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REGULAR ARTICLE

Stem Cells and the Microenvironment: Reciprocity with Asymmetry in Regenerative Medicine

Guglielmo Militello[1] · **Marta Bertolaso**[2]

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Abstract

Much of the current research in regenerative medicine concentrates on stem-cell therapy that exploits the regenerative capacities of stem cells when injected into different types of human tissues. Although new therapeutic paths have been opened up by induced pluripotent cells and human mesenchymal cells, the rate of success is still low and mainly due to the difficulties of managing cell proliferation and differentiation, giving rise to non-controlled stem cell differentiation that ultimately leads to cancer. Despite being still far from becoming a reality, these studies highlight the role of physical and biological constraints (e.g., cues and morphogenetic fields) placed by tissue microenvironment on stem cell fate. This asks for a clarification of the coupling of stem cells and microenvironmental factors in regenerative medicine. We argue that extracellular matrix and stem cells have a causal reciprocal and asymmetric relationship in that the 3D organization and composition of the extracellular matrix establish a spatial, temporal, and mechanical control over the fate of stem cells, which enable them to interact and control (as well as be controlled by) the cellular components and soluble factors of microenvironment. Such an account clarifies the notions of stemness and stem cell regeneration consistently with that of microenvironment.

Keywords Stem cells · Regenerative medicine · Stem cell microenvironment · Stem cell fate

✉ Guglielmo Militello
guglielmo.militello@ehu.eus

Marta Bertolaso
m.bertolaso@unicampus.it

[1] IAS-Research Centre, University of the Basque Country, San Sebastián, Spain

[2] University Campus Bio-Medico of Rome, Institute of Scientific and Technological Practice, Rome, Italy

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1 Introduction

Stem cell therapy plays a pivotal role both in transplant therapies and internal medicine[1], because it promotes the regeneration of tissues and organs anomalies through the introduction of stem cells into specific tissues. Stem cells are characterized by the so-called 'potency', which is the capacity to renew indefinitely and differentiate into specialized cell types through asymmetric division within an adequate microenvironment. Stem cells can be picked up from the same organism (autologous), another individual of the same species (allogeneic), an identical twin (syngeneic), or other species (xenogeneic). The stem cells that are injected into human tissues can be extracted from different types of tissues (e.g., adult, embryonic, fetal) and exhibit a distinctive potential to proliferate and differentiate (Ciccocioppo et al. 2019).

The in vitro cultivation of stem cells on engineered scaffolds (e.g., organoid models) has shown that they can proliferate and differentiate, thus being potentially suitable for tissue regeneration. Nevertheless, when engrafted in *in vivo models*, proliferation and differentiation capacities of stem cells are usually reduced or even lost, mainly due to the absence of blood vessels, lymphatics and neuronal innervation, which are required to function in vivo (Edgar et al. 2020). A major *risk* related to the introduction of adult, embryonic, and induced pluripotent stem cells is represented by the possibility of genetic instability, epigenetic aberrations, eventually leading to *malignancy*. Furthermore, the use of stem cells faces different problems that are biological (e.g., immunorejection, or the difficulty of proliferating and differentiating in vitro) and ethical (e.g., the extraction of embryonic cells) in nature (Ciccocioppo et al. 2019).

The limitations and the risks of stem cell therapy can be understood in the light of the components and architecture of tissue microenvironment into which they are inserted. The constituents and the topological features of tissue microenvironment play a role in stem cell fate, thus being essential to the success or the failure of stem cell therapies (Votteler et al. 2010; Wilems et al. 2019). However, the nature of the *causal* relationship between tissue microenvironment and stem cells has not been studied in detail. Seemingly, the ontological issues involved are far from being understood yet.

Accordingly, this paper pursues three main aims: first, to characterize the *causal relationship* between stem cells and their microenvironment; secondly, to clarify the notions of *stem cell microenvironment* and *stemness*; finally; to explore (and question) what makes stem cells *regenerative*. Our working hypothesis is that a clarification of the causal role[2] of the components, topology, and functional organization (e.g., diffusion processes, hormonal variations) of microenvironment[3] may help in

[1] Regenerative medicine for transplant therapies consists of decellularization, 3D printing, interspecies blastocyst complementation; whereas tissue engineering and gene therapy are regenerative therapies for internal medicine (Orlando et al. 2019; Ciccocioppo et al. 2019).

