CHAPTER

# 1

# The Need to Study, Mimic, and Target Stem Cell Niches

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OUTLINE



## 1. INTRODUCTION

### 1.1 The Stem Cell Niche in Health and Disease

As opposed to single-celled organisms, cells in complex multicellular organisms are associated with a tissue-specific physiological environment. Different cell types differ in morphology and function; yet, they are genetically identical. This variation, caused by differential gene expression, is controlled by intrinsic mechanisms and by extrinsic signals from the local environment, thereby controlling distinct cellular behavior, or "phenotype." The local physiological microenvironment supporting the cell and driving extrinsic cues from outside the cell is known as the "cell niche," which is composed of extracellular matrix (ECM) components for attachment/anchorage, diffusible biomolecules for cell signaling, cell surface ligands for signal transduction, and essential cell–cell interactions.

Studies of cell populations during embryonic development have led to the identification of stem cells that possess the capacity to produce a full organism from a fertilized egg.[1] Stem cells are functionally defined as undifferentiated embryonic or adult cells, which can self-renew and generate differentiated cell types with varying degrees of potency. The fundamental replicative feature of stem cells, along with their generation of differentiated progeny, accounts for the origin of the

word "stemness." However, whether stem cells need a special environment that controls stem cell renewal, maintenance, and survival, and what is the nature of such microenvironment are pertinent questions many researchers continue to explore. With growing evidence, there is a growing consensus that in vivo function and the fate of stem and progenitor cells are regulated by the interplay of various extrinsic signals of tissue-specific microenvironments, often referred to as "stem cell niches."

The concept of a stem cell niche was first proposed by Schofield in the late 1970s as a physiologically restricted microenvironment that supports stem cells.[2] The initial concept of anatomically distinct sites that regulate hematopoietic stem cell (HSC) activity and self-renewal was later extended to acknowledge the discovery of stem cells and their niches in multiple tissues.[3] Stem cells are often linked with asymmetrical cell division, and the niche maintains a stable number of stem cells during homeostasis, and removal of the niche induces differentiation. Extrinsic signals interact and integrate to ensure that one cell remains in the niche, while another escapes it by receiving a differentiation signal. It is now clear that in high-turnover systems, such as in the gut and blood, the behavior of stem cells is not uniformly quiescent, and the various niche components may govern their relative proliferative activity.[4–6] Also, it is emerging that stem cell performance is not only dependent on factors promoting stemness but is also a result of factors inhibiting differentiation pathways. Hence, in homeostasis, the underlying relationship between stem cell and niche accommodates nuances and involves various elements influencing the stem cell functional parameters: replicative capacity and potency. However, when tissue is injured or diseased, the niche actively engages stem cells; guides their proliferation, migration, and differentiation; and regulates their participation in tissue regeneration and repair. Therefore, the niche should be regarded as a dynamic participant controlling stem cell number, fate, and behavior in the health and disease of the tissue and the organism.

## 1.2 Components of Stem Cell Niche

The stem cell niche is a complex, heterotypic, and dynamic structure, which includes supporting ECM, neighboring niche cells, secreted soluble signaling factors (such as growth factors and cytokines), physical parameters (such as shear stress, tissue stiffness, and topography), and environmental signals (metabolites, hypoxia, inflammation, etc.) (Fig. 1.1).[7,8] Stem cell niches are highly innervated and densely vascularized, thus are directly or indirectly influenced by vascular and neural inputs.

