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Pluripotent Stem Cells and Their Dynamic Niche

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Abstract

Cell-seeded implants are a regenerative medicine strategy that aims to replace injured tissue and restore tissue function. Pluripotent stem cells are promising cell candidates for the development of regenerative medicine therapies as they have the ability to self-renew and commit towards numerous cell types. *In vivo*, stem cells reside in a dynamic niche, a stem cell-specific microenvironment that possesses chemical, biological and mechanical cues, which drive the stem cell fate and renewal. The connection between stem cells and their niche is a two-way relationship consisting of both cell–cell interaction and cell–extracellular matrix (ECM) interactions. An alternative regenerative medicine approach is the manipulation of the stem cell microenvironment. Hence, novel strategies have been developed including 3D biomaterials and bioreactor technologies providing topographical, chemical and mechanical cues to recreate the stem cell niche. Understanding the mechanisms controlling stem cell fate and the dynamic nature of the stem cell niche will enable researchers to replicate this stem cell-specific microenvironment, and therefore, harness and control the valuable attributes which stem cells possess. This chapter elucidates the importance of pluripotent stem cells and their dynamic niche in regenerative medicine. It further presents novel strategies to replicate chemical, topographical and mechanical stimuli which are essential for the regulation of stem cell fate and hence tissue regeneration.

Keywords: Pluripotent stem cells, Regenerative medicine, Stem cell niche, Mechanical cues, Bioreactor technologies

1. Introduction

Regenerative medicine is a promising field that aims to develop therapies for currently intractable diseases. These approaches include cell–cell and cell–scaffold implants. Self-renewal and the ability of pluripotent stem cells to commit towards particular cell lineages in response to

mechanical, chemical and physical stimuli makes them the ideal building block for such therapies. Embryonic stem cells (ESCs) are isolated during embryological development. Their utilisation in regenerative medicine is controversial, and therefore, adult stem cells have been more thoroughly investigated for their potential use for tissue regeneration. Currently, induced pluripotent stem cells (iPSCs) are being investigated as an alternative source for pluripotent stem cells due to their origin and clinical potential [1].

Stem cells *in vivo* reside in a dynamic, cell type-specific microenvironment, the so-called niche [2–5]. This microenvironment is composed of stem cells, supportive stromal cells and surrounding extracellular matrix (ECM) [5, 6]. The niche provides chemical, mechanical and topographical cues facilitating stem cell renewal and controlling stem cell fate [3, 4]. The ECM is an important component of the niche. It is the biological matrix directly surrounding stem cells *in vivo* and is composed of tissue-specific glycosaminoglycan (GAG), insoluble proteins (e.g. fibronectins, collagens, laminins) and inorganic hydroxyapatites [6, 7]. Research has shown that the biophysical properties of the ECM affect stem cell behaviour. Cells counteract external forces which result from their surroundings, through adjacent cells and ECM stiffness by altering the cytoskeleton tension and through the generation of internal forces [8]. These forces are exerted on and from each individual cell to their environment. The interaction between microenvironment and cells results in the regulation of stem cell behaviour [8–10]. The cellular response to external mechanical cues is defined as mechanotransduction.

An alternative strategy for tissue repair is the manipulation of the stem cell microenvironment to enable tissue repair through endogenous stem cells [11]. To recreate the stem cell niche, it is important to reproduce the physical and mechanical microenvironment stem cells experience *in vivo*. Hence, strategies including novel bioreactor technologies have been developed. By replicating the *in vivo* environment, bioreactors allow the study of mechanical stimuli in combination with chemical and biological signals on cell–cell and cell–biomaterial constructs [12].

This chapter will present the role of pluripotent stem cells and their dynamic niche in regenerative medicine, as well as the importance for the niche replication for the development of novel regenerative therapies. Finally, it will present novel strategies to replicate chemical, biological and mechanical stimuli which are essential for the regulation of stem cell fate and hence tissue regeneration.

2. Stem cell niches

In 1978, the term ‘niche’ was first described by Schofield [13] who theorised that stem cell self-renewal and character is dependent on their environment. Since then, the theory of the stem cell microenvironment has been expanded [14, 15]. Each stem cell population has a unique and specific environment, but there are features which stem cell niches have in common (**Figure 1**). Tissue-specific (e.g. osteoblasts) and non-specific (stromal cells) heterogeneous cell–cell interactions co-exist in stem cell niches [11]. Secreted-membrane bound factors bind stem cell surface receptors in order to direct stem cell self-renewal and fate (e.g. Wnt, chemokines,

Notch, SCF) [16–22]. During tissue injury and inflammation, immunological cells regulate the niche [23, 24]. The ECM provides structural support and orientation and serves as storage for soluble factors [25]. It further interacts with stem cells through gap junctions, soluble factors and surface receptors [5, 6]. In addition, physical and physiological parameters of the niche such as shape, elasticity, blood flow and oxygen tension influence stem cell differentiation and self-renewal and regulate the metabolic activity [22, 26–32]. The interactions between stem cells and their niche are reciprocal; since stem cells are able to remodel the niche and secrete ECM components in response to the signals they receive from it [33–35].

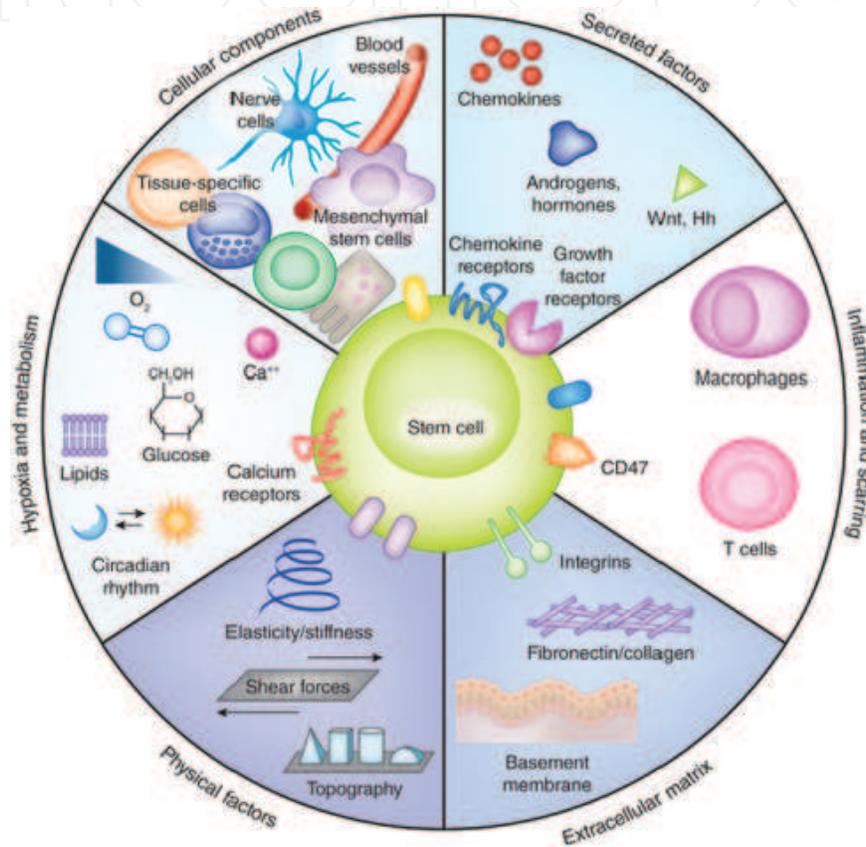


Figure 1. Components of the stem cell niche. Niches are complex dynamic heterogeneous microenvironments containing various cell types, extracellular matrix, soluble factors. The stem cell niche is influenced by a variety of factors including physical and metabolic parameters. The ECM and supportive stromal cells interact with stem cells through gap junction, soluble factors and surface receptors. Systematic signals are carried into the niche by blood vessels to facilitate the recruitment of inflammatory cells and neural signals convey distant physiological cues, such as shear stress, tissue stiffness and oxygen tension, to the stem cell niche. Image adapted with the permission from Lane et al. [11].

2.1. Stem cell niches: role

Niches have specific anatomical locations and form unique stem cells surroundings *in vivo*. This microenvironment regulates pathophysiological and physiological processes and directs cellular fate and function (**Figure 2**). The niche provides extracellular signals that maintain a balance between stem cell self-renewal and differentiation enabling stem cells to preserve a

dormant and low metabolic state in order to avoid stem cell exhaustion and the accumulation of gene mutations which might result in their transformation into cancer cells [4, 36, 37]. It has been shown that the destabilisation of the stem cell environment is involved in diseases connected to aging, tumorigenesis and degeneration [38]. In adjacent sites within the same tissue, stem cells co-exist in either quiescent or active state [39, 37]. For maintaining stem cell number and to meet the needs for differentiated cells in neighbouring tissues, the balance between asymmetric and symmetric stem cell division is essential [3]. Thus, in order to maintain a healthy stem cell pool, a crosstalk between tissue necessity and state is created by the stem cell niche [40, 41].

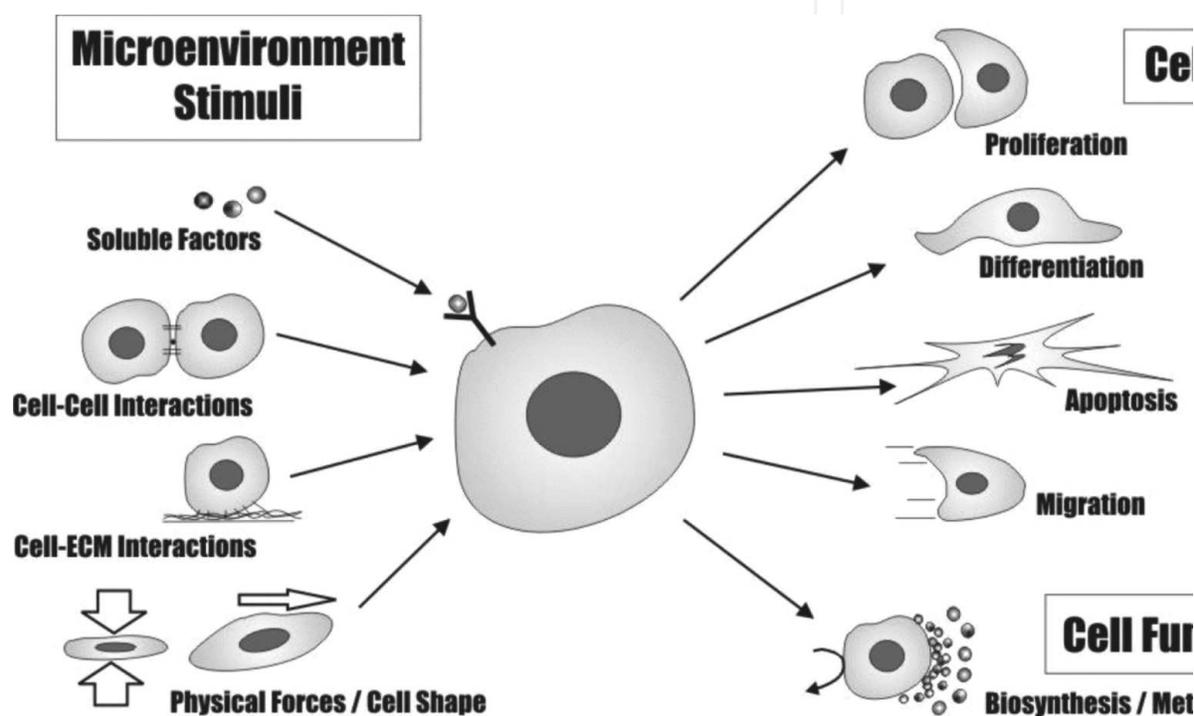


Figure 2. The stem cell microenvironment controls pathophysiological and physiological processes. Image adapted with the permission from Ou et al. [48].

2.2. Extracellular matrix

Due to its dynamic and diverse composition, the ECM provides structural and mechanical support and gives biochemical and physical characteristics to different stem cell niches, which are required for tissue morphogenesis and homeostasis as well as to facilitate stem cell renewal and control stem cell fate [3–5, 7]. It provides scaffolding to cells and stores soluble growth factors [11, 42, 43]. In addition, secreted or cell surface factors, signalling cascades and gradients, as well as physical factors, such as shear stress, oxygen tension and temperature, contribute to control stem cell behaviour in a well-orchestrated manner [40, 41].

The ECM is mainly composed of water, proteins and polysaccharides with each tissue exhibiting its unique composition and topology [42]. This diversity is caused by a combination

of specific molecular interactions between ratios, geometries and isoforms of its components [7]. Two main classes of macromolecules are found in the ECM. These are fibrous proteins (e.g. collagens, elastins, fibronectins and laminins) and proteoglycans (e.g. heparin sulphate, chondroitin sulphate, ketaran sulphate) [44, 45].

The most plentiful fibrous protein in the interstitial ECM is collagen. It contributes to approximately 30% of the total protein amount and provides tensile strength it controls chemotaxis, controls cell attachment and migration as well as influences tissue development [46]. In any given tissue, collagen is present as a non-homogenous mixture of collagen fibre types; however, one type is normally dominant (e.g. in bone: collagen type-I, cartilage: collagen type-II) [42]. In close connection to collagen are elastin fibres which recoil when tissues undergo repeated stretching. In fact, the degree of association with collagen is a limiting factor for tissue elasticity [47]. Besides directing the organisation of the interstitial ECM fibronectin also influences cell adhesion and migration [42, 46]. The majority of the extracellular part within tissues is filled by proteoglycans consisting of GAG chains, which are linked to a protein core and are classified according to the GAG and core protein arrangement [45]. These proteoglycans have numerous different purposes that mirror their hydration, binding, buffering and force-resistance properties [42, 48].

Furthermore, the ECM can be divided into two forms, the basal membrane (BM) and the interstitial matrix [49] both of which are composed of a collagen framework with glycoproteins (non-structural proteins) adhering to it to communicate with adjacent cells via integrins [50].

2.3. Niche cells and cell communication

Stem cell niches in adult tissues are populated by a number of different cell types with each having a particular function. This is shown in the adult hematopoietic stem cell (HSC) niche, which is located along the endosteal surface of trabecular bone, in close vicinity of osteoblasts and the endothelial cells of blood vessels [51]. Osteoblasts in the endosteal niche regulate the HSC number [51, 52] and preserve their quiescence by releasing signalling molecules [53–55]. The HSC niche is further inhabited by cell types including stromal cells, bone marrow adipocytes, osteal macrophages, CXCL12-abundant reticular (CAR) cells, nestin-positive mesenchymal stem cells, nestin-positive Schwann cells, endothelial cells [19, 56–59].

Besides cells, which reside permanently in the niche such as nerve cells, endothelial cells and connective tissue fibroblasts, cells of the innate and adaptive immune system and cells that are important for the repair of damaged tissues and promote protection against pathogens are also present [11].

When stem cells undergo cell death or apoptosis neighbouring cells belonging to the niche undergo dedifferentiation in order to replace the lost stem cells [11]. For example, the removal of hair follicle stem cells in mice results in the repopulation of the niche by epithelial cells which then sustain the hair renewal [60]. This reprogramming of endogenous differentiated cells into stem cell controlled by the stem cell niche [16], and the maintenance of stem cell sources [61] might hold important clinical promise [11]. The communication between niche cells and stem cells is facilitated either by indirect contact through the secretion of molecules or directly

through physical cell–cell interactions. The direct cell–cell contact is mediated by cell–cell adhesion molecules and receptors with membrane-bound ligands [11]. Indirect communication between cells in the HSC niche is clinically used to modify the HSC niche *in vivo*. For the treatment of bone marrow failure, genetic disorders and haematological malignancy cytokines (G-CSF, GM-CSF) are administered which result in the activation of hematopoietic stem cells, their expansion as well as diminished adhesion to their niche [11].

2.4. Physical and physiological parameters

Stem cell fate is greatly influenced by physical and physiological parameters (**Figure 2**). Niche topographies modify the stem cell cytoskeletal resulting in the activation of specific signalling pathways and stem cell differentiation [29, 62]. The modification of physical parameters such as substrate stiffness or elasticity, shape and shear forces has been utilised for clinical treatments but also for *in vitro* hematopoietic stem cell culture [26, 27]. Bone tumour and osteoporosis are treated clinically using drugs that change the balance between physical factors, for example rigid (bone) and elastic (arteriolar, dermal connective tissue). Shear forces and drugs which are used to promote blood flow for the development of embryonic HSC *in vivo* [28]. Physiological factors such as oxygen tension are important contributors for cell survival and maintenance. Many cells such as HSC inhabit hypoxic microenvironments [30]. Cells in these niches are carried out the glycolysis and express high levels of hypoxia inducible factor 1 α . Growing mammalian cells under hypoxic conditions positively influences cell proliferation, survival and function after engraftment [31].

3. Stem cells in regenerative medicine

Medical conditions such as tissue loss, organ failure, cancer abrasion, congenital structural anomalies can already be treated by clinical procedures such as autologous and allogenic organ transplantation and the use of artificial implants. However, these treatments are limited by organ shortages, impairment of healthy tissue during surgery and immune rejections. Breakthroughs in the field of regenerative medicine may enable the utilisation of stem cells and stem cell-based therapies for the restoration of tissue function [63].

Stem cells can be distinguished by their potency (multipotent versus pluripotent) and through their tissue source, that is ESCs, foetal stem cells (FSC), adult stem cells such as mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSC) [64, 65] (**Figure 3**). Stem cells are characterised by their ability to self-renew without senescence for extended culture periods and the potential to differentiate into multiple cell types [66]. Through their pluripotency and multipotency stem cells offer vast cell sources making them ideal for studying degenerative diseases and developing cell-based therapies [64, 65]. They have the capability to generate every tissue type and are essential to human development. However, due to regulatory, ethical and technical considerations involving genetic modification and cell isolation adult stem cells such as MSCs and adipose stem cells have been widely investigated as an alternative cell source to ESCs [63].

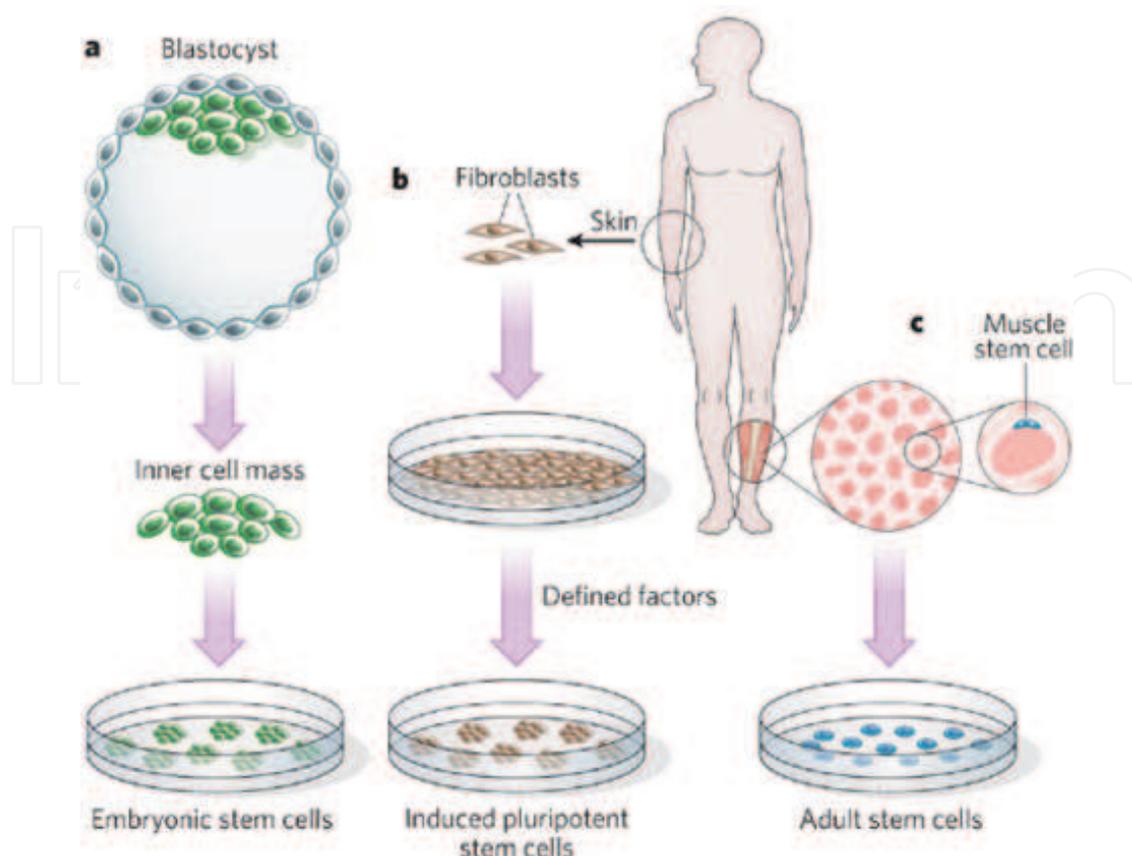


Figure 3. Stem cell types. (a) Embryonic stem cells derived from blastocysts were the first human pluripotent stem cells that could be differentiated to generate various cell types. (b) Induced pluripotent stem cells were first generated by reprogramming somatic adult cells such as skin fibroblasts. (c) Tissue-specific adult stem cells are primed for generating progeny that differentiate into specialized cell types (e.g. stem cells residing in the muscle). Image adapted with the permission from Lutolf et al. [225].

Tissue-specific adult stem cells which can be found in various niches such as the bone marrow or adipose tissue are multipotent and play a crucial role in tissue development, repair and growth [67–70]. Regenerative medicine approaches envision that these somatic stem cells could theoretically be harvested from the patient, be differentiated *in vitro* and injected back into the patient to regenerate impaired tissues without the need to suppress the immune system. Autologous chondrocyte implantation (ACI) for the repair of chondral defects in joints such as the knee is such a regenerative approach. In this two-stage process, the patient's chondrocytes are expanded *in vitro* and the subsequently implanted back into the patient [71–73].

Yet, low cell numbers, complications isolating adult stem cells from healthy tissues well as limited differentiation potential may require other stem cell types [66]. Latest developments indicate that directed differentiation and the development of differentiation protocols could result in the translation of cell therapies for presently incurable diseases [74–77]. The availability of patient-specific cells in required quantities and on demand could revolutionise stem cell therapies and further allow disease modelling and human pluripotent stem cell (hPSC)-based drug discovery [65].

3.1. Pluripotent stem cells

3.1.1. ESCs and FSCs

When ESCs were first isolated, they provided a model for the study of developmental biology but also opened up the possibility to exploit their pluripotency for stem cell-based therapy in order to treat organ damage or dysfunction allowing regenerative medicine to become a reality for the treatment of various diseases [66]. ESCs are harvested from the inner cell mass of the blastocyst-stage embryo, a hollow sphere of cells which is composed of an outer cell layer forming the placenta and an inner cell mass from which the ESCs are derived [66]. ESCs have the ability to proliferate, to maintain an undifferentiated phenotype for extended periods of time in culture and to develop into a large number of somatic cell types [78–80]. Early studies utilising ESCs were aimed at treating traumatic injuries of the CNS and degenerative diseases [81–84]. Implanting early stage and differentiated ESC into laboratory animals has been shown to improve function, behaviour and morphology, but also to cause teratoma formation and hyperproliferation [85]. The application of ESC has also shown great promise for the regeneration of cartilage, cardiac tissue and peripheral nerves [76–89].

The utilisation of tissue-specific stem cell lines isolated from foetal tissue is another possible strategy in regenerative medicine as these cells exhibit higher proliferation potential, more specific differentiation capacity, improved migration and regeneration after implantation [90, 91]. Functional integration of human FSC (hFSC)-derived dopamine neurons in a rodent Parkinson's disease model is one of the most significant examples for the use of hFSC. It became the basis for clinical trials demonstrating similar effects in patients [92–94]. Another study showed that the transplantation of human cortical neuroepithelial stem cells developed from foetal cortical brain did not result in tumour formation and facilitated the recovery of diminished tissue function in a rodent stroke model [95]. Despite associated ethical concerns and the risk for tumour development, these studies have shown great promise for the clinical application of ESC and FSC [64].

3.1.2. Induced pluripotent stem cells

In 2006, Takahashi and Yamanaka [96] discovered that the retroviral expression of pluripotency-specific transcription factors (Oct4, Sox2, Klf4 and c-Myc) reprograms adult somatic cells into a pluripotent state. These iPSCs showed epigenetic and transcriptional similarities to ESCs [97–99]. Over the years, the progress has been made in the generation of virus-free/vector-free reprogramming methodologies to avoid vector-induced tumour development [100–103]. In addition, iPSCs are now being derived from a variety of different cell types such as blood cells, dermal fibroblasts and keratinocytes at higher efficiencies [104–107].

To study the sporadic and genetically inherited diseases, patient-specific iPSCs have been generated (**Table 1**). These *in vitro* studies offer a proof-of-concept for the use of iPSCs for disease modelling with the goal to discover novel drugs and disease-specific pathways aiding their treatment. Since several studies were performed on very limited cell numbers, reproducibility of the observed phenotype still needs to be investigated [108].

Disease	Derived cells	Control cells	Result	References
Parkinson's disease	Dopaminergic neurons	hiPSC	No observed defect, not drug tested	[103]
Parkinson's disease	Dopaminergic neurons	hiPSC	Enhanced chemical sensitivity causes cell death, drug tested	[309]
Spinal muscular atrophy	Motor neurons	hiPSC	Loss of SMN gene expression and neuron formation, drug tested	[310]
RETT syndrome	Neurons	hiPSC	Reduced soma size and spine density, loss of synapse, drug tested	[311]
Familial dysautonomia	Neural crest cells	hiPSC, hESC	Loss of neural crest cells, drug tested	[312]
Long QT 1 syndrome	Cardiomyocytes	hiPSC	Depolarisation of cardiomyocytes, drug tested	[313]
Long QT 2 syndrome	Cardiomyocytes	hiPSC	Depolarisation of cardiomyocytes, drug tested	[314]
A1-antitrypsin deficiency	Hepatocytes	hiPSC	Downregulation of A1-antitrypsin expression, drug tested	[315]
Timothy syndrome	Cardiomyocytes	hiPSC	Depolarisation of cardiomyocytes, drug tested	[316]

Table adapted from Wu and Hochedlinger [108]

Table 1. Patient-specific disease models utilising human iPSC-derived cells.

The generation of autologous cells for cell therapy is another possible application for the iPSC technology as it minimises the challenges associated with human ESC-based therapies [108]. Researchers have described that iPSCs facilitate the reduction of the blood cell phenotype in a sickle cell anaemia mouse model [109]. iPSCs were derived from a transgenic mouse exhibiting a mutation in the human haemoglobin sequence. These iPSCs were genetically corrected and differentiated into haematopoietic progenitor cells. Subsequent implantation of these cells into the mouse model resulted in a normal haemoglobin level and restored phenotype [109]. Despite these remarkable results, haematopoietic stem cells with the ability for multilineage differentiation have not yet been generated. In addition, this study utilised retrovirus-derived iPSCs and it remains unclear if similar results can be achieved in a retrovirus-free approach. Comparable studies implanting iPSC-derived progenitors for mesodermal and ectodermal cells into animal models have been described (e.g. neurons, cardiomyocytes, blood) [110–112].

The conversion of somatic cells into iPSCs and the advances that have been made in their generation has enabled researchers to utilise disease-specific cells for disease modelling as well

as drug screening. Moreover, it opens up opportunities for the generation of custom-made iPSCs for cell therapies [108] (**Figure 4**). Patient-specific iPSCs are interesting autologous cell sources as they would eliminate the need to suppress the patient's immune system and could be generated in larger quantities. However, future studies are required to investigate the effect of genetic modifications of host and donor cells when injecting iPSC into patients [64].

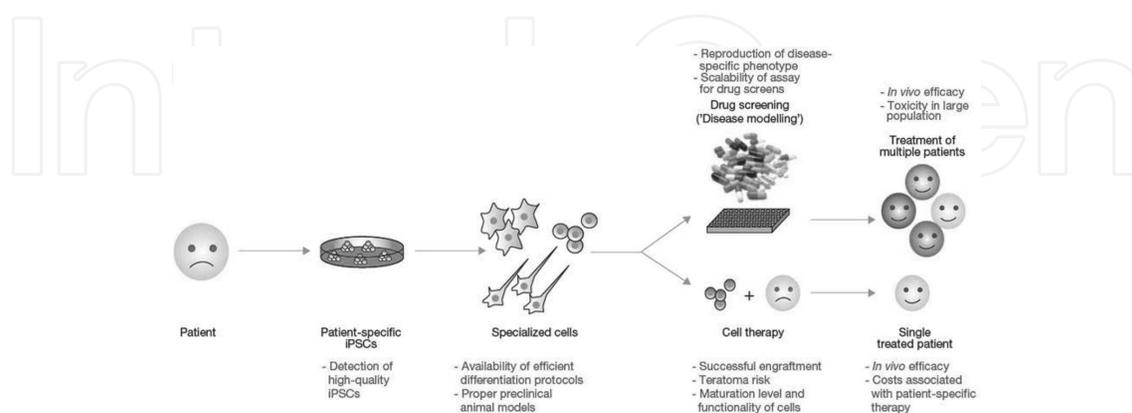


Figure 4. The potential of iPSC technology in regenerative medicine. Reprogramming a patient's somatic cells, for example blood cells or skin cells generates patient-specific iPSCs. These iPSCs can be differentiated into specific cell types which are subsequently used for either cell therapy or disease modelling. Cell therapy aims to fabricate autologous differentiated cells for implantation into a single patient. Disease modelling on the other hand is based on reproducing a cell phenotype from iPSC-derived specialist cells as present in the patient and utilising these cells for large-scale drug screening for the treatment of any patient with the same disease. Image adapted with the permission from Wu and Hochedlinger [108].

3.1.3. Comparison of ESCs and iPSCs

ESC and iPSC cell lines display biological differences between one another and how comparable the both cell types are might affect their functionality and safety. Researchers have shown that hiPSC and hESC are highly similar [113–116]. However, variations in gene expression [116], DNA methylation [117, 118], differentiation potential [119, 120] and teratoma-forming propensity [121] have been reported. Genetic background [122], passage number [116, 123], lab-to-lab differences and the use of vectors in their generation have extensive influence on the function and gene expression of PSC. Moreover, hiPSC and hESC have been shown to carry copy number variations (deletions and duplications) [124–126] and point mutations [127], which are consequences of their culture. Some mutations might also occur during reprogramming [108]. Since differences in transcriptional and DNA methylation profiles between 12 hiPSC and 20 hESC lines have been described, large sample numbers are required to robustly investigate potential differences and their influence on pluripotent stem cells [128].