[2] In the current literature, the causal role of microenvironment is sometimes referred as 'promotive' or 'protective', inasmuch as it can promote the activation or inhibition of cellular mechanisms that protect against tissue degradation.

[3] Throughout the paper, by 'tissue microenvironment', we mean a system of relations among the extracellular matrix, cellular, and non-cellular elements. Accordingly, we will mainly focus on the organization

understanding how microenvironment affects stem-cell therapy. We address the causal relationship between the tissue microenvironment and stem cells in two case-studies: the *cardiovascular* regenerative medicine and the *neuro-regenerative* medicine. We focus on these case-studies because they represent the most important (and promising) current applications of stem-cell therapy, and because they highlight the conceptual relevance of tissue microenvironment for the success or failure of stem cell therapy[4].

We argue that stem cells behavior and fate is intimately linked to tissue microenvironment and cannot be addressed separately from it, so that stem cells dynamics are controlled and regulated at the *system level* of the tissue. Furthermore, they constitute a network of *asymmetric mutual dependent constraints* where microenvironment (notably the extracellular matrix) establishes patterns of spatial, temporal, and mechanical control over stem cell fate and in turn stem cells constrain physiological processes of microenvironment such as vascularization or the modulation of the activity of immune cells. Such a framework sheds light on the nature of *stem cells* and their *regenerative potential* in regenerative medicine.

The paper is organized as follows. In Sect. 2, we critically review the current accounts of regenerative medicine, stem cells, and tissue microenvironment in biomedical literature. Sections 3 and 4 are respectively devoted to the study of stem cell therapy in the context of cardiovascular diseases and neurodegenerative and neurological diseases. Section 5 discusses the case-studies presented in the previous sections by focusing on the causal relationship between tissue microenvironment and stem cell fate. Finally, Sect. 6 offers some concluding remarks on the regenerative capacities of stem cells in the light of their relationship with microenvironment.

2 Regenerative medicine, stem cells, and the microenvironment: a critical review

Regenerative medicine is an interdisciplinary area that has begun to be acknowledged as a new emerging field in biomedical research in the last three decades. Until the ‘80s, the only way to re-establish damaged cells, tissues, and organs was organ transplantation. This trend has been changing since the ‘90s, as new technologies for regenerative medicine have been introduced: *engineered scaffolds*, implantation of *scaffolds seeded with cells*, and *cell-based therapy* (Sampogna et al. 2015).

Engineered scaffolds consist of natural (e.g., collagen and hyaluronic acid) and synthetic (e.g., synthetic polymers such as the polyglycolic acid) materials usually serving as three-dimensional (3D) extracellular matrix (ECM), playing the role of structural and functional support in tissues. Another approach is the use of *synthetic*

of the extracellular matrix in solid tissues and how it interacts with cellular and non-cellular elements.

[4] Another key target of stem-cell therapy is the bone marrow tissue for the treatment of leukaemia and multiple myeloma. As further pointed out in Sect. 5, the bone marrow extracellular matrix exerts a less rigid control on hematopoietic stem cells, thus not clearly elucidating the relationship between tissue microenvironment and stem cells. For this reason, we think that an in-depth analysis of solid tissues, such as those of the cardiac and nervous system, is more explanatorily relevant than that of the semisolid tissue of bone marrow.

scaffolds seeded with human cells[5], extracted through a patient's biopsy, expanded in vitro, and seeded onto a biomaterial scaffold (Atala 2012; Sampogna et al. 2015). After the isolation of human embryonic stem cells in 1998 and amniotic fluid-derived stem cells in 2007, *cell-based therapy* has turned out to be an important source of tissue regeneration (Sampogna et al. 2015). It consists in the introduction of novel and healthy cells in pathologic tissues by using differentiated endogenous primary cells or undifferentiated stem cells. Differentiated endogenous primary cells are obtained from specific tissues of patients (e.g., cardiomyocytes extracted from the cardiac tissue) and are engrafted after expansion in vitro. Although they can be implanted without any further manipulation, it is difficult to induce their proliferation in vitro, since they lack their original *microenvironment* needed to proliferate (Atala 2012; Sampogna et al. 2015).

