay SJ, Simmons PJ (1999). Differential cell surface expression of the STRO-1 and alkaline phosphatase antigens on discrete developmental stages in primary cultures of human bone cells. *J Bone Miner Res* 14(1):47-56.

Gruen L, Grabel L (2006). Concise review: scientific and ethical roadblocks to human embryonic stem cell therapy. *Stem Cells* 24(10):2162-9.

Haynesworth SE, Baber MA, Caplan AI (1992a). Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone* 13(1):69-80.

Haynesworth SE, Goshima J, Goldberg VM, Caplan AI (1992b). Characterization of cells with osteogenic potential from human marrow. *Bone* 13(1):81-8.

Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T (2002). Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc Natl Acad Sci U S A* 99(13):8932-7.

Hovatta O, Mikkola M, Gertow K, Stromberg AM, Inzunza J, Hreinsson J, Rozell B, Blennow E, Andang M, Ahrlund-Richter L (2003). A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. *Hum Reprod* 18(7):1404-9.

Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP (1997). Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *J Cell Biochem* 64(2):295-312.

Kalajzic I, Kalajzic Z, Clark S, Lichtler A, Rowe D (2002). Use of Col1a1GFP transgenes to identify subpopulations of cells at different stages of the osteoblast lineage. *Journal of Bone and Mineral Research* 17(1):15-25.

Kalajzic I, Staal A, Yang W, Wu Y, Johnson S, Feyen J, Krueger W, Maye P, Yu F, Zhao Y, Kuo L, Gupta R, Achenie L, Wang H, Shin D, Rowe D (2005). Expression profile of osteoblastic lineage at defined stages of differentiation. *J Biol Chem* 280(26):24618-26.

Kalluri R (2009). EMT: when epithelial cells decide to become mesenchymal-like cells. *J Clin Invest* 119(6):1417-9.

Karlsson C, Emanuelsson K, Wessberg F, Kajic K, Axell MZ, Eriksson PS, Lindahl A, Hyllner J, Strehl R (2009). Human embryonic stem cell-derived mesenchymal progenitors-Potential in regenerative medicine. *Stem Cell Res*.

Karner E, Unger C, Sloan AJ, Ahrlund-Richter L, Sugars RV, Wendel M (2007). Bone matrix formation in osteogenic cultures derived from human embryonic stem cells in vitro. *Stem Cells Dev* 16(1):39-52.

Karp J, Ferreira L, Khademhosseini A, Kwon A, Yeh J, Langer R (2006). Cultivation of human embryonic stem cells without the embryoid body step enhances osteogenesis in vitro. *Stem Cells* 24(4):835-43.

Knowles LP (2004). A regulatory patchwork--human ES cell research oversight. *Nat Biotechnol* 22(2):157-63.

Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM (2000). Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol* 18(2):307-16.

Kopher RA, Penchev VR, Islam MS, Hill KL, Khosla S, Kaufman DS (2010). Human embryonic stem cell-derived CD34(+) cells function as MSC progenitor cells. *Bone*.

Krebsbach PH, Kuznetsov SA, Satomura K, Emmons RV, Rowe DW, Robey PG (1997). Bone formation in vivo: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. *Transplantation* 63(8):1059-69.

Krebsbach PH, Kuznetsov SA, Bianco P, Robey PG (1999). Bone marrow stromal cells: characterization and clinical application. *Crit Rev Oral Biol Med* 10(2):165-81.

Kuznetsov S, Cherman N, Gehron Robey P In Vivo Bone Formation by Progeny of Human Embryonic Stem Cells. *Stem Cells Dev*.

Le Blanc K, Pittenger M (2005). Mesenchymal stem cells: progress toward promise. *Cytotherapy* 7(1):36-45.

Lian Q, Lye E, Suan Yeo K, Khia Way Tan E, Salto-Tellez M, Liu TM, Palanisamy N, El Oakley RM, Lee EH, Lim B, Lim SK (2007). Derivation of clinically compliant MSCs from CD105+, CD24- differentiated human ESCs. *Stem Cells* 25(2):425-36.

Olivier EN, Rybicki AC, Bouhassira EE (2006). Differentiation of Human Embryonic Stem Cells in Bipotent Mesenchymal Stem Cells. *Stem Cells* 24(8):1914-22.

Owen M, Friedenstein AJ (1988). Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found Symp* 136:42-60.

Pittenger M, Mackay A, Beck S (1996). Human mesenchymal stem cells can be directed into chondrocytes, adipocytes or osteocytes. *Mol Biol Cell* 7:305a.

Romieu-Mourez R, Francois M, Boivin MN, Stagg J, Galipeau J (2007). Regulation of MHC class II expression and antigen processing in murine and human mesenchymal stromal cells by IFN-gamma, TGF-beta, and cell density. *J Immunol* 179(3):1549-58.

Smith LA, Liu X, Hu J, Wang P, Ma PX (2009). Enhancing osteogenic differentiation of mouse embryonic stem cells by nanofibers. *Tissue Eng Part A* 15(7):1855-64.