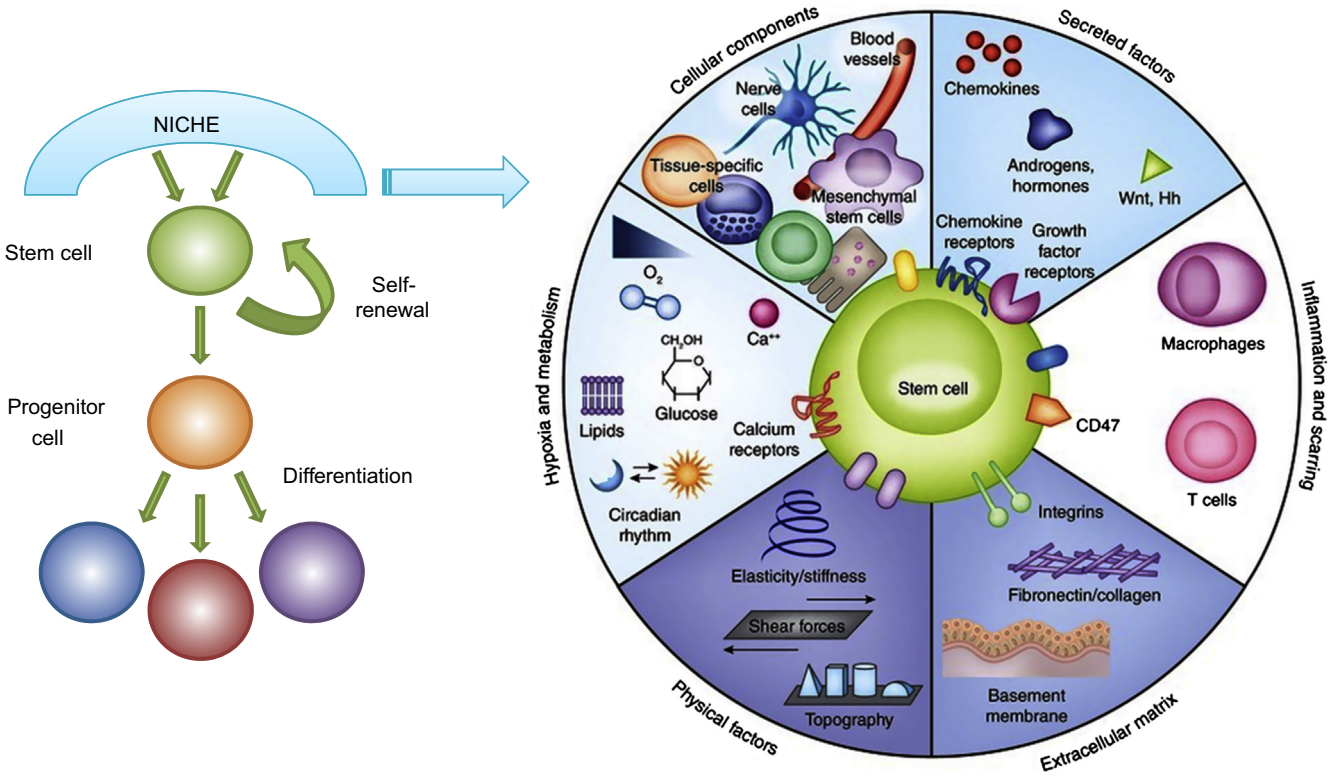


FIGURE 1.1 Components of stem cell niche. *Adapted from Lane SW, Williams DA, Watt FM. Modulating the stem cell niche for tissue regeneration.* Nat Biotechnol *2014;**32**(8):795–803.*

In addition to matrix and cell signaling elements mentioned above, niche cells form functional units within the stem cell niche. These are neighboring tissue-specific stem or somatic cell populations that interact with resident stem cells to regulate cell fate. For example, mesenchymal stromal/stem cells in the HSC niche or parenchymal hepatocytes in liver. In addition to stem cell themselves, niche cells provide a source of physical and biochemical signals within the niche microenvironment by building extracellular matrix and producing cell surface or soluble signaling factors.

Importantly therefore, stem cell microenvironments are highly dynamic and display temporal variations. Such variations in direct cell–cell contacts and ECM components, as well as their interaction with regulatory molecules secreted by stem or niche cells and the spatial organization of niche components, ultimately enable the regulation of stem cells to render tissue homeostasis and regeneration.[9]

## 2. BIOLOGY OF THE STEM CELL NICHE

### 2.1 Behavior of Stem Cells: Hierarchical Versus Stochastic Model

Understanding developmental biology is an important approach to fully comprehend the structure and function of the human body developed from a single totipotent stem cell, the zygote. The potency of a given cell to differentiate into many specialized cells is defined by the degree of its plasticity and versatility at various stages. Totipotent stem cells are those with the greatest differentiation potential and can differentiate into any and all cells in an organism, plus the extraembryonic or placental cells. Pluripotent stem cells can differentiate into any cell within the three germ layers (endoderm, mesoderm, and ectoderm). Embryonic stem cells (ESCs) are pluripotent and can divide and differentiate into cells of various types found in the body. Multipotent stem cells are progenitor cells that can differentiate into numerous cell types but within a similar "family" or lineage. Lastly, unipotent stem cells, the most restricted precursor, can only result in one cell fate. Unlike ESCs, stem cells from adult tissues are multipotent or unipotent.

During development and in the healthy body, stem cells can divide to produce new cells. This is a carefully controlled process that allows the body to grow and to replace lost or damaged cells during adult life. For the body to maintain homeostasis, stem cells proliferate before differentiating into a specific lineage, such that the generation of differentiated cells and the maintenance of stem/progenitor pools are balanced. Two distinct models have been proposed to explain the lineage choices of stem cells (Fig. 1.2). The hierarchical model suggests a discrete arrangement of cells consisting of slow-cycling stem cells that can self-renew extensively, which also give rise to short-lived transit amplifying progenitor cells that then further differentiate into committed nondividing cells. The stochastic model suggests that each stem cell chooses at random between self-renewal and differentiation. In this model, each individual clone will vary in size.

Recent lineage tracing studies have supported the findings of the hierarchical model of stem cell behavior,

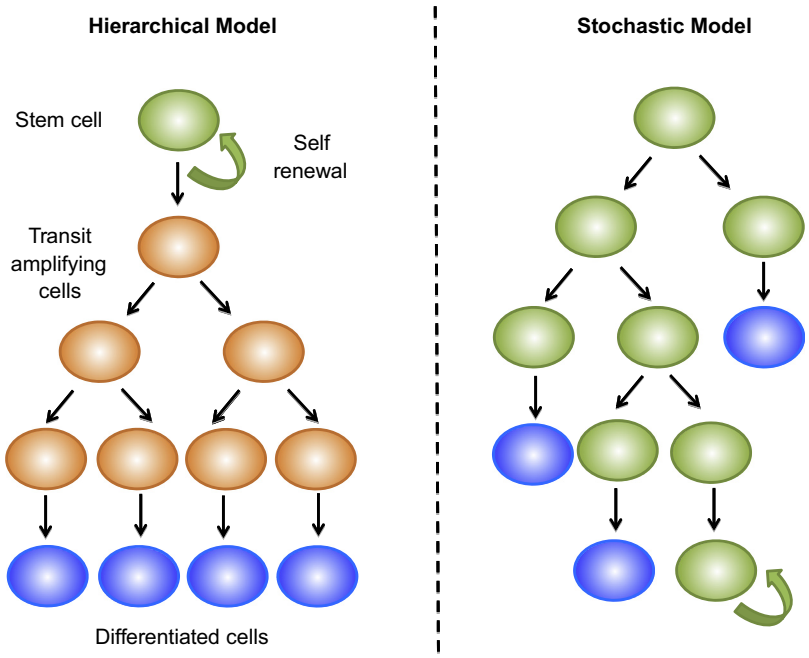


FIGURE 1.2 Hierarchial versus stochastic model for behavior of stem cells.