Compared to differentiated endogenous primary cells, undifferentiated stem cells are a promising way to induce the regeneration of damaged human tissues and organs. Stem cells are characterized by two main hallmarks: *cell potency* and *self-renewal* (Trebinjac and Nair 2020). *Cell potency* designates the ability of a cell to *differentiate* into other cell types. We can distinguish four main kinds of cell potency: *totipotency*, *pluripotency*, *multipotency*, and *unipotency* (Fortier 2005). Totipotency is the ability of a cell to differentiate into any kind of cell (like the cell potency of a blastocyst). Pluripotency is the ability to differentiate into a cell of all three germ layers. Multipotency is the ability to differentiate into a limited number of cell types. Unipotency is the ability to differentiate into one single cell type. The other fundamental property of stem cells is their *self-renewal* capacity, which is the process of asymmetric or symmetric cellular division[6], leading to new daughter cells that can develop and reproduce as the mother cell (He et al. 2009)[7]. Such hallmarks occur simultaneously, as far as a stem cell divides into a new stem cell and a new differentiating cell. The distinction of the two hallmarks has therefore an epistemic and a physical relevance.

Stem cells can be collected from *adult*, *embryonic*, and *fetal tissues*. *Adult* stem cells, which are usually extracted from bone marrow, adipose tissue, umbilical cord, and skin[8], exhibit *multipotency* and differentiate into a specific cell type[9]. *Embryonic* stem cells, which derive from the blastocyst inner cell mass of developing embryos, are *pluripotent* and differentiate into all the cells derived from the three germ layers.

[5] An example is provided by scaffolds consisting of collagen and polyglycolic acid and seeded with patient's urothelial and smooth muscle cells (Sampogna et al. 2015).

[6] In the asymmetric division, a stem cell generates one differentiated cell and one stem cell. In the symmetric division, a stem cell gives rise to two differentiated cells or two stem cells (Shahriyari and Komarova 2013).

[7] Self-renewal and proliferation, in spite of being closely linked to cell division, are not the same: the former refers to a regulated process of cellular division leading to daughter cells with reproductive capacities; the latter designates the production of daughter cells that are not necessarily tightly regulated (as in the case of tumor proliferation) and not necessarily able to reproduce (He et al. 2009).

[8] Other adult tissues (extracted, for example, from liver, skeletal muscle, kidney, and urothelium) can be potential sources of adult stem cells, as their progenitors can proliferate and differentiate into specific organ cell types (Yalcinkaya et al. 2014).

[9] An interesting example of adult stem cells is provided by mesenchymal stem cells, which may differentiate in vitro in a variety of cell types such as hematopoietic stem cells, adipocytes, osteocytes, and chondrocytes (Uccelli et al. 2008).

Amniotic fluid-derived stem cells are *multipotent* stem cells taken from chorionic villi. They represent a promising path for cell therapy and tissue engineering and for the treatment of congenital malformations, because they can easily proliferate and do not present risks of tumorigenicity, do not pose ethical problems, and can be easily preserved (Ghionzoli et al. 2010; Sampogna et al. 2015). Finally, *induced* pluripotent stem cells are obtained through the reprogramming of adult cells[10]. This kind of stem cells is *pluripotent* and could represent an alternative to embryonic stem cells (Takahashi and Yamanaka 2006; De Los Angeles et al. 2015).

In spite of being a promising route for contemporary cell therapy, stem cells reveal major limitations. A first problem is represented by the *reduced capacity to proliferate and differentiate in vitro*: this is the case of adult stem cells, which proliferate and differentiate only in their specific tissue microenvironment (Scadden 2006; Votteler et al. 2010). Furthermore, recent studies conducted on mice and cohorts of patients have indicated that adult mesenchymal and hematopoietic (Karaöz and Tepeköy 2019; Molina et al. 2019), embryonic (Blum and Benvenisty 2008; Fujimori et al. 2012), and induced pluripotent stem cells (Kamada et al. 2014) are associated to a *tumorigenic risk*. For example, embryonic stem cells and induced pluripotent stem cells could give rise to teratomas, which are tumor-like formations consisting of tissues of the three germ layers (Nussbaum et al. 2007; Hentze et al. 2009). Another fundamental weakness of stem cell therapy lies in the *immunorejection*. Embryonic stem cells, for example, are highly successful when they are autologous. In all those tissues (e.g., the tissues of brain, heart, and pancreas) that do not provide an available source for autologous primary cell expansion, allogeneic and syngeneic embryonic stem cells could give rise to immunorejection (Yalcinkaya et al. 2014; Khan et al. 2018). A further problem, mostly related to embryonic stem cells, is *ethic*: since embryonic stem cells are extracted from in vivo embryos, their supply entails the killing of the embryos (Sampogna et al. 2015).

The potential and limitations of stem cell therapy are thus closely linked to the features of the *microenvironment* -or niche- in which stem cells are embedded[11]. By stem cell 'microenvironment', the current scientific literature designates a three-dimensional organization consisting of *cells* and *soluble factors* (e.g., growth factors, cytokines, hormones) that are surrounded and connected by the *extracellular matrix* and with specific *physical features* (e.g., oxygen tension, matrix stiffness) (Wang and Wagers 2011; Wagers 2012). Microenvironment provides stem cells with *chemical* (e.g., cell-cell interactions) and *physical cues* (e.g., the mechanical cues associated to matrix stiffness) that influence how stem cells respond to external environment, support and coordinate their activities, thus determining the success or the failure of proliferation and differentiation of stem cells. (Wagers 2012; Vining and Mooney 2017).

Recent studies have underlined the importance of targeting therapeutically the stem cell microenvironment in order to manipulate stem cell fate (proliferation, dif-

[10] The reprogramming of human somatic cells into induced pluripotent stem cells is obtained by inducing abnormal gene expression of a core of transcription factors (OCT4, SOX2, and NANOG) that governs pluripotency (De Los Angeles et al. 2015).

[11] Stem cell niches have been characterized in many different tissues such as the bone marrow, skeletal muscle, central and peripheral nervous system (see Votteler et al. 2010).

ferentiation) (Wagers 2012; West-Livingston et al. 2020). For stem cells transplantation to be viable, the cues of microenvironment need to trigger stem cell expansion. The reconstitution of stem cell microenvironment in vitro provides stem cells with external cues that favor stem cell differentiation (Wagers 2012). Moreover, the components of the microenvironment can be targeted to restore lost niches or reverse niche dysfunction, thus restoring or enhancing endogenous regenerative potential. In anti-cancer therapy, tumor microenvironment can be treated with stem cells to revert malignant phenotypes (process of tumor reversion) (Proietti et al. 2020).

Contemporary descriptions of stem cell microenvironment have focused on how single components of the niche (e.g., the extracellular matrix, signaling molecules, or cells) *directly* affect stem cell fate, implicitly assuming the idea of an *efficient causation* (i.e., mechanisms) of local components on stem cell fate. Nevertheless, this theoretical framework reveals two main shortcomings: first, the nature of the *causal relationship* between the microenvironment and the stem cell determination (from the microenvironment to stem cells and vice versa) has not been investigated in detail. In other words, the idea that single components of the microenvironment *directly* affect stem cell determination is considered as a given and not conceptualized as such. This tendency is reinforced in current techniques of tissue engineering, which identify and mimic *specific* aspects of the three-dimensional microenvironment to improve stem cell fate engraftment (Wilems et al. 2019; West-Livingston et al. 2020; Caianiello et al. forthcoming).

Secondly, stem cell fate is the result of systemic conditions of the microenvironment and current accounts usually focus on single (local) aspects of it, thus facing the risk of losing sight of a systemic perspective on stem cell microenvironment. As argued by Inman et al. (2015) and Vining and Mooney (2017), the whole tissue microenvironment -and not just its single components- *enables* stem cells to exert forces and be subject to external forces, which *regulate* intracellular signaling pathways, thus controlling stem cell fate, behavior, and development. In order to fill this theoretical void, the following sections explore how microenvironment acts on stem cell fate and how stem cells modify and shape the microenvironment.