3.2. Clinical potential of pluripotent stem cells

Translation of pluripotent stem cell-based therapies into the clinic will depend on several factors such as cell purification, the efficiency of cell-lineage-specific differentiation leading to functional cells, eliminating tumour generation and finally the generation of novel organs [108]. Even though iPSCs have been differentiated into a number of cell types including

cardiomyocytes [129–133], neurons [134, 135], blood [133, 136, 137] and pancreatic cells [137, 138], purities over 95% have not been reported and isolating these cells from a heterogeneous cell population is difficult. Furthermore, ESC-/iPSC-derived cells are mostly immature and whether their stage will affect their clinical performance remains to be investigated in a cell- and disease-specific manner [108].

Another important issue for the application of PSC in regenerative medicine is their integration into the host tissue. Organs exhibit a balance between the numbers of each cell type, their geometrical arrangement and their developmental stages. Whether the injection of cell suspension will auto-regulate cell type numbers to generate an endogenous tissue and whether the transplanted cells will function in synergy with existing cells still needs to be investigated [108]. In addition, regulatory requirements for pluripotent stem cell–derived therapies remain high as shown by a clinical trial performed by Geron, a biopharmaceutical company that tested human ESC–derived oligodendrocytes for spinal cord injury [139]. Microscopic cysts found in cell-transplanted mice resulted in extensive studies for the batch-to-batch assessment of cyst formation as well as follow-up safety study on these cyst containing grafts were requested by the FDA [139].

Concerns over the use of hiPSCs for cell therapy are also arising due to studies reporting on genetic and epigenetic modifications during the reprogramming process such as protein coding and DNA methylation [127, 140]. To-date, progress in the development of iPSC protocols has eliminated the need for vectors reducing the risk of tumour formation [113, 141, 142]. Furthermore, whether iPSC-derived cell types retain their epigenetic ‘memory’ remains under investigation. Studies have shown that both mouse and human iPSCs preserve an epigenetic profile which is related to the donor cell source and this may affect subsequent differentiation [143–146]. Drawbacks for the application of patient-specific iPSCs also include regulatory, cost and time requirements for the generation of patient-specific iPSC-based treatments as well as scalability and good manufacturing practice (GMP)-compliant cell therapies.

Despite all this, in recent years, the focus has been on translating hESC research into the clinic such as the trial sponsored by Geron which has highlighted the obstacles but also promises of pluripotent stem cell therapy. Furthermore, Japanese researchers were applying for regulatory approval to use patient-specific iPSC-derived RPE cells to treat macular degeneration [147]. Pluripotent stem cell research has evolved from the isolation of hPSC to the development of differentiation protocols and early clinical trials. Less than two decades into hPSC research, the utilisation of human pluripotent stem cell–derived treatments are under clinical investigation [65].

3.3. Multipotent stem cells as alternative cell sources

Limited availability of embryonic and foetal tissue as well as ongoing research into the development of improved protocols yielding matured differentiated cells may be limiting factors for the use of ESC, FSC and iPSC. Alternatives to pluripotent stem cells are multipotent stem cells such as MSCs and adipose-derived stem cells which have been extensively studied for regenerative medicine therapies.

3.3.1. Mesenchymal stem cells

Non-hematopoietic multipotent MSCs are derived from the mesoderm and are present in a variety of connective tissues and postnatal organs [63]. Their discovery was reported over 130 years ago when research suggested that bone marrow is the source collagen depositing cells that can develop into various cell types including bone and cartilage [148]. Caplan [149] then named these marrow cells MSCs after their capability to differentiate into cells of mesenchyme origin such as bone, cartilage, tendon, muscle and adipose tissue. The clinical routine to treat haematological diseases with bone marrow makes MSCs interesting contenders for cell-based therapies. MSCs isolated from bone marrow are probably among the most characterised and clinical utilised stem cell types [150]. The multipotency of MSC allows them to differentiate into bone, cartilage, muscle and neural cells [151–153]. They offer the possibility for autologous cell transplantation and at the same time eliminating the risk for graft-versus-host diseases. MSCs are non-immunogenic, affect the maturation and response of immune cells and do not cause tumour formation when implanted in allogeneic hosts [64]. Finally, the injection of MSC leads to the secretion of growth factors by host cells including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) among others. In summary, the capacity of MSC to change their default state and their potential to promote tissue regeneration may surpass their application for haematopoietic diseases and might make them a suitable tool to treat degenerative diseases such as neurological and neurodegenerative disorders [154]. Employing MSC in combination with biomaterials or alone resulted in functional regeneration of paralysed limbs, diminished cavity formation in the spinal cord and axonal regrowth [155–158]. MSCs have also been utilised in first clinical trials to treat myocardial infarcts, stroke and diseases of the CNS [159, 160]. These studies have demonstrated that bone marrow-derived MSCs are a promising stem cell type for clinical application and stem cell therapies [64]. However, limited integration into the host tissue was observed in clinical trials indicating that the detected benefit in these trials were most likely due to the secretion of cytokines and soluble factors [161].

MSCs with similar properties to bone marrow-derived MSC have been isolated from trabecular bone [162], periosteum [163], synovial membrane [164], skeletal muscle [165], skin [166], pericytes [167], peripheral blood [168] and umbilical cord [169, 170]. Due to their low number upon isolation, adult stem cells require *in vitro* expansion and modification before being employed therapeutically [63].

3.3.2. Adipose-Derived stem cells

Adipose-derived stem cells (ASCs) are a promising cell type as they can be easily isolated in large numbers from adipose tissue without causing severe donor site morbidity and discomfort [171, 172]. Over 80% of adipose tissue is composed of mature adipocytes and the stromal vascular fraction consisting of vascular smooth muscle cells, fibroblasts, preadipocytes, endothelial cells, resident immune cells and ASCs [173, 174]. Many of the ASC properties and density differ according to the harvest location of the adipose tissue [63]. Studies have shown that the ASCs proliferation rate is dependent on age, tissue location and type, the culture conditions and isolation procedure, but is in general higher compared to the doubling rate of

bone marrow–derived MSCs [175–178]. Recent studies have shown that their application is not restricted to mesodermal tissues instead they can be employed for endodermal and ectodermal tissue regeneration [63]. ASCs have been successfully differentiated into adipogenic [179–181], osteogenic [182], cardiomyogenic [183, 184], chondrogenic [182, 185, 186], angiogenic [187, 188], tenogenic [189], hepatogenic [190, 191] lineages. Moreover, studies utilising scaffolds in combination with growth factors have been carried out to assess their tissue regenerative potential [192, 193]. In summary, ASCs are a valuable cell source for the development of cell-based therapies and have been shown to be safe and effective in clinical and preclinical studies [171, 172]. However, since ASCs are characterised as multipotent adult stem cells, their differentiation potential is restricted compared to ESC and iPSC. Even though limited numbers of clinical studies have investigated the therapeutic potential of ASCs and their lineage-specific differentiation depends on the site of harvest, gender and age of donor [194, 195], there are practical advantages of using ASCs in regenerative medicine [63].

4. Regenerative medicine strategies: engineering artificial niches for the control of stem cell fate

Adams and Scadden [196] elucidated the concept that the stem cell niche is ‘dynamic’ and its properties change during development and with varying physiological conditions. These changes affect stem cell fate, but could also be utilised as potential therapeutic tool in regenerative medicine. Consequently, the control of nanotopography, mechanical and chemical properties of the ECM among others in engineered constructs as well as their mechanical loading is essential for directing stem cell fate in bioartificial systems and for the development of regenerative therapies (Figure 5) [197].

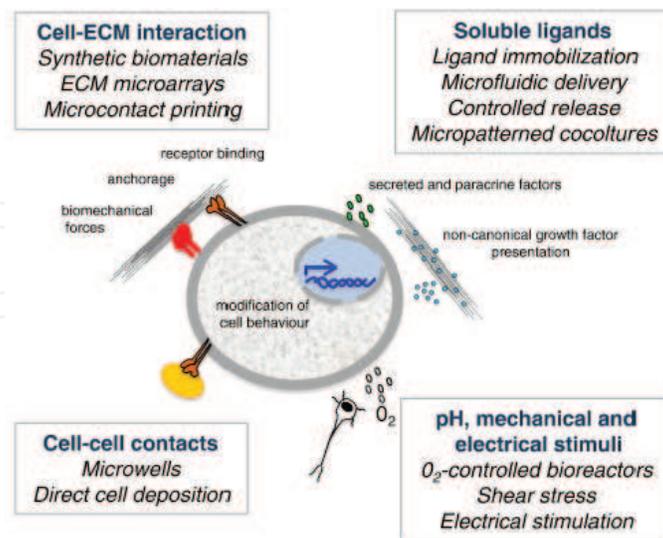


Figure 5. Engineering stem cell niches for the development of regenerative medicine strategies. To replicate niches in order to control stem cell fate chemical, topographical and mechanical properties are being mimicked using engineering techniques. Image adapted with the permission from Gazzatto et al. 2014 [5].

4.1. Biochemical signals as stem cell fate regulators

Chemical signals that cells are exposed to in the ECM activate signalling cascades determining cell proliferation, differentiation, migration and apoptosis [198, 199]. Hence, it is essential to mimic chemical cues of the ECM to control stem cell behaviour. Growth factors, which regulate cell adhesion, proliferation and lineage development, are an important type of chemical cue [200]. Growth factors are either added to the culture medium or secreted by niche cells and stem cells. They are crucial for stem cell fate decision and are spatiotemporally regulated during embryonic development [201]. A chemically defined protocol for the directed differ-

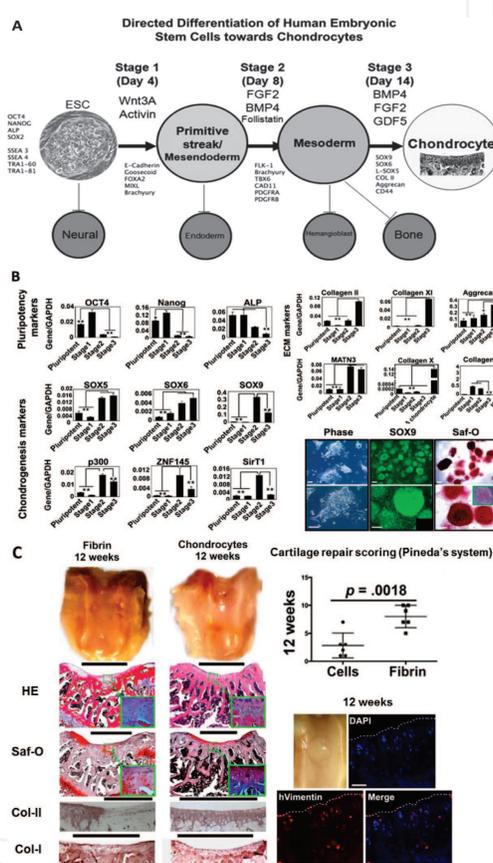


Figure 6. Differentiation of hESC and *in vivo* cartilage formation of hESC-derived chondroprogenitors. (A) hESC underwent a 14-day defined protocol for promoting chondrogenic differentiation. (B) Characterisation of chondroprogenitor cells by quantifying the upregulation of pluripotency, chondrogenic and cartilage ECM markers using qPCR. Results are shown as means \pm SD (n = 3). Chondrogenic cells derived from MAN7 showed high expression of SOX9 (inset shows IgG control) and Safranin-O staining (inset shows pre-treated with chondroitinase ABC) at the end of the protocol. Scale bars = 100 mm. (C) Assessment of *in vivo* cartilage repair capacity of pre-differentiated hESC after 12 weeks by macroscopic observation of the gross appearance of the RNU rat patella groove. Histological sections through the knee were stained for HE, Saf-O immunological assessment through Col-I and COL-II staining. Cartilage repair was scored using Pineda's system (0-worst to 14-best). Human cells were detected 12 weeks after implantation by immunohistochemistry staining. Scale bars black = defect area; white = 500 mm. Abbreviations: alkaline phosphatase (ALP); extracellular matrix (ECM); fibronectin (FN); glyceraldehyde-3-phosphate dehydrogenase (GAPDH); gelatine (GEL); Safranin-O (Saf-O); collagen type-I (Col-I); collagen type-II (Col-II); haematoxylin (HE). Figures were modified with the permission from Cheng et al. [202] and Oldershaw et al. [203].

entiation of hESC towards chondrocytes in 2D culture was developed where the pluripotent stem cells undergo intermediate developmental stages by supplementing the culture medium with exogenous growth factors and culturing cells on substrates of known matrix proteins over a culture period of 14 days [202, 203] (**Figure 6A**). The cartilage repair capacity of these chondroprogenitors was assessed by incorporation into fibrin hydrogels and implantation using an osteochondral defect model in the patellar groove of athymic RNU rats. Results showed that chondrogenic cells derived from hESC using a chemically defined differentiation protocol promoted cartilage repair [202] (**Figure 6B**).

Furthermore, the importance of cell–cell interactions in soluble factor signalling has revealed that certain cell types will respond only to locally secreted growth factors when in direct contact with adjacent cells [204]. Growth factors affect secreting cells (autocrine) and other cells (paracrine) *in vivo*. Soluble factors are often bound to the ECM limiting their diffusion and enhancing their efficiency. This can be replicated by tethering these to a biomaterial substrate [205]. Kuhl et al. [200] demonstrated that growth factors in their matrix-bound state were more effective than their soluble form. When proteins are incorporated or linked to biomaterials, they are commonly denatured or degraded. Consequently, short peptide sequences mimicking growth factors and chemokines are integrated to control stem cell fate [206]. The RGD sequence present in ECM proteins has been widely linked to biomaterial surfaces demonstrating enhanced osteogenesis and chondrogenesis compared to non-modified surfaces [206–208]. Bone morphogenic proteins have been shown to play a key role in stem cell activity and osteoblastic differentiation [209, 210]. Another approach is a surface modification of natural and synthetic biomaterials with specific functional chemical groups [211]. Examples include the functionalisation of PEG hydrogels with phosphate groups for bone mineralisation, carboxyl groups resembling GAG for cartilage and tert-butyl groups to mimic lipids for adipose tissue [212]. Moreover, self-assembled monolayers (SAM) functionalised with methyl-, hydroxyl-, amino- and carboxyl groups have been shown to promote osteogenic differentiation of MSC with the amino group being the most effective chemical group [213].

Growth factors and reactive chemical groups have been successfully used to guide stem cell differentiation. It was shown that immobilised chemical cues are more effective than soluble factors. However, improved control over bioactivity, spacing and orientation of the immobilised growth factors is required to guide-/direct-specific stem cell differentiation [214]. Further studies investigating the effect of chemical signals on stem cell fate are summarised in **Table 2**.

Cell type	Material	Chemical group	Result	References
<i>Soluble factors</i>				
hMSC	PLGA	BMP-2	Enhanced osteogenic differentiation	[317]
hMSC	PLLA	BMP-2	Enhanced osteogenic differentiation	[318]
hMSC	Chitosan/collagen IV	VEGF	Endothelia differentiation	[319–321]

Cell type	Material	Chemical group	Result	References
hESC	TCP coated with FN, gelatine and both	Sequential addition of Wnt3a, Activin-A, BMP-4, Follistatin, GDF5, FGF2, NT4	Chondrogenic differentiation	[202, 203]
<i>Peptides</i>				
hMSC	Alginate	Osteopontin peptide	Osteogenic differentiation	[322]
hMSC	PLGA	Osteocalcin peptide	Osteogenic differentiation	[323]
hMSC	BCP/PLA	RGD	osteogenic differentiation	[324]
rMSC	HA-PLG	BMP-2 peptide	Increased ALP expression, ectopic bone formation in vivo	[325]
<i>Chemically reactive groups</i>				
hMSC	Silk fibroin	-COO-, =C=O, SO ₃ H, NH ₂ , CH ₃	Enhanced osteogenic differentiation	[326]
hMSC	PEG	PO ₃	Increase of osteogenic markers at protein and gene level	[212, 327, 328]
hMSC	Glass	COOH, CH ₃ , OH, NH ₂ , SO ₃ H, SH	-NH ₂ and -SH group promoted and maintained osteogenesis, -OH and -COOH promoted chondrogenesis	[329-331]

Table adapted with the permission from Griffin et al. [214]

hMSC: human mesenchymal stem cells; hESC: human embryonic stem cells; PLGA: poly (lactic-co-glycolic acid); PLLA: poly(L-lactic acid); PEG: polyethylene glycol; HA-PLG: hydroxyapatite (HA)/poly(lactic-co-glycolic acid); BMP: bone morphogenetic protein; PMMA-g-PEG: poly(methyl methacrylate)-graft-poly(ethylene glycol); BCP: biphasic calcium phosphate; TCP: tissue culture plastic; EGF: epidermal growth factor; PDGF-AA: VEGF: vascular endothelial growth factor.

Table 2. Effect of chemical signals on stem cell differentiation.

4.2. Topographical signals as regulators of stem cell fate

Cells in their native in vivo environment engage with a variety of macro (tissues)-, micro (cells)- and nano-sized (proteins and ligands) topographical features. The basement membrane with its nanoscale pores, ridges and fibres is the most crucial ECM structure providing tissue organisation and support [215]. Micro- and nanopatterning techniques including soft lithography, electrospinning, layer-by-layer microfluidic patterning, three-dimensional printing, reactive ion etching and ion milling have resulted in the fabrication of scaffolds with controlled porosity, geometry and rigidity and texture [216-218]. The production of specific surface topography scales (nano, micro), types (ridges, pit, pillar, grooves) and distributions (random,

regular) has enabled researchers to study the influence of topographical signals on stem cell differentiation [214]. Structural cues greatly affect gene expression as they determine cell shape, elongation, positioning of focal adhesion and cell–cell interactions [29, 219–221]. Cell shape is a crucial regulator for cell physiology and function as well as dictator for stem cell differentiation [222]. Manasek et al. [223] reported that cell shape regulates myocardial development, whereas ECM-induced cell shape changes are responsible for the proliferation and differentiation of capillary endothelial cells [224]. Numerous studies have demonstrated that stem cell fate can be guided through controlling their shape by artificial extracellular matrices [197]. Traditionally, stem cells are expanded two-dimensionally on rigid, flat-coated or non-coated tissue culture plastic and are exposed to soluble factors in the liquid growth medium. These plastic culture substrates are usually coated with collagen or laminin, feeder cell layers or with hydrogels such as Matrigel [225]. However, this culture approach is very different from the native microenvironment cells are experiencing *in vivo*, where they are residing in the stem cell niche anchored to ECM through adhesion molecules [225]. Two-dimensional culture approaches provide simplified methods to expand stem cells and to study individual cues influencing their fate decisions *in vitro*. This has allowed the identification, patterning and concentration of soluble to tethered ECM molecules regulating stem cell niche and its inhabitants. ECM arrays have been utilised to screen for molecules individually or in combination that induce fate changes [226, 227]. The development of 3D-culture systems compared to traditional 2D culture systems results in a more rounded cell morphology. Culturing chondrocytes in 2D, for example resulted in a dedifferentiation and change from chondrogenic to fibroblastic phenotype [228], whereas pellet culture or incorporation of chondrocytes in hydrogels maintained their native phenotype [229, 230]. It was further demonstrated that for bone marrow–derived MSCs to undergo chondrogenic differentiation cells would have to be cultured with a spherical shape either as spheroid culture or encapsulated in hydrogels [221, 231–233]. However, when MSCs were cultured in hydrogels facilitating their adhesion and spreading stem cells exhibited a fibroblastic phenotype [234]. **Table 3** provides a summary of studies examining the correlation between surface topography and stem cell differentiation. Oh et al. [235] found that MSCs cultured on ~100-nm nanotubes differentiated towards the osteogenic lineage, whereas MSCs seeded on 30-nm nanotubes did not differentiate [235]. Moreover, surface topography can also cause cell elongation on material surfaces resulting in the distortion of the nuclei shape as the nuclei are mechanically integrated into the cells, which has been shown to influence osteoblastic stem cell differentiation [237]. When ESCs are aggregated into embryoid bodies (EB), which mimic early stages of embryonic development, it becomes clear that geometrical signals also affect cell–cell interaction and hence guide stem cell differentiation [66]. The investigation of hexagonal patterns, random nanopits, disordered and ordered squares utilising on hMSCs electron beam lithography (EBL) showed that highly asymmetric structures resulted in highest osteogenic expression in the absence of growth factor supplemented medium [237] indicating that nano-sized topographical induction of stem cell differentiation is as effective as chemical induction [238]. Micropatterns have also been shown to affect cell shape. Changes from rounded (on small islands) to flattened (on large islands) shapes result in modifications of the actin cytoskeleton and focal

adhesions and hence controlling the lineage commitment of MSC into a variety of cell phenotypes [29].

Cell type	Material	Topographical feature	Result	References
hMSC	TiO ₂	Nanotubes (15, 100 nm)	Cells grown on 15 nm exhibited enhanced integrin clustering, cell spreading, osteogenesis	[332]
hMSC	TiO ₂	Nanotubes (30, 50, 70 and 100 nm)	Osteogenic differentiation promoted on 70–100 nm nanotubes	[235]
hMSC	PDMS	Islands	1000 μm ² facilitated osteogenesis	[29]
hMSC	PDMS	Grafting (350 nm)	Neurogenesis	[333]
hMSC	PDMS	Micropattern, stripped grooves collagen type-I coated	Neuronal differentiation enhanced	[334]
hMSC	PMMA	Hexagonal pattern, nanopits, disordered and ordered squares	Enhanced osteogenesis on disordered squares	[237]
hBMSC	Hydrogenated amorphous carbon	Grooves (80/40, 40/30, 30/20 μm-width/spacing; 24 nm depth)	Neurogenesis, absence of growth factor supplemented medium	[335]
hESC	PDMS	Square shaped fibronectin surrounded by pluronic-F127, micropattern	Myogenesis and chondrogenesis	[336]
hESC	PDMS	Grooves	Neuronal	[337]
mESC	PLLA	Fibrous grating (50–500 nm), TCP	Enhanced osteogenesis on fibrous gratings	[338]

Table adapted with the permission from Griffin et al. [214]

hMCS: human mesenchymal stem cell; hBMSC: human bone marrow-derived stem cells; hESC: human embryonic stem cell; mESC: mouse embryonic stem cell; PDMS: polydimethylsiloxane; PMMA: polymethyl methacrylate; PLLA: poly(L-lactide); TiO₂: titanium dioxide, TCP: tissue culture plastic.

Table 3. Effect of surface nanotopography and stem cell fate.

4.3. Biomaterials affecting stem cell fate

The control of stem cell fate through biomaterial nanotopography is promising as these approaches are not subjected to short-term degradation as are chemical cues. In addition, an

individual biomaterial could exhibit various nanotopographies and gradients which would allow for further clinical applications [214]. Biomaterials are being designed and utilised to mimic the microenvironment and its cues stem cells experience *in vivo*. Nano- and microtopography replicating the ECM signals have been shown to direct stem cell differentiation. ECM components linked to the biomaterials surface in form of small peptides or through the incorporation of growth factors have been successful approaches for guided stem cell differentiation and provide advanced stem cell-based clinical approaches [214, 225]. To overcome limiting factors for the clinical use of pluripotent stem cells, new culture systems based on advanced biomaterials are required which more closely mimic the native *in vivo* milieu and support application related stem cell fate decisions [225]. In summary, simplifying a complex three-dimensional stem cell niche into a two-dimensional biomaterial approach is a potent tool to study control machineries regulating stem cell biology. In order to reconstruct the complexity and interplay of the stem cell niche and its components it is, however, necessary to utilise 3D culture approaches [225]. Hence, there have been numerous strategies to create 3D biomaterial matrices with a variety of structural, chemical and mechanical properties as artificial growth environments for cells each with its advantages and disadvantages [239]. Since the investigation of niche components and their effect on stem cell fate is a complex undertaking, 3D high-throughput approaches similar to 2D ECM protein arrays are being developed [240–242]. These could be produced by printing or liquid-dispensing technologies [263]. For example, 3D PEG gel arrays were prepared to study the effect of gel degradation and cell adhesion ligand concentration [264]. Once ideal biomaterial candidates have been identified, it is essential to examine their *in vivo* performance by implantation into hosts, some of which have been reviewed elsewhere [239].

Biomaterials have also been utilised to study the interplay between stem cells and support cells such as vascular cells, neural cells and stromal cells [225]. Electropatterning of living cells within PEG hydrogels resulted in the deposition of multicellular aggregates of known size and shape, which was shown to regulate biosynthesis of chondrocytes by increase of sulphated GAG in larger cell aggregates [245]. Moreover, 3D angiogenesis was studied combining microfluidics with gel patterning [246]. Gradients are known to be crucial for the regulation of dynamic processes during development and during tissue regeneration and homeostasis. Hence, the development of biomaterial gradients has found wide interest in regenerative medicine [225, 247–249]. It has been shown for bone/marrow [52, 250], prostate [251] and breast [252] that cell–cell interactions in those stem cell niches are influenced by paracrine hormone signalling [197].

Furthermore, cell–cell interactions have been studied in co-culture approaches, which on the other hand, do not allow for the identification of the particular function of soluble or immobilised molecules. *In vivo*, cytokines and growth factors are mostly immobilised to the ECM suggesting that tethered chemical factors are more stable and their signalling is longstanding. So has for example, the immobilisation of FGF2 to a synthetic polymer resulted in an increase in its potency and stability and subsequent enhancement in ECS proliferation and ERK1 activation [253]. Additionally, the tethering of EGF to a biomaterial scaffold has demonstrated an increased effectiveness compared to its soluble equivalent [254]. These studies have

demonstrated that investigating chemical cues individually is informative, but testing growth factors in a high-throughput manner on polymer arrays is more desirable for identifying pluripotent stem cell regulators [255].

4.4. ECM elasticity as stem cell fate regulator

ECM subjects cells to multiple physical signals including mechanical signals due to its stiffness [256–258]. Cells adhering to their surrounding employ contractile forces resulting in tensile stresses within their cytoskeleton [259]. The connection between these forces and ECM stiffness has a huge influence on cell migration [260, 261], proliferation [262] and apoptosis [263] (Table 4). Stem cells have shown to respond to mechanical properties of their surrounding microenvironment [214, 264]. Engler et al. [264] observed that when MSCs were cultured on collagen-coated polyacrylamide hydrogels with varying stiffness, cells differentiation without the use of supplemented medium. On soft gels mimicking brain tissue (0.1–1.0 kPa), MSCs underwent neurogenic differentiation. Stiffer gels resembling muscle tissue enabled myogenic development and very stiff gels (25–40 kPa) replicating the bone properties resulted in osteogenic differentiation of MSC [264]. Similar observations were made by other researchers for two-dimensional and three-dimensional culture [265–269]. MSC incorporated into collagen-GAG scaffolds with stiffness of 0.5, 1 and 1.5 kPa exhibited different chondrogenic differentiation. Softer gels allowed for chondrogenic and stiffer gels for osteogenic differentiation [269]. It is assumed that the substrate stiffness induces alterations in the activity of focal adhesion and causes remodelling, which triggers a cascade of signalling pathways enabling cell differentiation [270, 271]. In addition, integrins are thought to be a central cell structure for sensing mechanical stimulation [270, 271]. A limitation to these studies is the fact that different tissues may have similar stiffness, and hence, it might not be possible to direct stem cell fate by a single mechanical property of the surrounding. Instead, it is necessary to consider a more complex interplay of extrinsic and intrinsic factors influencing stem cell differentiation [197].

Cell type	Material	Mechanical cues	Result	References
<i>ECM stiffness</i>				
MSC	Polyacrylamide gel, collagen coated	Stiffness-0.1–1.0, 25–40 kPa	Lineage commitment according to substrate stiffness; softer gel-neuronal, stiffer gel-osteoblastic differentiation	[264]
ANSC	Interfacial hydrogel	Substrate moduli 0.01–10 kPa	Self-renewal, cell spreading and differentiation inhibited on soft gels (0.01 kPa); cell proliferation and neuronal differentiation maintained on ≥ 0.1 kPa; glial differentiation on gels 1–10 kPa	[339]
hMSC	Polyacrylamide	Marrow and	Cells were quiescent but	[35]

Cell type	Material	Mechanical cues	Result	References
	gel, collagen and fibronectin coated	adipose tissue 0.25 kPa	maintained multilineage potential,	
MSC	Collagen-glycosaminoglycan	0.5, 1 and 1.5 kPa	Softer gels triggered chondrogenic differentiation; stiffer gels resulted in osteogenic differentiation	[269]
<i>Mechanical stimulation</i>				
MSC	Protein-coated membranes	1, 5, 10, 15% cyclic uniaxial stretch	Myogenic differentiation for 5 and 10%; 1 and 15% failure of myogenic lineage	[340–342]
AMSC		10% uniaxial cyclic strain at 1 Hz for 7 days	Decreased expression of myogenic markers	[343]
MSC		Uniform biaxial strain	Enhanced osteogenesis and calcium deposition	[344–347]
AMSC		Pulsatile fluid flow	Enhanced osteogenesis	[348]
mESC		4–12% strain, 1 Hz, 24 h	Differentiation into vascular smooth muscle cells; increase in proliferation	[349]
hESC		Cyclic strain	promoting of self-renewal, inhibition of differentiation,	[350]
MSC	Agarose	Cyclic confined compression	Chondrogenic differentiation	[351]
MSC	Spheroids	IHP	Chondrogenesis	[352]
hBMSC	PCL	IHP, 270 kPa, 1 Hz, over 21 days	Enhanced metabolic activity, upregulation of osteogenic markers and calcium deposition	[295]
FCSC	<i>Ex vivo</i> cultured chick femurs	IHP, 270 kPa, 1 Hz, over 21 days	Enhanced mineralisation	[287]

MSC: mesenchymal stem cells; ANSC: adult neuronal stem cells, AMSC: adipose-derived mesenchymal stem cells, hBMSC: human bone marrow-derived mesenchymal stem cells, FCSC: fetal chick stem cells, kPa: kilo Pascal, Hz: hertz, IHP: intermittent hydrostatic pressure.

Table 4. Effect of ECM stiffness and mechanical stimulation on stem cell differentiation.

4.5. Biomechanical regulation of stem cell fate

The body's cells are constantly subjected to a variety of mechanical stimuli through muscle action, blood flow, gravity and other physical and physiological processes [197]. To ensure tissue health and function, the interplay between cells and mechanical cues is essential. It is assumed that these mechanical factors are involved in diseases including osteoarthritis,

osteoporosis and atherosclerosis [277]. Furthermore, there is mounting evidence that mechanical forces are crucial for development [278, 279] stem cell lineage commitment and fate decisions [197]. Despite early *in vivo* studies by Glückmann et al. [280], Fang and Hall [281], Hall and Herring [282] and Murray and Drachman [283] on the effect of mechanical factors on development little was known on how biomechanical cues affect gene expression and stem cell fate [280–283]. One difficulty in studying these interactions is the complexity by which cells sense mechanical signals. Mechanical loading of tissue can result in a variety of stresses, strains, pressures, fluid flows, osmotic pressures and electric charges on biological molecules [284]. These modifications of the cell's microenvironment result in structural changes of ECM proteins, as well as the activity of immobilised or soluble growth factors. For this reason it is challenging to differentiate the effect of direct mechanical forces *in vivo* from indirectly driven effects in adhesive and paracrine signals and resulting changes such as cell shape [197, 285]. Nevertheless, mechanical forces are influencing cellular processes directly and the mechanism by which cells perceive external mechanical stimuli have been described by Liedtke and Kim [285]. Cellular responses to physical stimuli are not simply a reaction to the input stimuli, instead they are also linked to cytoskeletal changes, ECM interactions and the production of cellular forces [29, 264]. Stem cell response to controlled physical forces as well as the biomechanical mechanism and signalling pathways that direct stem cell lineage commitment are being investigated [286]. Several research groups have attempted to isolate the effect of applied mechanical stimuli such as fluid shear, strain and compression. **Table 4** summarises studies on the effect of externally applied mechanical stimuli and ECM stiffness on stem cell fate. These studies demonstrate that the effect of physical stimulation is dependent on stem cell source, type and state of pre-differentiation. Dynamic mechanical compression, for example increases chondrogenic marker expression of BhMSC. However, ESC-derived EB exhibit downregulation of chondrogenic markers when subjected to the same stimulation regime [197]. It is also under investigation whether mechanical cues provided by bioreactors alone or in combination with other cues can induce lineage commitment in PSC [287, 288].

5. Bioreactor technologies promoting mechanical stimuli

As elucidated, earlier physical forces *in vivo* are essential cues during development and for regulating stem cell fate. Furthermore, it has been widely acknowledged that *in vitro* mechanical cues (e.g. stress, strain, shear, compression, hydrostatic pressure) are greatly influencing the cell morphology, cell adhesion, proliferation and gene regulation [214, 289]. In order to provide physiological relevant growth environments for cells and tissue-engineered constructs, various types of bioreactors have been developed [287, 290]. Bioreactors are devices that utilise mechanical forces to influence biological processes under closely controlled and monitored conditions [291, 292]. They offer biophysical cues encouraging cells to differentiate or/and produce ECM prior to implantation *in vivo* [293]. In general, bioreactors meet the following applications in regenerative medicine-1: they provide spatially uniform cell distribution, 2: they deliver physiological relevant concentrations of oxygen and carbon dioxide as well as nutrients in the culture medium, 3: they support mass transport to the core

of the tissue engineered construct facilitating cell survival throughout tissue-engineered constructs, 4: they provide physical stimuli to regulate stem cell differentiation and proliferation, 5: they facilitate tissue development and accelerate construct maturation [287, 294–297]. Studies utilising mechanical forces for the direction of stem cell fate and tissue development are summarised in **Table 4**.

5.1. Bioreactor designs and their application for tissue regeneration

Many attempts in regenerative medicine employ traditional cell culture approaches. These, however, are associated with numerous disadvantages. To overcome limitations in cell culture techniques and to provide mechanical stimulus for tissue development, various types of custom-made and commercially available bioreactors have been manufactured [293] (**Figure 7**).

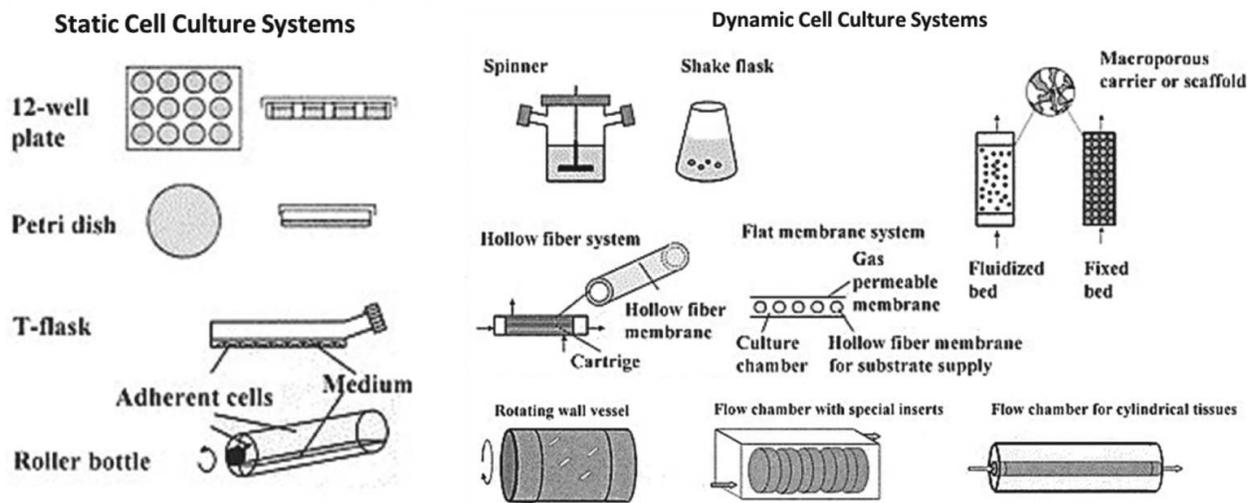


Figure 7. Static and dynamic cell culture systems. Traditional cell culture systems include well plate formats, petri dishes, T-flasks and roller bottles (left). To overcome disadvantages of static culture dynamic culture systems have been developed such as spinner flasks, rotating vessels, hollow fibre systems (right). Image adapted with the permission from Pörtner et al. 2005.

5.1.1. Rotating wall bioreactors

The rotating wall vessel bioreactor was originally developed by Schwarz and colleagues at Nasa’s Johnson Space Centre to protect cell culture experiments from high forces during space shuttle take-off and landing [290, 298]. This bioreactor consists of a cylindrical chamber in which the scaffolds are free to move within the cell culture media. A permeable membrane is inserted in the vessel wall to allow for sufficient gas exchange. The vessel is rotated so that the downward gravitational force and the upward hydrodynamic force are balanced so that the scaffolds remain suspended in the media experiencing microgravity. This bioreactor has been utilised for regenerative studies on bone, cartilage, human ovarian surface epithelial cells among others [299–301].

5.1.2. Flow perfusion

A fluid pump is used to pump media through a sample chamber which is designed so that fluid flow is directed through the centre of cellular scaffolds [293]. Superior fluid transport was obtained for flow perfusion bioreactors when comparing spinner flasks, rotating wall bioreactors and flow perfusion bioreactors. For the comparison of the three bioreactor types, consistent flow rate, scaffold type and cell densities were utilised resulting in homogenous cell distribution throughout the scaffold only for the flow perfusion bioreactor [302, 303]. In order to obtain optimum experimental outcome, fluid flow rate needs to be adjusted to facilitate a balance between mass transfer of waste products and nutrients and to retain newly produced ECM [293].

5.1.3. Compression bioreactors

Compression bioreactors are commonly used for cartilage and bone tissue engineering and can be adjusted for both dynamic and static loading as studies have exhibited shown that dynamic loading is more suitable for certain tissues such as cartilage, but negative for others [291, 292]. A compression bioreactor commonly consists of a motor, a controlling mechanism providing various magnitudes of displacements and frequencies as well as a tool to provide linear motion [304]. Evenly distributed load is applied to cellular scaffolds via flat plates [305].

5.1.4. Strain bioreactors

Strain bioreactors are normally utilised for the development of tendon and ligament tissues, but also for bone, cartilage and cardiovascular tissues [293]. The bioreactor design is similar to compression bioreactors whereas instead of applying force through flat plates, scaffolds are held in place via clamps when tensile force is employed.

5.1.5. Hydrostatic pressure bioreactor

Hydrostatic pressure bioreactors have been utilised for the development of bone and cartilage tissues among others. Professor El Haj's group has developed a novel hydrostatic bioreactor allowing for ease of handling and scale up of sample numbers that can be mechanically stimulated simultaneously [287, 288] (**Figure 8**). This bioreactor system is composed of a sealed aluminium chamber suitable for standard tissue culture well plates. By compressing incubator air and subsequently pumping it into the bioreactor chamber, hydrostatic pressure is applied to samples creating a gas-liquid interface between cell culture medium and the air. A temperature controller maintains the temperature of the compressed air at 37°C. The gas phase is removed from the bioreactor chamber, pumped back into the incubator and reused for the next stimulation cycle. A system accompanying software controls continuous and sinusoidal waveforms at various pressures (0–280 kPa) and frequencies (0.0001–2 Hz).