yet also support that progenitors can behave in a stochastic manner.[10,11] Different types of stem cells at various body sites with distinct niches may work in different ways, and it is possible that both these theories are correct. In addition, based on recent discoveries, it has been proposed that the stem cell niche has a bicompartmental organization[12]: one compartment that engages in immediate, rapid new growth and one that contributes later to long-term growth. In this way, stem cells might work cooperatively with their progeny to sustain tissue regeneration. Although the precise mechanisms mediating these phenotypic changes may be complex, these lineage tracing studies demonstrate that the niche is a critical component for the stem cells.

## 2.2 Embryonic and Adult Stem Cell Niches

The first example of a stem cell niche was demonstrated by Kimble and White in 1981, who showed that the mesenchymal distal tip cell provides the essential microenvironment for the maintenance of the germ line stem cell in *Caenorhabditis elegans*.[13] Later, *Drosophila melanogaster* became the best characterized system to analyze the relationship between stem cells and their niche through studies in *Drosophila* germline stem cells.[14] These studies have provided an extensive understanding of the molecular basis of stem cell niche regulation. Meanwhile, several niches of mammalian adult tissue stem cells were discovered, for example, the HSC niche in bone marrow, hair follicle stem cells at the follicle bulge, and skin interfollicular epidermis stem cells located in clusters at the tops of rete ridges in the basal layer,[15–17] among others. This book covers in-depth many of the studied examples of embryonic and adult stem cell niches, along with molecular mechanisms, in Section 1.

Schofield's 1978 mammalian stem cell niche hypothesis was based on the observation that colony-forming stem cells were less capable of replacing blood cells compared with bone marrow cells when injected in vivo into irradiated animals.[2] Since his discovery, the field of bone marrow HSCs has exploded. Schofield's study postulated that HSCs reside in specific loci of bone marrow and receive support from multiple cellular components of their microenvironment. Since then, there have been several studies that aimed at recognizing such components and determining interactive signaling mechanisms of the HSC niche.[18–20] **Chapters 4 and 5** comprehensively review how microenvironmental cues from the bone marrow and intrinsic signals from the HSC dictate its fate to remain quiescent, become active, differentiate, migrate, or participate in regeneration. These two chapters detail advances in genetically modified mouse models and high-resolution, real-time imaging to identify HSC niche components and the molecular signaling emanating from them. They discuss recent findings on how the niche network maintains different states of HSCs by providing multiple signaling inputs from different cellular sources during homeostasis and mobilization/homing.

Compared with other somatic stem cell microenvironments described since the late 1970's, the microenvironment of pluripotent cells had not been characterized until relatively recently. This is largely because of the transient nature of pluripotent cells in vivo and the characterization of their regulation being restricted to in vitro methods. Also, pluripotent cells seem to be mutually interacting with their neighboring cells, which are dynamically changing in a short period of time relative to somatic stem cells. **Chapter 3** describes the microenvironment in early embryogenesis, which provides robust information about cellular interactions required for the acquisition of a pluripotent state. It also describes the transcriptional and functional heterogeneities observed within established ESC cultures and their emerging significance for self-renewal and maintenance of differentiation potentials of ESCs.

Mesenchymal stem cells (MSCs) are multipotent stem cells found in stromal or connective tissue and are distinct from stem cells found in parenchymal or "functional" tissue. The MSC niche will not be discussed in this edition of the book, since our understanding of what constitutes an MSC niche at different tissue sites is limited due to few in vivo studies and heterogenous population variation of MSCs in vitro. Further chapters in this section will discuss stem cell niches in specific organs or organ systems.

In the adult mammalian brain, the two major stem cell niches that support neurogenesis are the ventricular–subventricular zone that lies along the lateral walls of the lateral ventricles and the dentate gyrus subgranular zone found within the hippocampus. **Chapter 6** focuses on the development of the brain's neuronal stem cell niches and further reviews key characteristics and molecular regulators, including molecular pathways that help support the mammalian brain's two neurogenic stem cell niches.

Stem cell niches play a critical role in cardiac homeostasis and myocardial repair after injury by replication of a self-sustained pool of preexisting immature myocytes. In the myocardium, cardiac progenitor cells are clustered in interstitial microdomains with an architectural organization distinct from the surrounding non–stem cell tissue. The structural and molecular properties of this niche condition the response of the tissue to physiologic and pathologic cues by creating a favorable environment for the interaction of stem cells with the surrounding cells and the interstitial space. **Chapter 7** emphasizes the relevance of cardiac progenitor cells and their niches to cardiomyogenesis. Also, the chapter discusses alternative stem cell–independent

mechanisms that may account for the global replenishment of lost cardiomyocytes.