3 Stem cells and the microenvironment in cardiovascular regenerative medicine

Myocardial infarction determines a global change in the organization and composition of cardiac muscle tissue microenvironment. The transition from healthy to diseased cardiac microenvironment is characterized by a number of key events. First, the heart is prevented from receiving oxygen, leading to *cardiomyocyte*[12]*death*. In order to repair cardiac tissue, *immune cells* are *activated*, and fibroblasts[13] turn into

[12] Cardiomyocytes are tubular muscle cells that contains myofibrils (protein fibres sliding past each other) organized into sarcomeres (the fundamental contractile units of muscle cells). The membrane and the interior of cardiomyocytes are connected through protrusions of cardiomyocytes membrane (T-tubules).

[13] Fibroblasts are cells that synthesize the components of the extracellular matrix (e.g., collagen) and produce the structural framework (the stroma) for animal tissues.

myofibroblasts, which exhibit an intermediate capacity between a fibroblast and smooth muscle cells. Myofibroblasts repair an injury by producing collagen and joining the edges of the injured area. The appearance of myofibroblasts determines an overproduction of ECM components (e.g., proteins such as collagen and elastin, and polysaccharides such as glycosaminoglycans), scar formation, and the increase in the ECM stiffness[14], thus affecting the contractility of cardiac tissue (Humeres and Frangogiannis 2019). Finally, in response to low oxygen tension, the activation of hypoxia-inducible factors[15] promote vascular remodeling (Abe et al. 2017). The injection of stem cells into infarcted hearts has offered therapeutic benefits, even though its real effectiveness is still debated. In this section, after having presented the potential therapeutic benefits of stem cells for cardiac regeneration, we analyze how infarcted myocardial microenvironment affects stem cell fate determination.

Stem cells employed in cardiac tissue regeneration can be of *endogenous* and *exogenous* origin. The former includes *cardiac progenitor cells*, which are stem cells residing in specific niches of cardiac tissue (Jones and Wagers 2008; Amini et al. 2017). The latter entails all those stem *cells extracting from other tissues* (e.g., bone marrow, peripheral blood, skeletal myoblasts) and exhibiting *different kinds of cell potency* (pluripotency, induced pluripotency, and multipotency) (Laflamme and Murry 2011; Müller et al. 2018). Both endogenous and exogenous stem cells have the capacity to differentiate into cardiomyocytes, endothelial and smooth muscle cells through *direct* and *indirect* mechanisms. Direct mechanisms include (trans)*differentiation*[16] into cardiomyocytes, endothelial and smooth muscle cells. Indirect mechanisms are essentially mediated by *paracrine signaling pathways*[17] that allow stem cells to communicate with each other at relatively short distances even in absence of cell-cell interactions contacts with the host tissue (Gallina et al. 2015).

Stem cells contribute to cardiovascular regeneration in different ways. First of all, the secreted molecules of stem cells trigger *neovascularization* in infarcted hearts through the release of pro-angiogenic factors (e.g., vascular endothelial growth factor, hepatocyte growth factor, and insulin-like growth factor-1), thus enabling the perfusion of blood to heart (Chimenti et al. 2010; Johnson et al. 2019)[18]. Stem cells can also have *immunomodulatory properties*, as they can interact with cells of the innate and adaptive immune system. Mesenchymal stem cells have an anti-inflammatory action by suppressing the proliferation and activation of immune cells (e.g., T-cells, B cells, dendritic cells) and inducing their apoptosis through direct cell-cell contact-dependent mechanism and the releasing of soluble factors (Glennie et al. 2005; Plumas et al. 2005). Stem cells can influence the polarization of macrophages to

[14] In physics, stiffness is the strain of an elastic body expressed as a function of the force producing the strain. The stiffness of healthy tissue microenvironments requires a balance between rigid-like (elastic) and dissipative (viscous) components (Cameron et al. 2011).

[15] Hypoxia-inducible factors are transcription factors promoting vascularization.

[16] Transdifferentiation is an artificial process in which one mature somatic cell is transformed into another mature somatic cell without undergoing an intermediate pluripotent state or progenitor cell type.

[17] Paracrine signals include all those molecules travelling over a *relatively short distance* (local action) and modifying nearby cells.

[18] The different types of stem cells produce different degrees of neovascularization in infarcted hearts (Müller et al. 2018).

facilitate their shift from pro-inflammatory to *anti-inflammatory phenotypes*, which can accelerate wound healing processes (Hasan et al. 2016). Stem cells can also activate *endogenous regenerative processes* through the recruitment of resident stem and progenitor cells, the stimulation of cardiomyocyte proliferation, and the reactivation of the normal cell cycle of cardiomyocytes (Weil and Canty 2013). A further aspect of stem cells is that they trigger *cardiac remodeling processes* consisting in molecular, cellular, and interstitial changes (e.g., adverse cardiomyocyte organization and altered extracellular matrix homeostasis) in cardiac structure and function (Azevedo et al. 2016)[19].

Despite the effectiveness observed in pre-clinical studies over animals, clinical trials on humans have not confirmed the substantial benefits of stem cell therapy for cardiac regeneration (Müller et al. 2018). Important limitations of stem cell engraftment have been recognized: fusion with host cardiomyocytes and inability to proliferate and differentiate (Nygren et al. 2004; Andrade et al. 2005), transient improvement of cardiac fibrosis and cell survival without long-term amelioration of cardiovascular conditions (Menasché 2018; Liang et al. 2019). The main obstacles to the effective functional engraftment of cardiac stem cells are represented by the *infarcted cardiac microenvironment*, which consists of *immune cells and infiltrated myofibroblasts*, *extracellular matrix proteins*, and *soluble factors*. The infarcted cardiac environment is characterized by altered *mechanical forces* exerted on stem cells. All these factors provide stem cells with a *hostile environment* (Macrí-Pellizzeri et al. 2015; Mauretti et al. 2017).

Myocardial infarction produces a condition of hypoxia that induces the secretion of *pro-inflammatory cytokines* (e.g., tumor-necrotic factor α and interleukin-6) and *immune cells*[20] (Khodayari et al. 2019). The interaction between stem cells, cytokine receptors (e.g., IL-1R, APO1, TRAIL-R)[21] and their ligands triggers *apoptotic* processes of stem cells (Spaggiari et al. 2006). The apoptotic processes of stem cells are stimulated by different molecular mechanisms such as injured heart tissue inflammatory response, hypoxia and substrates delivery, loss of the cell-cell contact, cytotoxic and/or proapoptotic factors (Khodayari et al. 2019). In such an environment, hypoxia and inflammation can induce cell death and local cellular degeneration of the endogenous and injected stem cells.

A consequence of inflammatory processes is the transformation of fibroblasts into myofibroblasts. This determines an overproduction of type 1 collagen that increases the *stiffness* of the extracellular matrix of cardiac tissue, generate *higher cell-matrix tension* and a more definitive *pro-contractile tissue* environment, and *interrupt* the *electrophysiological activity* of implanted stem cells through gap junctions or mechanical coupling (Liang et al. 2019). These ECM changes determine a *failure* in

[19] Although it is known that stem cells modulate the extracellular matrix of cardiomyocytes, the exact mechanisms underlying it is not fully known.

[20] The production of immune cells is promoted by free radicals, which favor chemotactic migration of inflammatory cells into the injured tissue microenvironment, and by the infiltration of leukocytes into the infarcted tissue in response to the chemokines' expression (Khodayari et al. 2019).

[21] IL-1R stands for interleukin 1 receptor; APO1 for the apoptosis antigen 1; TRAIL-R for the TNF-related apoptosis-inducing receptor.

mechanotransduction[22], a condition characterized by an alteration of the mechanical forces transmitted from the ECM to the cytoskeleton and nucleus of cardiac cells.