Sotille V, Thomson A, McWhir J (2003). In vitro osteogenic differentiation of human ES cells. *Cloning and Stem Cells* 5(2):149-55.

Stewart K, Walsh S, Screen J, Jefferiss CM, Chainey J, Jordan G, Beresford JN (1999). Further Characterization of Cells Expressing STRO-1 in Cultures of Adult Human Bone Marrow Stromal Cells. *Journal of Bone and Mineral Research* 14(8):1345-1356.

Stojkovic P, Lako M, Stewart R, Przyborski S, Armstrong L, Evans J, Murdoch A, Strachan T, Stojkovic M (2005). An autogeneic feeder cell system that efficiently supports growth of undifferentiated human embryonic stem cells. *Stem Cells* 23(3):306-14.

Sze SK, de Kleijn DP, Lai RC, Khia Way Tan E, Zhao H, Yeo KS, Low TY, Lian Q, Lee CN, Mitchell W, El Oakley RM, Lim SK (2007). Elucidating the secretion proteome of human embryonic stem cell-derived mesenchymal stem cells. *Mol Cell Proteomics* 6(10):1680-9.

Thomson J, Itskovitz-Eldor J, Shapiro S, Waknitz M, Swiergiel J, Marshall V, Jones J (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282(5391):1145-47.

Tong W, Brown SE, Krebsbach PK (2007). Human Embryonic Stem Cells Undergo Osteogenic Differentiation in Human Bone Marrow Stromal Cell Microenvironments. *Journal of Stem Cells* 2(3):139-47.

Tremoleda JL, Forsyth NR, Khan NS, Wojtacha D, Christodoulou I, Tye BJ, Racey SN, Collishaw S, Sottile V, Thomson AJ, Simpson AHWR, Noble BS, McWhir J (2008). Bone Tissue Formation from Human Embryonic Stem Cells In Vivo. *Cloning and Stem Cells* 1(10):119-131.

Trivedi P, Hematti P (2007). Simultaneous generation of CD34+ primitive hematopoietic cells and CD73+ mesenchymal stem cells from human embryonic stem cells cocultured with murine OP9 stromal cells. *Exp Hematol* 35(1):146-54.

Ullmann U, In't Veld P, Gilles C, Sermon K, De Rycke M, Van de Velde H, Van Steirteghem A, Liebaers I (2007). Epithelial-mesenchymal transition process in human embryonic stem cells cultured in feeder-free conditions. *Mol Hum Reprod* 13(1):21-32.

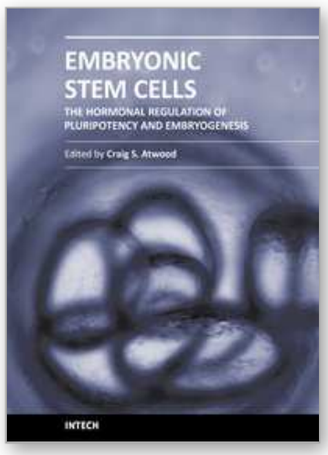
Villa-Diaz LG, Nandivada H, Ding J, Nogueira-de-Souza NC, Krebsbach PH, O'Shea KS, Lahann J, Smith GD (2010). Synthetic polymer coatings for long-term growth of human embryonic stem cells. *Nat Biotechnol* 28(6):581-3.

Wagers A, Weissman I (2004). Plasticity of adult stem cells. *Cells* 116(5):639-48.

Wang Q, Fang ZF, Jin F, Lu Y, Gai H, Sheng HZ (2005). Derivation and growing human embryonic stem cells on feeders derived from themselves. *Stem Cells* 23(9):1221-7.

Xu C, Jiang J, Sottile V, McWhir J, Lebkowski J, Carpenter MK (2004). Immortalized fibroblast-like cells derived from human embryonic stem cells support undifferentiated cell growth. *Stem Cells* 22(6):972-80.

Zeisberg M, Neilson EG (2009). Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest* 119(6):1429-37.



Embryonic Stem Cells: The Hormonal Regulation of Pluripotency and Embryogenesis

Edited by Prof. Craig Atwood

ISBN 978-953-307-196-1
Hard cover, 672 pages
Publisher InTech
Published online 26, April, 2011
Published in print edition April, 2011

Pluripotency is a prerequisite for the subsequent coordinated differentiation of embryonic stem cells into all tissues of the body. This book describes recent advances in our understanding of pluripotency and the hormonal regulation of embryonic stem cell differentiation into tissue types derived from the ectoderm, mesoderm and endoderm.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Shelley E. Brown and Paul H. Krebsbach (2011). Derivation of Mesenchymal Stem Cells from Human Embryonic Stem Cells, Embryonic Stem Cells: The Hormonal Regulation of Pluripotency and Embryogenesis, Prof. Craig Atwood (Ed.), ISBN: 978-953-307-196-1, InTech, Available from: http://www.intechopen.com/books/embryonic-stem-cells-the-hormonal-regulation-of-pluripotency-and-embryogenesis/derivation-of-mesenchymal-stem-cells-from-human-embryonic-stem-cells

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen