Stem Cells: Cellular and Extracellular Requirements for Generation and Use

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1 Stem Cells—Definition

Stem cells are defined by their capacity to self-renew and their ability to give rise to one or more type of differentiated progeny [4, 34]. Stem cells can be isolated from numerous sites and from numerous stages of development, each site and stage providing specific characteristics to the stem cell.

Cells that can differentiate into all three germ layers of a human (endoderm, mesoderm, and ectoderm) and into extra-embryonic (placental) cell types are called totipotent or omnipotent stem cells. These cells are the immediate descendants of a zygote, which is produced from the fusion of an egg cell and a sperm cell [42].

Embryonic stem (ES) cells are derived from the inner cell mass of a blastocyst, which represents a pre-implantation stage of embryogenesis. ES cells can differentiate into all three germ layers of an organism as well as their descendant cells. However, the potential of an ES cell is confined to the embryo proper. Hence, ES cells are described as being pluripotent [51].

During the development of an organism, ES cells lose their pluripotency to differentiate along different cellular lineages. In other words, these pluripotent cells lose their stem cell properties and develop into more specialized cells. Within the developing embryo, lineage-commitment is guided by extracellular cues, such as signals from the extracellular matrix (ECM), which are dependent upon the position of a certain ES cell within the developing blastocyst. Typically, a miniscule fraction of tissue-specific cells persist that do not fully complete maturation and remain in an undifferentiated, yet lineage-committed, developmental state throughout the life-

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time of an organism. These cells are referred to as adult stem cells and are considered multipotent.

2 History of Stem Cell Discoveries and Milestones

In 1981, two research groups, one led by Martin Evans and Matthew Kaufman and another led by Gail R. Martin, published the isolation and in vitro culture of pluripotent cells; the term embryonic stem cell was coined at this time by Dr. Martin [18, 32]. These ES cells were isolated from the inner cell mass of developing mouse embryos at the blastocyst stage. Special measures were taken to maintain the ES cells in an undifferentiated state in the petri dish. Combined with gene targeting, this was the first step toward generating a genetically altered animal.

In 1989, just eight years after their isolation ES cells were used to create the first genetically engineered mouse through gene targeting [13, 49]. The capability to isolate, grow, and genetically modify mouse ES cells and subsequently generate genetically distinct animals sparked an entire field of research [insert reference here]. In the following years, ES cell culture and the generation of genetically altered mice using DNA recombination became a standardized technique in countless labs around the world ([23] and references therein).

The research stemming from these early studies revolutionized the life sciences as it was used to understand gene function in development and disease within a mammalian system; mice were developed to model human conditions including cancer, heart disease, diabetes, hearing loss, and countless more. For these reasons, the 2007 Nobel Prize in Physiology or Medicine was awarded to Capecchi, Evans, and Smithies for the development of genetically engineered mice using ES cells.

Embryonic stem cell research indeed led to a better understanding of gene and protein function and promoted knowledge in the basic sciences. While these animal models could suggest pathways for new therapeutic approaches and these therapies could even be tested in the mice, the translation of mouse studies to human conditions lagged behind for a variety of reasons, with one major reason being the unanticipated difficulty of isolating and culturing human ES cells.

But in 1998, James Thomson spearheaded a research group was able for the first time to isolate and grow human ES cells in culture [50]. This accomplishment represented a tremendous advance toward tissue engineering for medical purposes as the ability to culture and sustain human ES cells in vitro provided a theoretically unlimited source of these precious cells. However, despite further technical improvements to establish human ES cell lines in culture, ethical difficulties regarding the isolation cells from pre-implantation stage human embryos left its use problematic. Additionally, while ES cells bear the potential to differentiate into all types of tissue-specific cells, in practice, the challenge to induce and control directed cell-type specific differentiation for therapeutic use remained unmet.

Meanwhile, another approach to cellular programming was being used. In 1996, the concepts and innovative work of Ian Wilmut, Keith Campbell, and colleagues

came to fruition with the birth of Dolly [11, 55]. This famous sheep stemmed from a second line of research in regenerative medicine that discovered the DNA from a differentiated cell contains all of the information necessary to give rise to a new organism. Dolly was created from the contents of a single cell isolated from the udder of an adult sheep inserted to the ovum of another sheep and transplanted to a third sheep for surrogate gestation and birth. This approach, called somatic cell nuclear transfer (SCNT), utilized a series of epigenetic changes of the DNA induced upon transfer of the DNA into an enucleated oocyte, an oocyte with it's nuclear content removed. This method resulted in reprogramming of donor cell nucleus. The cloning of Dolly sparked a hot ethical debate about the possibility and use of cloning human beings. (The assembly of recombinant DNA molecules and their replication within host organisms, often *E. coli*, using techniques and methods of molecular biology is also referred to as 'cloning', which is a technique ubiquitous in research labs around the world.) Ethical concerns surrounding cloning and stem cell use are discussed later in this chapter. While Dolly proved the principal that cloning a mammal was possible, SCNT was found to be very inefficient and instead of developing into a widely used method, sparked the field of cellular reprogramming.

In 2006, induced pluripotent stem (iPS) cells were generated and published for the first time by Shinya Yamanaka, presenting a major milestone in stem cell research and opening up the field of regenerative medicine [48]. The use of iPS cells has the potential to circumvent the major ethical hurdles faced by ES cell research and cloning attempts, as mature (adult) cells are induced to de-differentiate or are reprogrammed into a pluripotent, ES cell-like state without SCNT. In order to accomplish this, Yamanaka and his colleague Takahashi expressed different combinations of candidate transcription factors in a cellular system designed to test for pluripotency. Starting with 24 candidate genes, the researches pared it down to just four genes, Oct3/4, Sox2, Klf4, and c-Myc, that were determined to be indispensable reprogramming factors. These four factors are commonly referred to as the 'Yamanaka factors'. The explosion of studies that immediately followed this publication highlights the impact of this discovery.

Within the following years, numerous research groups, including those led by Yamanaka [47], Jaenisch [33], Hochedlinger [31], Zhou [58], Gao [26] and Thomson [57] adopted and improved this approach, resulting in the generation of iPS cell-derived mice and human iPS cells.

With these accomplishments, iPS technology appeared to overcome principal ethical concerns and possible immunological barriers of ES cells and SCNT, making the clinical use of stem cells appear attainable for the first time since their discovery more than four decades earlier. Ideas about clinical uses of iPS cells, including tissue repair, in vitro tissue and organ generation for subsequent transplantation, and the possibility of generating disease or patient-specific iPS cells for drug testing and therapy development were emerging. However, these prospective goals were hindered by the current state of the technique, which required the DNA sequence of the reprograming Yamanaka factors to be inserted into the host genome. This presented two major issues. First, the integration sites could potentially disturb endogenous gene function, as integration was random. Second, the inserted

genetic sequence could transcribe a protein known to drive cancer, an oncogene, like c-Myc, required for iPS cell generation. Additionally, the efficiency of the reprogramming process proved low and sometimes incomplete, i.e. did not fully erase epigenetic imprints.

One emphasis of the last decade's stem cell research was increasing iPS reprogramming efficiency and developing alternative delivery methods of the reprogramming factors. To date, integration-free vector delivery methods have proven successful, as have protein delivery systems requiring no integration of genetic material. In 2013, the research group surrounding Hongkui Deng achieved a successful mouse somatic cell reprogramming solely by small molecule compounds. This approach utilized seven small compounds and achieved a reprogramming frequency of 0.2% [56]. Today, stem cells arising from different sources in the human body are being tested in clinical studies (Tables 1 and 2).

Table 1 Sampling of clinical trials using HSCs as intervention including the disease/condition and the clinical developmental stage

Disease/purpose/condition	Intervention	Status
Alpha thalassemia major; hemoglobinopathy	In utero HSC transplantation	Phase I
Stiff person syndrome	Autologous HSC transplantation	Phase I/II
Inflammatory bowel diseases	Autologous HSC transplantation	Phase I/II
Neuromyelitis optica (Devic's Disease)	HSC transplantation	Phase I/II
End stage renal disease	Kidney and HSC transplantation	Pilot
Multiple sclerosis	Autologous HSC transplantation	Phase I
Crohn's disease	HSC transplantation	Pilot
Pancreatic adenocarcinoma	Allogeneic HSC transplantation	Phase I/II
Beta-thalassemia	Autologous HSC genetically modified with lentiviral vector encoding for the human beta-globin gene	Phase I/II
Triple-negative invasive breast carcinoma	Pharmacologic and autologous HSC transplantation	Phase II
Sickle cell anemia	Fludarabine and HSC transplantation	Phase I/II
Fanconi anemia	Pharmacologic and HSC transplantation	Phase II
Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)	Allogeneic HSC	Phase I
Immunodeficiency	HSC transplantation	Phase II
Systemic lupus erythematosus	Immunoablation and autologous HSC transplantation	Phase II
Pancreatic adenocarcinoma	Allogeneic HSC transplantation	Phase I/II
Wiskott-Aldrich syndrome	Autologous CD34 positive cells transduced with a lentiviral vector containing human WAS gene	Phase I/II

Table 2 Sampling of clinical trials using non-HSC stem cells populations as intervention including the disease/condition and the clinical developmental stage

Disease/purpose/condition	Intervention	Status
HSC transplantation	MSC infusion, cyclophosphamid	Phase III
Facial rejuvenation	Adipose-derived stem cells	Pilot
Urticaria	Autologous MSC therapy	Phase I
Hair restoration therapy for androgenetic alopecia	Adipose tissue derived autologous MSC	Phase II
Non-obstructive azoospermia male infertility	Autologous MSC transplantation	Phase I/II
Diabetes mellitus type 1	Autologous CD34+, CD133+ MSC transplantation	Phase I/II
Respiratory distress syndrome	MSC therapy	Phase II
Limbal stem cell deficiency	Limbal epithelial stem cell graft	Phase II
Amyotrophic lateral sclerosis	Injection of adipose derived MSCs	Phase I
Chronic myocardial ischemia	Autologous Bone Marrow-derived MSC Administration	Phase I
Type 1 diabetes with diabetic ketoacidosis	umbilical cord MSC transplantation	Phase II
Liver cirrhosis	MSC transplantation	Phase I/II
Peripheral vascular disease; ischemia; diabetic foot	Adipose-derived stem cell transplantation	Phase I
COPD	Adipose derived stem cell transplantation	Pilot
Full thickness rotator cuff tear	MSC augmentation in rotator cuff repair	Pilot
Cystic fibrosis	Allogenic MSC infusion	Phase I
Stroke	MSC transplantation	Phase I
Age related macular degeneration	Human embryonic stem cell derived retinal pigmented epithelium	Phase I/II
Spinal cord injury	Human spinal cord-derived neural stem cell transplantation	Phase I/II
Parkinson disease	Human neural stem cell injection	pilot
Heart failure	Intracoronary injection of autologous cardiac stem cells	Phase II
Glioma	Neural stem cells loaded with an oncolytic adenovirus	Phase I
Muscle dystrophy	Intramuscular injection of muscle derived stem cell and adipose derived MSC	Phase I
Hepatic cirrhosis	Human umbilical cord-MSC transplantation	Phase I

3 Types of Stem Cells

3.1 Embryonic Stem (ES) Cells

ES cells arise from the cell division of a fertilized egg in the inner cell mass of a developing embryo. During normal development, ES cells give rise to the embryo proper and differentiate into three germ layers, endoderm, mesoderm, and ectoderm. Later in development, cells from the endoderm give rise to the gastrointestinal and respiratory tracts and tissues forming the liver and thyroid. Mesodermal cells contribute to the development of organs such as the heart, blood vessels, lymphoid tissues, and blood. Furthermore, the kidneys, skeletal muscle, connective tissue, and bone are derived from the mesoderm. Finally, the ectoderm differentiates to form the skin and the neuronal system. Due to the pluripotency of ES cells, their potential for scientific research and eventually for therapeutic purposes is tremendous.

As outlined above, the capability of expanding ES cells in culture dishes without the cells differentiating paved the way for the experimental genetic alteration of these cells by homologous recombination, called 'gene targeting', and the subsequent selection of individual (targeted) ES cell clones, resulting in the generation of genetically modified mice. More recently, the workflow of cellular gene editing has been accelerated considerably by the development of the CRISPRCas9-System [16]. These technological developments revolutionized the entire field of life sciences and boosted the knowledge of gene function. Translating these findings from murine models to the human system has proven difficult and it was not until 1998 that human ES cell culture techniques were established to maintain these cells in culture in an undifferentiated state. As ES cells are derived from the pre-implantation stage embryo, the development of patient-matched stem cell lines to support precision medicine is currently out of reach using ES cells. Additionally, as pre-implantation stage embryos are repurposed to develop ES cell cultures, their use remains highly controversial.

3.2 Induced Pluripotent Stem (iPS) Cells

iPS cells are cells that are either de-differentiated or reprogrammed from adult somatic cells to harbor the characteristics of ES cells, having the ability to then differentiate into numerous cell types; they are pluripotent. In order to reprogram adult cells, certain transcription factors, the Yamanaka factors, need to be expressed. Expression vectors, a plasmid or virus designed to express a certain gene or set of genes, were first used to introduce these factors into the adult cells. This created a hurdle as these gene delivery systems (expression vectors) remained in the genome of the reprogrammed cells. Because the expression vectors could theoretically insert anywhere in the host genome, deleterious mutations could be induced.

With this in mind, several research groups have developed methods of generating iPS cells without the use of stably introduced expression vectors, in a transgene-free manner [44]. Around the same time, it was shown using a tratraploid complementation assay, the most rigorous assay available, that iPS cells are comparable to ES cells in their potential to contribute to all cells of an organism including germ cells [26, 58].

This technique has the potential to address ethical concerns about human ES cell isolation and provided ways to generate unlimited amounts of patient specific pluripotent cells. These cells have been used with great advantage for studying mechanisms of human disease or drug effects.

3.3 Adult Stem Cells

Adult stem cells are generally multipotent cells and are present in numerous adult tissues. Unlike the pluripotent ES and iPS cells, adult stem cells have the potential to develop into a restricted line or a family of closely related cells. Examples of adult stem cells include hematopoietic stem cells (HSCs), which give rise to all lineages of the blood system, mesenchymal stem cells (MSCs), which are capable of differentiating into bone, cartilage, and adipose tissue, hepatic stem cells, which can differentiate into cells of the liver, satellite cells of the muscle, and neuronal stem cells, which serve as precursors of neurons, astrocytes, and glia. Techniques have been developed to isolate certain stem cell populations, sorting out the cells of interest from the rest of the cells in the tissue. Of the many types of adult stem cells, MSCs including muse cells and HSCs are the best characterized.

3.4 Mesechymal Stem Cells (MSCs)

MSCs have first been described as colony forming units (CFUs) from ex vivo mouse bone marrow stroma cells [5]. These cells are defined by their ability to differentiate into bone, cartilage, and adipose tissue. MSCs have also been suggested to have the potential to differentiate into muscle [38]. MSC populations are accessible from bone marrow and adipose tissue and can be isolated using the cell surface markers CD34 and CD133, which are receptor proteins expressed specifically by these cells. MSCs have immuno-modulatory properties and avoid immune rejection upon allo-transplantation, meaning they themselves are unlikely to be recognized as foreign cells and when transplanted together with HSCs, make the other cells less likely to be rejected as foreign cells. These properties make them an exquisite candidate for medical use in numerous transplantation settings. A number of clinical trials are currently testing MSC use in autoimmune and other diseases (Table 1).

Muse cells

In 2010, research guided by Mari Dezawa led to the discovery and isolation of a rare subpopulation of MSCs that was termed Muse (multilineage differentiating stress enduring) cells. These cells have been shown to express pluripotency markers including SSEA3, TRA1-60, Nanog, Oct3/4, and Sox2 at low levels and to self-renew. They are able to differentiate into cells from all three germ layers and have the capacity to home to damaged tissue and differentiate into the tissue at the site of damage, contributing to functional tissue repair [28]. Implications of this discovery are discussed below in the transdifferentiation section.

3.4.1 Hematopoietic Stem Cells (HSCs)

HSCs describe a small population of cells within the bone marrow. HSCs are also found in and isolated from umbilical cord blood, which is the blood that remains in the placenta and umbilical cord after a child is born. All blood cells derive from HSCs, which have the potential to self-renew and to differentiate into all hematopoietic cell lineages, which can be distinguished by their expression of characteristic cell surface markers (Fig. 1). During normal development, HSCs are generated outside the embryo proper in the yolk sac and within the embryo in the para-aortic splanchnopleura (PAS)/aorta-gonad mesonephros (AGM) region. HSCs give rise to lymphatic, myeloid, and erythroid precursor cells. The stem cells themselves can be sorted out from the milieu of other bone marrow cells in a laboratory with a combination of surface proteins using the technique of flow cytometry. In addition to cell surface markers, HSCs are functionally defined by their ability to give rise to long-term multi-lineage reconstitution in lethally irradiated mice. In other words, a single HSC can be injected into mice that have had their bone marrow cells completely eliminated by radiation and, if the cell has been properly sorted, this single cells is capable of reconstituting the entire immune system of the mouse, demonstrating both self-renewal capacity and multipotency of HSCs.

Much research has been invested in determining the molecular cues guiding hematopoietic and other stem cells to either differentiate or to self-renew [19, 22, 27, 53]. Our own research identified the transcription factor, nuclear factor Y (NFY), to act upstream of a signaling cascade critical to these decisions in HSCs [8]. This work helped decipher the nature of these molecular pathways and to better understand how this network is altered during diseases such as leukemia and how it can be manipulated for therapeutic purposes. We took this work one step further and demonstrated that NFY is instrumental in expanding CD34+ cord blood cells in vitro [14]. This is an important discovery as cord blood cells, like isolated HSCs, are used to treat cancers of the blood system and different forms of anemia; methods to expand their number in culture promote their clinical utility.

The comparatively easy accessibility and the established clinical use of HSCs, which will be discussed later in this chapter, propelled their investigation and

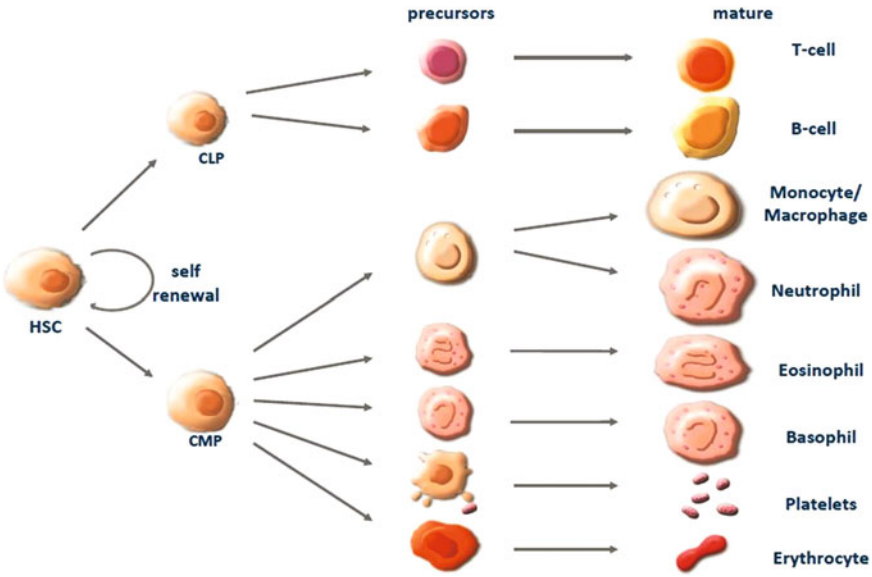

Fig. 1 Simplified depiction of the hematopoietic hierarchy. HSCs give rise to common lymphoid precursors (CLPs) and common myeloid precursors (CMPs), which further differentiate into different mature cell types. While lineage committed cells can be described upon their expression of certain markers on the cell surface, HSCs and precursor cells are usually identified by a combination of several surface markers [12]

additionally made them a model of stem cell research. In fact, concepts about cellular differentiation hierarchies and cellular self-renewal stem from HSC research.

3.4.2 Cancer Stem Cells

The concept that a small number of stem cells are able to support a tissue comprised of millions of different cells and perhaps dozens of different cell types can be translated from HSCs to other stem cell populations. When properly controlled, this exquisite system is beneficial for a developing or injured organism. However, unbalanced cell proliferation and differentiation can result in metaplastic or dysplastic growth and cancer. Metaplasia refers to the replacement of one differentiated cell type with that of another differentiated cell type. Dysplasia refers to abnormal development, often meaning an abnormally large number of immature cells in a tissue.

In 1994 and 1997, John E. Dick and colleagues published research demonstrating that the same principles of self-renewal and differentiation discovered for HSCs hold true for cancer cells [6, 29]. The underlying experiments evaluated the number and properties of leukemic cells necessary to transplant leukemia from one

mouse to another. Interestingly, only a small specific population of cancer cells was necessary and sufficient to transmit leukemia. These cells were termed leukemic stem cells. In perfect analogy to HSCs, cancer stem cells are capable of self-renewal and differentiation, thus feeding the tumor without being exhausted. Like HSCs, cancer stem cells likely divide slowly and give rise to rapidly dividing cells. Thus, cancer therapies targeting rapidly dividing cells may temporarily ease the symptoms of cancer, but are doomed to fail as the cancer stem cells continue to slowly divide. This concept necessitates new strategies for the treatment of cancer, many of which are currently being tested in clinical studies (Table 1).

4 Extracellular Matrix and the Stem Cell Niche

When cells are removed from their natural environments, they behave differently than when they remain within their natural environments. Much effort has been concentrated on developing external environments for cells, including stem cells, that simulate the natural environment.

Stem cells thrive in particular microenvironments referred to as niches. The stem cell niche is an environment that fosters proper growth, proliferation, and differentiation of stem cells.

The idea of a specific niche for stem cells was proposed four decades ago by Schofield [41], who proposed the stem cell niche [10].

It is now known that stem cell niches do properties of hematopoietic stem cells are due to their association with other cells within a particular niche. Since this time, niches have been proposed for stem cells of numerous different origins, including skin, hair follicle, and intestine, to name a few [1, 2, 20, 30, 36, 46]. Cancer not only consists of cells, but the extracellular matrix (ECM) is a critical component contributing to uncontrolled cellular proliferation [39]. In addition to providing structure and support to tissue and anchorage to cells, the ECM is involved in receptor signaling and plays a dynamic role in establishing growth factor gradients and other secreted factors that serve as modulating cues that determine cell fate [15, 35]. Only the most prominent ECM molecules which we also reviewed earlier [40] will be discussed below, including collagen, proteoglycans/glycosaminoglycans, laminins, and fibronectin.

As the name implies, ECM proteins exist outside of the cell. They are secreted molecules that are highly modified, meaning they undergo post-translational processing in which certain residues are covalently phosphorylated, acetylated, glycosylated, or otherwise modified. These modifications impart a tremendous degree of diversity and allow for exquisite spatial and temporal functional regulation of these proteins. The ECM proteins will be discussed below all undergo such post-translational modification and, while classified as glycoproteins, possess a number of other covalent modifications that are dependent on their tissue location and the particular stage of cellular development.

The most abundant ECM molecule is also the most abundant protein in the body, collagen. Collagen provides a large degree of structural support and contains

epitopes found to influence cellular processes such as angiogenesis, or blood vessel growth. Like other ECM molecules, collagen binds to transmembrane receptors such as integrins and helps to mediate both inside-out and outside-in signaling between the cell proper and its extracellular environment [9].

Heparan sulphate proteoglycans (HSPGs) stand out as additional key players in the development and maintenance of the stem cell niche [21, 35]. HSPGs consist of specific core proteins with a variable number of polysaccharide chains, called glycosaminoglycans (GAGs) attached [7, 25, 37]. Unique combinations of core proteins and GAG side chains imparts a tremendous degree of diversity to the ECM and provides a single proteoglycan the ability to play diverse roles within a tissue in time-dependent manner [7].

Laminin and fibronectin are additional ECM components that provide structure and individuality to specific stem cell niches. Laminins comprise a family of glycoproteins consisting of alpha, beta, and gamma chains that combine to form various functional heterotrimers. Fibronectin is another large glycoprotein found within the ECM. Like collagens, laminins and fibronectins bind integrin receptors as one means of regulating cellular behavior.

4.1 Three-Dimensional Scaffolds

In the organ system, cells reside in highly specialized niches consisting of supportive cells and ECM. These components provide mechanical stability, generate and transduce biophysical signals via cellular receptor binding to intracellular and extracellular ligands, and sequester and release soluble growth factors to promote growth and differentiation. These specific and interdependent cell-matrix interactions provide instructive cues for stem cells that determine their behavior. For these reasons, growing, expanding, and differentiating cells outside of the body has proved challenging. A flat plastic petri dish cannot provide the optimal conditions for stem cell growth and expansion.

Three-dimensional scaffolds represent an artificial microenvironment aimed at imitating the natural niche and come in as many varieties as do stem cells themselves. The emergence of three-dimensional (3D) scaffolds to mimic the natural stem cell environment has enriched the field of stem cell research and advanced the field of regenerative medicine. Most scaffolds consist of biodegradable materials that can be loaded with cells and supplied with nutrients and morphogens within a bioreactor, an engineered system used to support a biological environment, providing a home away from home in order to obtain the desired behavior from the cultured stem cells 24,570,851, 27,851,739. Thus, the desired cellular behavior dictates the design and properties of the scaffold.

Research led by Dennis Discher provided the first evidence that the nature of the matrix strongly influences the lineage into which stem cells differentiate [17]. Using identical culture serum conditions, this group found soft matrices to support neurogenic differentiation, stiffer matrices myogenic differentiation, and solid matrices

osteogenic differentiation. The results of these studies demonstrate how physical factors influence stem cell differentiation.

Work stemming from our own research contributed to show that physical factors affecting stem cell behavior are not limited to the stiffness of culture matrices. Mechanical stimulation using extracorporeal shock waves additionally has the potential to modify migration and proliferation of stem cell populations [45]. In addition to the stiffness of the matrix and physical stimulation, ligand-receptor interactions, autocrine and paracrine signals, as well as oxygen, cytokine, and nutrient concentrations need to be considered synergistically in the design of a bioreactor [52].

In order to be clinically relevant, biologically relevant culture of stem cells and even culture of entire organ grafts is necessary. One avenue taken in rodent models and a few single human cases has been to de-cellularize a donor organ, meaning to take out all cells and small molecules, leaving only the natural three-dimensional extracellular matrix behind. Recipient cells seeded onto this matrix have been successfully grown into transplantable organs. Studies of such organ transplant research are ongoing, including studies of trachea, lung, kidney, and heart. Advances and risks including immunological concerns surrounding this line of research have been reviewed [54].

4.2 Stem Cell Therapies in Clinical Development

HSC culture has been established for the treatment of blood cancer, anemia, and autoimmune diseases for decades. However, techniques are constantly improving. The number of studies and trials involving HSCs highlights the interest and potential clinical impact of research in this field. As of today, more than 700 interventional clinical studies involving HSCs are listed at clinicaltrials.gov (access at clinicaltrials.gov, Apr. 26th, 2017). Table 1 lists a representation of ongoing interventional clinical trials involving HSC, with therapies targeting blood cancers omitted from the list.

In recent years, treatment options beyond HSCs have been designed and are being tested clinically using new approaches that employ different stem cell populations. Currently, there are also around 700 such trials listed (access at clinicaltrials.gov, Apr. 26th, 2017). Table 2 provides examples of ongoing stem cell based interventional approaches utilizing stem cells other than HSCs that are currently in clinical trials for a variety of disease.

4.2.1 Transdifferentiation

For many stem cell populations, the central limitation hindering clinical use remains the accessibility of large numbers of differentiated cell types. While ES cells but not differentiated/mature cells can be expanded infinitesimally in culture, this shortage

can only be overcome if patient-specific pluripotent cells could be differentiated into the target cells. This entails, first, the accessibility of such cells and second, ways of controlled, directed differentiation.

The first challenge has been overcome by the generation of induced pluripotent cells (iPSC) from basically any differentiated cell type [48].

The second obstacle, i.e. directed differentiation of these cells in vitro, remains challenging. The intracellular factors and extracellular stimuli are not yet sufficiently defined to deliver these factors or design 3D-culture systems that adequately mimic an appropriate microenvironment (see above, [52]). In 2008, a group led by Douglas Melton achieved to convert pancreatic exocrine cells in adult mice into cells indistinguishable from endogenous β-cells [59]. Besides the obvious importance for diabetes research, this study proved that a differentiated cell can be transdifferentiated into another differentiated cell type without being de-differentiated into the state of pluripotency. However, it does not seem entirely clear what happens during this process. As to the best of our knowledge, it cannot be excluded at that time that differentiated cells during the process of lineage conversion resemble a common progenitor or even a pluripotent cell. However, while this is an important question in stem cell research, it appears less relevant for clinical purposes as long as the lineage converted cells take over the desired functions.

From the medical point of view, the use of Muse cells (discussed above) might outperform trans-differentiated cells because of their relative accessibility, expandability, and applicability. It remains to be seen if these cells hold their promise in clinical trials.

4.3 Ethics in Stem Cell Research

Given the sensitivity of the topic of stem cell research, ethical considerations are addressed in this chapter. While oocytes are not fertilized for the specific use of research, there are numerous fertilized oocytes that remain unutilized from in vitro fertilization (IVF). ES cell cultures have been obtained from supernumerary (excess) products of IVF. Discussions about IVF, a preceding scientific innovation, include arguments similar to those raised in the debate about ES cell research. Likewise, abortion the cloned sheep, Dolly, and genetically modified crops caused a fundamental debate over the limits of human intervention in all matters of life [3]. Interestingly, today the majority of the population in Western societies is in favor of IVF, given its undisputable medical usefulness. Discussions over GM crops endure with different outcomes concerning their acceptance in different parts of the world despite a scientific consensus for their safety [3]. This highlights the complexity of ethics in the field of research, which is further complicated by political, cultural, and religious confounders.

The major ethical position against ES cell research is the isolation of ES cells from supernumerary pre-implantation stage embryos from fertility clinics. The argument is that using these embryos for research purposes prevents the development of a human being. Follow-up questions include how ethical the unlimited storage of these embryos is or what should be done with the supernumerary fertilized oocytes. The position in favor of using ES cells for research argues that failing to use this powerful tool that has the potential cure serious diseases would be unethical. Different political and governmental administrations in different countries have adopted different positions concerning the use of ES cells for research, highlighting that a global agreement is not in sight.

As a bright spot, while there is a need for research on human ES cell lines [24] their widespread use for clinical purposes can be omitted as the iPS technology has provided an elegant way around this ethical dilemma. Furthermore, the discovery of additional adult stem cell populations may provide plentiful opportunities for the development of cellular therapies and tissue grafts.

4.4 Summary

The field of stem cell research continues to advance at a remarkable pace and holds tremendous promise for clinical applications. From the discovery of embryonic stem cells to the creation of Dolly to the ever-increasing use of induced pluripotent stem cells, this exciting field continues to surprise and challenge our understanding of biology and tissue repair as well as our beliefs regarding autonomy and life.

Stem cell research has now entered a new phase; one that includes clinical studies in human subjects where this wealth of knowledge gained through years of innovative research can be tested for therapeutic utility. Reaching this lofty goal required the combined efforts of diverse groups of scientists, including cell biologists, extracellular matrix experts, and bioengineers.

Additional possibilities for cell based patient specific therapies may arise once combined with contemporary technologies of gene editing, such as CRISPR/Cas9, which has itself progressed into the clinical development stage [43].

As stem cell research continues, it will be important to continue an open dialogue concerning ethical matters surrounding the techniques used in this filed. Discussions about gene editing and the possibility of creating germline changes that would affect future generations will certainly join those concerning the use, origin, development, and disposing of various types of stem cells. With the potential to effectively treat countless medical conditions on an individual bases, so called precision medicine, comes the responsibility to define and follow the developing code of ethics.

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Author Biography

Dr. Gerd Bungartz (Germany) Dr. Bungartz has a diverse and international career in science and research. After his studies at the University of Cologne and the German Sport University, he focused his research on adhesion molecules, at Lund University in Sweden. He then continued this work at the Max Planck Institute for Biochemistry where he expanded his interests to include research on hematopoietic (stem) cells. After receiving his Ph.D. with honors in 2005, Dr. Bungartz relocated to work with Professor Emerson at the University of Pennsylvania and later to Massachusetts General Hospital/Harvard Medical School to work with Professor Scadden of the Harvard Stem Cell Institute. In 2012, he moved to the German Sport University in Cologne and later into industry working for Pfizer. Dr. Bungartz' research was awarded several grants and published in high-ranking journals. Currently, he holds positions at 'Hochschule Döpfer' and Merck Serono.