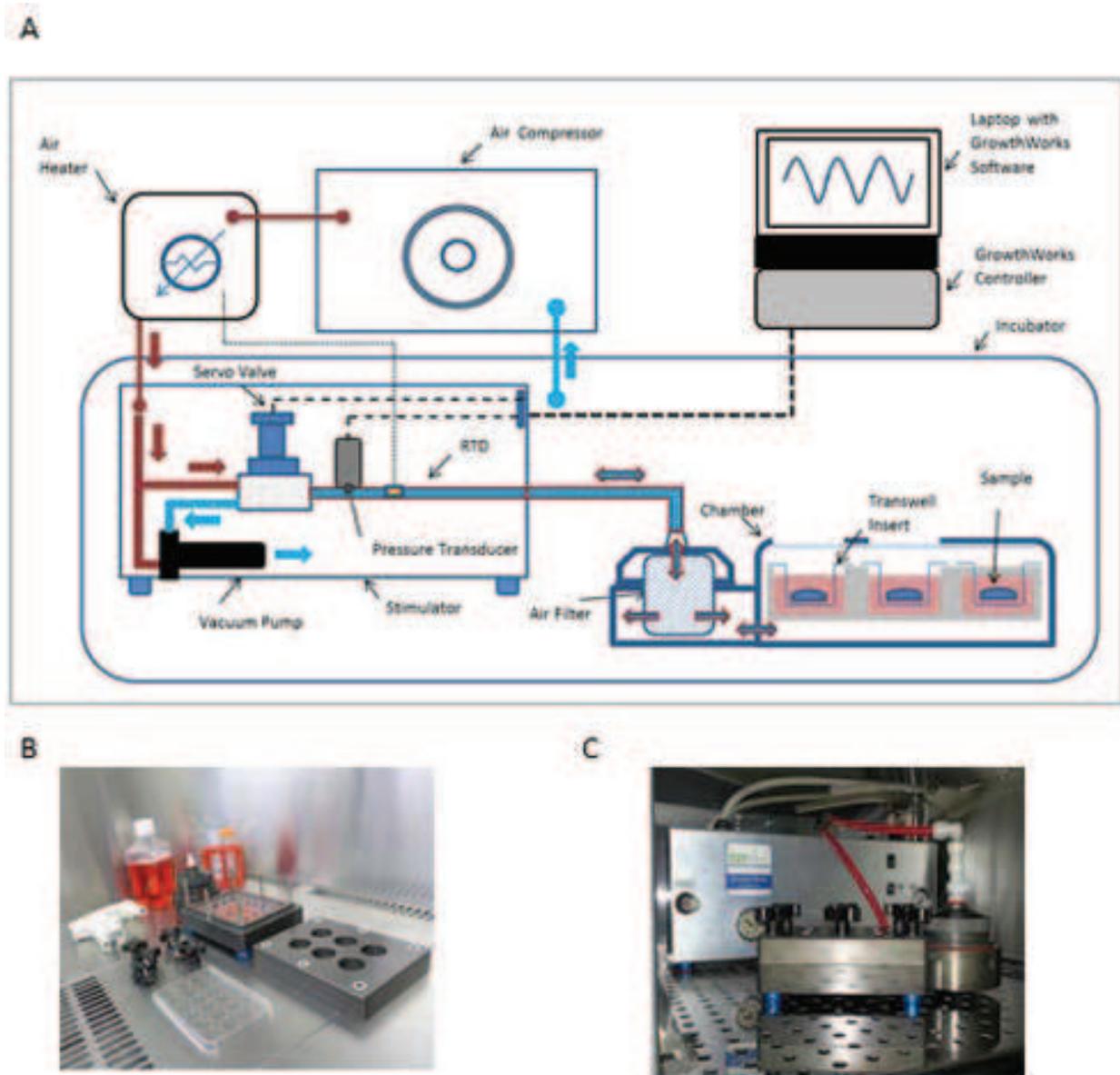


Figure 8. Hydrostatic force bioreactor. The bioreactor system (A) consists of a compressor, computer for monitoring of applied pressures and a bioreactor chamber (B) fitting standard tissue culture well plates, which is connected to a stimulator within a standard cell culture incubator (C). Image was adapted with the permission from Reinwald et al. 2015 [287].

Pluripotent stem cells are interesting cell types due to their capability to differentiate into numerous cell types. A limitation, however, is that hPSCs develop their functionality at later stages in development. At present, protocols yield in differentiated cells that match the embryonic stages of development. Protocols are needed that result in cells with adult-like functionality [65]. The investigation of signalling pathways controlling cell development has led to successful strategies to direct stem cell fate [306], and small molecules have been utilised for the creation of differentiation protocols [202, 203, 307, 308]. In addition, bioreactors have been successfully employed to direct stem cell fate [288, 295]. Recently, a collaborative

approach between Manchester University and Keele University started to investigate the effect of hydrostatic pressure on the maturation of hESC-derived chondroprogenitors and hMSC. Chondroprogenitors were obtained following a defined 2 weeks of differentiation protocol (Figure 6A) [202, 203]. To enhance maturation of these cells and to differentiate hMSC towards chondrogenic lineage, they were cultured in growth factor supplemented medium as either spheroids or embedded in fibrin hydrogels and subjected to intermittent hydrostatic pressure at 270 kPa, 1Hz for 1 h daily. Results suggested that a combination of mechanical and chemical cues resulted in the production of matrix proteins collagen and GAG as well as the upregulation and maintained expression of chondrogenic markers Aggrecan and SOX9 (Figure 9).

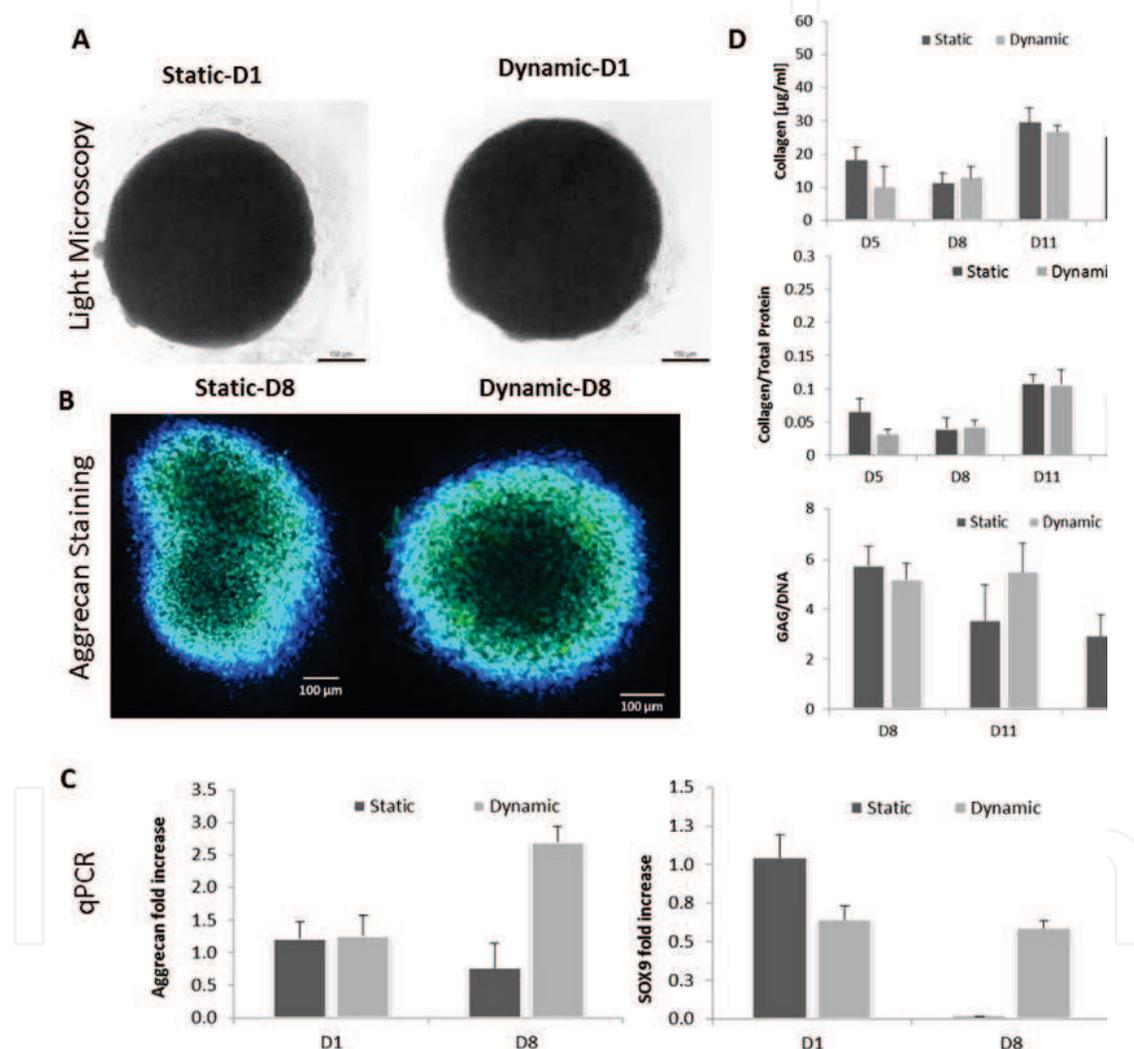


Figure 9. Stem cell differentiation was promoted by mechanical and chemical cues. hMSC spheroids (A) were cultured in growth factor supplemented medium either under static conditions or when subjected to hydrostatic pressure at 270 kPa, 1 Hz and 1 h daily over a period of 14 days. Positive immunocytochemistry staining for Aggrecan was observed for both culture conditions (B). To quantify changes in the expression of chondrogenic markers, Aggrecan and SOX9 quantitative polymerase chain reactor was performed revealing 2.5-fold Aggrecan upregulation and maintained SOX9 expression compared to static controls ($n = 5$). Increased levels of matrix proteins collagen and GAG and total protein were detected from biochemical assays (D) ($n = 5$). Scale bars = 150 μm (light microscopic images) and 100 μm (immunocytochemistry stains).

6. Conclusion

This chapter highlights the valuable role of multipotent and pluripotent stem cells and the importance of replicating their niche *in vitro* to develop novel regenerative therapies. Despite the ethical and safety concerns associated with PSC, they offer valuable differentiation and proliferation potential and could possibly offer a valuable cell source for clinical applications.

The stem cell niche influences stem cell fate in many ways, including mechanical support, elasticity, topography, biochemical signals, oxygen tension and cell communication. By artificially engineering the dynamic stem cell niche, stem cell fate can be directed. Advanced 3D biomaterials are being harnessed to mimic the *in vivo* environment by providing physical and chemical support and by allowing for cell–cell interactions. Bioreactor technologies are able to replicate the mechanical stimuli and mimic the physiologically relevant environment.

Whilst promising advances have been made in regenerative medicine, significant obstacles have been identified and these must be overcome before novel cell and tissue engineering therapies are clinically established.

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