The adult intestinal epithelium represents one of the most suitable models to study tissue stem cells in vitro. It is a rapidly self-renewing tissue, and the design of its basic unit, the crypt–villus, is highly stereotypical. Since the discovery of $Lgr5^+$ stem cells at the base of intestinal crypts, it has become possible to unveil the molecular mechanisms that control the homeostasis of these stem cells, including the extrinsic signaling cues from the crypt niche. Based on these insights, in vitro propagation of murine and human intestinal stem cells in the form of ever-expanding organoids has also become possible.[20a] **Chapter 8** discusses such recent developments in the field of the intestinal stem cell niche. Additionally, the chapter focuses on a three-dimensional (3D) organoid culture system, originally established for murine small intestine that has now been adapted for other types of tissue stem cells by adding additional small molecules and/or growth factors. Organoids offer numerous possibilities for the study of basic research questions on tissue development and maintenance, stem cell characteristics, the detailed description of endogenous niche factors, gene function, etc. Furthermore, in vitro organoids have the potential to contribute to drug development and hold promise for regenerative medicine, through the possibility to transplant these organoids back into their original organs.

Skin is a primary protective barrier and is being constantly renewed. As such, skin stem cells play a key role in maintaining epithelial homeostasis, similar to the intestinal epithelium. Likewise, $Lgr5^+$ cells in hair follicles has been show to comprise an actively proliferating and multipotent stem cell population.[20b] **Chapter 9** describes the location and cellular hierarchy of each epithelial stem cell of the skin within the epidermis, hair follicle, sebaceous gland, and sweat gland and highlights the intrinsic regulators, which maintain their stemness. The chapter reviews the extrinsic regulators of epithelial stem cell function and sheds light on recent findings that introduce new actors in the epithelial stem cell niche in the skin. Also, it discusses how the niche components in the skin may vary depending on the body location.

In the skeletal muscle, a specialized population known as satellite stem cells contribute to its regenerative capacity. Owing to their ability to generate both stem cells and committed myogenic progenitors, satellite stem cells allow self-renewal of the satellite cell reservoir and provide myogenic progenitor cells to repair the muscle tissue. **Chapter 10** highlights the indispensable role of the satellite cell niche in the regulation of the stem cell functions. This niche maintains the muscle stem cell in a quiescent state; however, in response to muscle injury, the niche actively generates signals for satellite cell activation, proliferation, and differentiation. The chapter describes the cross talk between satellite cells and their niche along with regulation of satellite cell functions, namely commitment and self-renewal, in resting, injured, and pathologic muscle.

Cancer stem cells (CSCs) reside in a microenvironment that comprises various other cell types such as tumor-associated endothelial cells, mesenchymal cells, and immune cells. In addition to these nonmalignant cells, ECM, metabolites, endocrine signals, waste products, and other secreted factors contribute to the proliferation, survival, and dissemination of tumor cells.[21] It is clear from recent reports that the niche plays an important role in triggering stem cell–like programs in subpopulations of cancer cells and at each different stage of tumorigenesis such as tumor initiation, progression, metastasis, and drug resistance. **Chapter 11** reviews the current knowledge on the CSC niche and its contribution to tumorigenesis.

Understanding stem cell–niche interactions requires the elucidation of a complex microenvironment with possibly hundreds of biochemical and biophysical cues acting in concert, perhaps synergistically, to control stem cell fate. The chapters in the following section focus on key niche factors that regulate stem cell behavior.

## 3. BIOCHEMICAL AND BIOPHYSICAL REGULATION OF STEM CELL BEHAVIOR

### 3.1 Extracellular Matrix and Biochemical Cues

ECM is a natural substrate manufactured by the cells themselves providing multiple biochemical and biophysical cues to stem cells residing on or in it, thus maintaining stem cell pools. It is a mixture of long biopolymers consisting of proteins (e.g., collagen, fibronectin, vitronectin, elastin, and laminin) and glycosaminoglycans (e.g., heparin, chondroitin sulfate, keratin sulfate, and hyaluronic acid). ECM was once considered an inert support structure, but research has revealed it to be a signaling core, with a critical role in the niche for developing and maturing stem cells. Matrix provides the necessary chemical and mechanical signals to modulate cellular processes such as cell renewal, morphogenesis, differentiation, repair, and homeostasis. Stem cells bind to matrix adhesion ligands by integrins and other receptors localized in their plasma membrane. Integrins are heterodimeric proteins consisting of $\alpha$ form and $\beta$ form, and the combination of their subtypes, such as $\alpha1\beta1$, specifies the type of ECM proteins they bind (collagen or laminin in case of $\alpha1\beta1$, for instance) and provides intracellular signals, leading to cellular responses.[22] In addition to the effect of cell–matrix adhesion on directing the behavior of cells, cells are also able to degrade and remodel matrix by providing enzymes into their surroundings, such as

metalloproteinases.[23] Thus, the communication between cells and matrix is bidirectional and dynamic. Furthermore, matrix is able to provide biochemical cues to cells effectively, because it sequesters various biomacromolecules, such as growth factors, through specific and nonspecific bindings, and makes residing cells more accessible to these molecules.[24] Cells recognize and respond to secreted signaling factors through multiple pathways: for instance, cells express receptors to their agonists in their microenvironment and initiate intracellular signal transduction once binding between a receptor and an agonist is established.

The chemical composition of the ECM can vary widely among tissues. Fig. 1.3 shows four different stem cell niches, together with their cellular and ECM components. ECM molecules playing major roles in the different niches are indicated. Looking closely at the matrix, it is possible to identify the major structural and chemical entities as patterns and combinations of functional groups, present in proteins, proteoglycans, and peptide sequences. To date, many studies have examined the relationship between stem cells and matrix in vitro using biomaterial scaffolds that mimic the natural ECM.[26] In a particularly interesting approach, researchers applied printing techniques using automatic pipettes or robots to generate a library of artificial niches in a single experiment.[27] Another group created microarrays of artificial niche components comprising ECM proteins for probing single stem cell fates in high throughput.[28] These studies clearly demonstrated a robust proof-of-concept that adding known ECM molecules or functional groups to synthetic

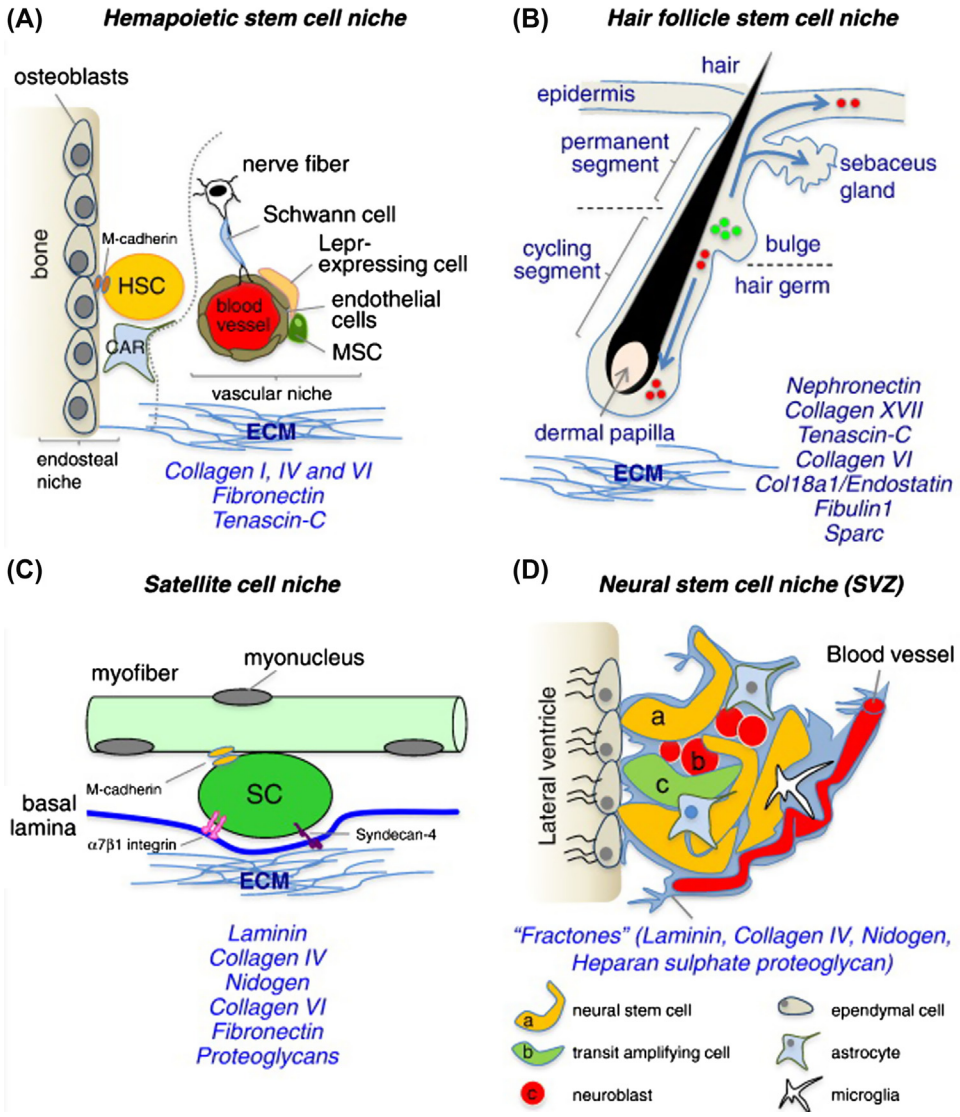


FIGURE 1.3 Extracellular matrix composition of four different stem cell niches. *Adapted from Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche.* Biochim Biophys Acta (BBA) *2014;**1840**:2506–19.*

constructs enables the development of controlled environments regulating stem cell behavior. It is critical to further understand such relationships to fully appreciate the effect of chemical functional groups in the stem cell niche on cell behavior. The small molecular patterns found throughout the body can have a significant influence on stem cell behavior when found in high numbers within a niche. Studying each unit in isolation helps to delineate their individual effects on the stem cells. **Chapter 13** discusses some of the chemical functional groups that have been extensively investigated to understand their role in modulating stem cell behavior. In addition to matrix in the extracellular surrounding, niches may consist of cells, either a single type or a range of interacting multiple cell types. Cell–cell communication can be mediated by interactions via direct contact between cells carrying distinct functional group, such as cadherin-based adherens junctions in epithelial cells and endothelial cells, affecting their behavior. The prospect of replicating the complex ECM chemistry using engineered natural or synthetic biomaterial substrates would be highly valuable, not only to understand complex synergies guiding stem cell behavior but also to develop novel translational technologies, such as regenerative scaffolds.

## 3.2 Soluble Growth Factors and Ligands

Niches contain secreted or cell surface factors that can be secreted by stem cells themselves or by other cells that reside in the niche. Cellular communication employs different sets of secreted soluble hormones, growth factors, and cytokines to regulate cellular functions, including proliferation, differentiation, or migration. A few key biomolecules that control stem cell renewal, maintenance, or survival include Notch, Wnt, fibroblast growth factor, epidermal growth factor, transforming growth factor-β, stem cell factor, and chemokine families.

A vast majority of the currently applied methods for ex vivo stem cell expansion, maintenance, and differentiation rely on the addition of soluble microenvironmental components, namely cytokines and growth factors. The action of signaling molecules is highly interconnected and activation steps downstream of receptor binding can depend on the mode of factor presentation. Hence, it is important to consider their pleiotropic actions in the design of engineered stem cell microenvironments. Contrary to the conditions of standard in vitro cell culture, growth factors do not randomly diffuse in vivo but are spatially and temporally organized by the neighboring cells and the ECM within the niche. Also, many ligands that influence stem cell fate decisions in vivo are tethered to the cell membrane or reversibly bound to the ECM. Thus, the control of the spatiotemporal presentation of soluble signaling molecules and insoluble, matrix-bound cues is essential for effective stem cell bioengineering schemes and critically depends on the development of advanced, multi-biofunctional biomaterials. **Chapter 14** summarizes biomaterials-based approaches for the spatial and temporal control of factor and ligand presentation. The chapter focuses on the presentation of growth factors from versatile 3D polymeric matrix platforms for the biomimetic presentation of multiple molecular effectors and for the adjustment of physical constraints.

## 3.3 Physical Cues and Matrix Mechanics

It is now well understood that not only the biochemical properties of the ECM but also the biophysical properties of the microenvironment—stiffness and elasticity, electrostatic charges, wettability, and structural information—regulate cellular functions. One of the biophysical properties that has been intensively studied is the stiffness of the microenvironment. A variety of cells recognize the stiffness of their surroundings and respond to this. For example, MSCs become quiescent on polyacrylamide gels with a stiffness of approximately 200 Pa, which is comparable with the stiffness of bone marrow.[29] Myocytes and cardiomyocytes cultured ex vivo exhibit striation, when they contact polyacrylamide gels of which the stiffness mimics the stiffness of skeletal muscle or heart tissue, respectively.[30,31] The lineage of MSC differentiation is also affected by the stiffness of the microenvironment: MSCs are able to differentiate into adipocytes most effectively on approximately 200-Pa polyacrylamide gels, which is the stiffness of adipose tissues,[29] whereas osteogenic differentiation becomes more effective as the stiffness of polyacrylamide gels increases.[32,33] Thus, mimicking the stiffness of the microenvironment, represented by the stiffness of tissues in many cases, may be a prerequisite for stem cells to reenact in vivo cellular functions. Along with stiffness, diverse substrate patterns generated using innovative fabrication processes have also been shown to alter stem cell behavior by applying mechanical constraints on cells. They can be regulated by careful design of the microscale and nanoscale features of the substrate geometry. **Chapter 15** focuses on the recent advances in exploitation of mechanical stimulations to differentiate stem cells. It also discusses several mechanisms that underlie the stem cell's response to mechanical stimuli, including changes in the cell cytoskeleton, nuclear alterations affecting gene expression, and cell adhesion site reconfigurations. Each of these biophysical elements mediates mechanical forces, and together guide cell behavior, organization, and differentiation.

Interfacial wettability and its effect on cellular behaviors were first proposed in a study by Lampin in 1997, which established a correlation between matrix wettability and cell adhesion and migration.[34] Over the years, many wettability-mediated matrices have been fabricated to modulate a wide range of cellular functions from cell adhesion and proliferation[35] to cell pattern.[36] The matrix wettability effect on stem cell behaviors can be extremely dependent on the cell type. To better understand and evaluate the interfacial wettability stem cells need according to their types, **Chapter 16** discusses how interfacial wettability affects stem cell adhesion, proliferation, and differentiation.

Stem cells, like several other cell types, are subjected to fluid flow in the body. In particular, stem cells in vivo experience shear and chemotransport from fluid flow within mechanically active tissues and while migrating from niches to homing targets in the body. **Chapter 17** introduces this concept describing fluid flow devices used for cell stimulation. It further discusses fluid flow mechanical stimulus applied to stem cells as a regulator of proliferation and quiescence, differentiation into various lineages (osteogenic, cardiovascular, neural, etc.), migration, and tissue remodeling, with a brief discussion of mechanically active pathways.

### 3.4 Oxygen and Metabolism

Of the many metabolic factors influencing cell behavior, oxygen tension in the cellular microenvironment plays a pivotal role, serving as both metabolic substrate and a signaling molecule regulating stem cell fate. In vivo, low oxygen tension or hypoxia is a common feature of stem cell niche shared among different types of stem cells and linked to their plasticity. **Chapter 18** summarizes the physiological relevance of hypoxia in regulating stem cell metabolism and biological properties, including self-renewal, multipotency in differentiation, ischemic resistance, cellular senescence, and paracrine secretion. It also discusses hypoxia preconditioning as a therapeutic strategy to enhance efficacy during stem cell transplantation under the context of disease treatment, including stroke, ischemic heart injury, and kidney injury.

### 3.5 Immune Cells, Inflammation, and Immunomodulation

Immunological cells provide dynamic biochemical regulation of the stem cell niche during homeostasis. The presence of innate and adaptive immune cells to help maintain the integrity of the stem cell microenvironment is best characterized in the bone marrow. The HSC niche is laden with immune cells implicated in complex cell, ECM, and cytokine interactions, which is vital for the development of the lymphohematopoietic system.[37–39] Researchers are also now able to reveal specific immune regulatory elements involved in regulating marrow function in concert with stromal cells. For example, macrophages have been implicated in HSC mobilization through the production of granulocyte-colony stimulating factor,[40] whereas neutrophils are seen to indirectly influence the MSC niche through macrophages.[41] Furthermore, inflammation in response to injury causes a transient increase of immune cells in tissues to protect against pathogens and promote tissue healing. The transient stem cell–immune niche interactions mediate endogenous tissue repair and regenerative mechanisms.[42] The function of immune cells can be modulated to promote stem cell function in cases of continuous tissue injury and scarring. Application of immunomodulation remains an interesting aspect of tissue regeneration strategies.[43] Designing and controlling for this is a current challenge, and greater appreciation is needed between the interactions of the immune system, the cells involved in tissue healing, and biomaterials.

## 4. MIMICKING THE STEM CELL NICHE: BIOENGINEERING TOOLS AND TECHNIQUES

The stem cell niche is a complex, dynamic microenvironment, which is best characterized in an in vivo model. However, it is this complexity that makes understanding the specific function of individual niche components difficult. At the same time, decoding the effect of specific niche components at the single-cell level is challenging. Studying stem cells clonally, as individual cellular entities able to self-renew and differentiate, is emerging as a major focus to understand their origin and key features. Population-based analyses of stem cell behavior often fall short in defining mechanisms that may be unique to these specialized cells. Similarly, niche interactions at an individual cell level can reveal significant information on a cell's microenvironment and behavior at specific locations when compared with studies performed with cell aggregates. Although the implementation of clonal assays for the analysis of stem cells and its niche interaction is experimentally challenging, it is crucial for understanding the complex mechanisms that govern stemness.

Although traditional two-dimensional (2D) culture systems provide a simple means to study stem cell niche interactions, they suffer from inherent limitations to replicate the complexity of the native niche and in

providing greater insights into in vivo cell−cell and cell−matrix interactions. This has motivated the need to develop 3D platforms for stem cell culture and for engineering artificial stem cell niches. The 3D culture technology opens new avenues for studying fundamental questions regarding stem cells and their niches. Interestingly, it has also allowed several advanced applications in personalized medicine,[44,45] such as tissue-engineered constructs, biomanufacturing approaches, and platforms for drug discovery and toxicity testing. To recapitulate the in vivo niche environment and further examine the roles of the extrinsic cues in controlling the behavior of a stem cell, artificial niches with tunable physical, biochemical, and cellular parameters have been prepared.[9] Bioengineering tools along with novel fabrication techniques provide useful ways to tune niche properties of these stem cells. Bioengineering tools include synthesizing novel natural, synthetic, or hybrid biomaterials and micro- or nanofabrication of niche components in 2D or 3D. Together with the application of sophisticated bioengineering techniques and analysis methods, manufacturing synthetic stem cell niches will allow us to (1) culture stem cells under defined conditions, thereby improving reproducibility; (2) facilitate mechanistic studies to reveal specific roles of various niche cues in regulation of stem cell fate; and (3) develop novel strategies to engineer stem cell fates in vivo for tissue regeneration.[46]

A range of synthetic and natural polymers is currently used to fabricate scaffolds. Broadly, bioactive materials can be classified into natural and synthetic biomaterials. Some of the commonly used natural materials used to mimic ECM include biopolymers like collagen, hyaluronic acid, chondroitin sulfate, fibronectin, alginate, chitosan, and silk fibroin. Synthetic polymers, such as poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), and copolymer poly(lactic-glycolic acid) are some of the most commonly employed synthetically engineered scaffolds. **Chapter 19** highlights recent progress in polymer design and development for 3D stem cell culture, comparing the advantages of both natural- and synthetic-based precursors. Additionally, special attention is given to smart synthetic polymer systems that exhibit responsiveness to environmental stimuli (e.g., electricity, temperature, enzyme, light, heat, pH).

Although various natural and synthetic bioactive polymers have been engineered to improve the environmental conditions of a cell, artificially, the choice of the material depends on the selected application, the cell type used, and the tissue type. For example, bioceramics have been considered as one of the most suitable materials for the repair and reconstruction of diseased or damaged parts of the skeletal system. Ceramic materials are inorganic, nonmetallic solids, which include crystalline ceramic and amorphous glass compounds. **Chapter 20** overviews the development of the most commonly used bioactive ceramics for tissue repair and regeneration. It highlights understanding in the relationship between their structure and biocompatibility toward designing next-generation bioceramic materials for stem cell niche applications.

Stem cells can be regulated or controlled by manipulating their microenvironment, and therefore, controlling the interactions between biomaterials and stem cells is a critical factor for exploring the complete potential of biomaterials. There are a variety of methods and technologies available to fabricate and modify biomaterials according to the choice of the cell or tissue properties that needs to be regenerated. For example, surface functionalization or modification is one of the approaches that can create biomimetic microenvironments, which are able to control stem cell fate and functions. **Chapter 21** highlights the different processes involved in modifying the surface of a biomaterial by attaching molecules or substances via physical or chemical methods, or both. It focuses on the biological relevance of surface-functionalized biomaterials in the context of stem cell research.

Fabricating biomaterial scaffolds in 3D with controllable topographies and stiffnesses and conjugating signaling factors to scaffolds to regulate stem cell fates are critical to simulating in vivo conditions.[9] Hydrogels, which are hydrated polymer networks, share many key physical properties with native tissues and can be designed to include elements to control cell fate and function. **Chapter 22** comprehensively describes hydrogel design criteria, including source material, degradation, topography, adhesion, and growth factor presentation, in the context of 3D stem cell culture. To complement, **Chapter 23** explicitly discusses the structural features that control the mechanical properties within hydrogel networks of different types. It also discusses how gels with different mechanical properties can be used clinically to control cell differentiation and function.

Recent advancement in fabrication technologies like nano- and microfabrication has provided opportunities to design biomaterials with intricate topographical structures. In addition to functional tissue formation, some of the in vitro applications include (but are not limited to) identifying suitable ECM candidates as substrates for stem cell culture through micropatterning of ECM in 2D; high-throughput ECM microarrays; and synthesizing novel biomaterials. **Chapter 24** reviews the principal techniques used to generate micro- and nanotopographical features in substrates suitable for stem cell culture and niche engineering. It describes use of micro- and nanotopographic patterning as a means to probe the stem cell niche, and ultimately to

guide stem cell fate through mechanical cues that govern processes such as adhesion, proliferation, and differentiation. Self-assembly in particular is one of the most promising fields in building materials and structures at the nanoscale. It takes a bottom-up approach for fabrication, where small molecular components interact with each other under specific conditions to spontaneously organize into more complex 2D or 3D structures.[47,48] **Chapter 25** provides a comprehensive background on self-assembly nanofabrication techniques and their applications in bioengineering stem cell niches.

The in vivo ECM in particular possesses a nanoscale fibrous topography. **Chapter 26** discusses the role of this topography in modulating stem cell fates in vitro and details nanofiber fabrication techniques to produce matrices that have a morphological resemblance to fibers naturally found in the ECM. Their similar characteristics suggest that nanofibers could be used as a supportive matrix for creating artificial niches for stem cells, upon which additional functionalities could be incorporated to further modulate stem cell fates.[49]

Stem cells are exposed to a multitude of biochemical gradients across a niche, subjecting them to different signals in different locations in the niche, which shapes the way the cells divide. Hence, engineering a modulated niche with spatial complexity would better reflect the in vivo situation. Microfluidics allows for the controlled delivery of signals to specific locations within the niche, thus modulating the properties of an artificial stem cell niche. It provides ways to manipulate single stem cells to better understand behavior across a diverse population of stem cells. In addition, fabricating microfluidic channels within biomaterials has been shown to be a promising tool to mimic nutrient and gas transport for stem cell niche engineering.[50] **Chapter 27** reviews microfluidic concepts used to generate a wide variety of biomolecule gradients and presents the most successful applications of microfluidic device–induced gradients for pluripotent stem cell patterning.

## 5. BIOENGINEERING SPECIALIZED ARTIFICIAL STEM CELL NICHES FOR CLINICAL THERAPIES

Stem cells are promising cell source candidates for use in regenerative medicine. With the recent discovery of induced pluripotent stem cells (iPSCs), emerging combinations of biomaterials and iPSCs are bringing unprecedented opportunities for treating debilitating human diseases.[51] Potential knowledge from the biomaterials-mediated approaches for enhancing stem cell–based tissue repair is being applied for achieving many therapeutic and nontherapeutic goals. Engineering specialized artificial stem cell niches helps researchers to elucidate the mechanisms by which stem cells receive information from the multifactorial microenvironment. Promoting desirable stem cell phenotypes allows for the exploitation of their unique property of stemness and their ability to home and differentiate, thus contributing to tissue repair. Additionally, in vitro engineered niches can be used to screen for molecules that can regulate niche biology and thus avoid the need for exogenous cell therapy.

The currently available therapeutic strategies for end-stage debilitating disease such as congestive heart or liver failure are all invasive surgical approaches, including implantable devices and, ultimately, organ transplantation. However, these surgical treatments are unable to completely restore damaged tissue. For example, all current therapeutic procedures for heart failure to date only modulate hemodynamics and none are available to regenerate heart tissue. Regenerative therapies based on stem cells hold promise as a treatment to overcome this limitation, but to date have achieved only modest outcomes in clinical trials. To enhance potential stem cell behavior and exploit its maximum functional effect, numerous studies have taken great effort to elucidate the tissue-specific stem cell and progenitor cell niches. **Chapters 28–37** demonstrate such current advances in the construction of biomimetic niches to modulate cell–matrix interactions or cell–cell interactions for enhanced repair or regeneration in different tissue types, namely cardiac, bone, cartilage, skin, and liver. For vascular tissues and organs, de novo blood vessel formation, also known as vasculogenesis, and the subsequent expansion of the nascent vascular network via angiogenesis, constitutes a complex vascular niche essential to tissue repair. Coordinating blood vessel generation along with parenchymal tissue development is critical so that an adequate supply of nutrients and oxygen is maintained along with the removal of waste metabolites. Harnessing niche biology and in vitro model systems to engineer a regenerative therapeutic is the ultimate goal.

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