Experiments conducted on polyacrylamide gel systems[23] have shown that stiffness gradients, and not stiffness alone, drive the migration[24] of mesenchymal and somatic stem cells towards specific niches of the cardiac tissue, where they undergo a differentiation process (Rowlands et al. 2008; Tse and Engler 2011). However, it has not yet been clarified whether a stiffer ECM, typical of infarcted tissue, inhibits or rather favors stem cell differentiation into contractile myogenic lineages. Some studies suggest that mesenchymal stem cells tend to migrate to a stiffer region and then develop into a more contractile myogenic lineage (Tse and Engler 2011) and that myofibril organization and function are not affected by substrate stiffness (Hersch et al. 2013). Other researches have stressed that mesenchymal stem cells cultivated on a stiff substrate underwent osteogenic differentiation, but modest myogenic differentiation (Rowlands et al. 2008), and that induced pluripotent stem cell-derived cardiomyocytes were affected by the degree of stiffness of the substrate (Heras-Bautista et al. 2019).

Soluble factors (e.g., vascular endothelial growth factors, epidermal growth factors, fibroblast growth factors, growth hormones) play an important role in stem cell fate, binding to cell surface receptors and modulating stem cell survival, growth, and differentiation (Murtuza et al. 2009; Ghafar-Zadeh et al. 2011). For example, vascular endothelial growth factors and fibroblast growth factors (e.g., FGF2 and FGF10) have been shown to promote cardiac stem cell proliferation and differentiation (Yamakawa et al. 2015). Furthermore, in response to *low oxygen tension*, cells express hypoxia-inducible factors that inhibit[25] cardiac stem cell differentiation (Mas-Bargues et al. 2019; Mennen et al. 2020).

To conclude, the benefits of stem cells to cardiac regeneration are controversial: on the one hand, pre-clinical studies have shown regenerative capacities of stem cells in murine infarcted hearts; on the other, clinical trials have underlined that stem cells produce absent or short-term effects on human hearts. The potentialities and limitations of cardiac stem cells can be therefore understood in the light of the *cardiac infarcted microenvironment*, which affects stem cell fate via *molecular interactions* (e.g., molecular interaction with immune cells, cytokines, soluble factors), *altered mechanical forces*, and the *topography* of the ECM (e.g., stiffness) that globally constrain the survival, proliferation, and differentiation of stem cells.

[22] A more detailed characterization of the process of mechanotransduction and how it affects stem cell fate is done in Sect. 5.

[23] Since it is very difficult to experimentally manipulate the ECM stiffness in vivo, the effects of the stiffness/flexibility of the ECM on stem cells are usually studied on artificial matrixes that mimic tissue elasticity (Gattazzo et al. 2014).

[24] 'Durotaxis' is the name given to the process of cell migration driven by stiffness differences in the substrate.

[25] In some cases, although some murine induced pluripotent stem cells and embryonic stem cells have been shown to differentiate to cardiomyocytes at 2% or 5% of oxygen tension, either they do not survive or generate ineffective beating cardiomyocytes (Brodarac et al. 2015).

4 The influence of tissue microenvironment on stem cell fate in neuro-regenerative medicine

The loss of neural connections and the death of neural cells is a hallmark of many neurological disorders ranging from neurological trauma to neurodegenerative diseases. The regrowth or repair of neural tissue and cells (*neural regeneration*) is a process that may occur naturally in *peripheral nervous system* suffering from injury[26] or be artificially induced in *central nervous system*[27] suffered trauma (e.g., spinal cord injury and brain ischemia) through the use of *stem cells* (Goncalves and Przyborski 2018). Stem cell therapy has attracted increasing interest for the treatment of neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis), which are characterized by a progressive loss of neural cells in the brain and in the spinal cord[28]. In this section, we examine the contribution of stem cells to neuroregeneration and how neural microenvironment of damaged neural tissue affects stem cell fate.

Different kinds of stem cells are employed for neuroregeneration: *embryonic*, *induced pluripotent*, *mesenchymal*, and *neural* stem cells (Sivandzade and Cucullo 2021). Human *embryonic stem cells* are extracted from blastocysts and can self-renew indefinitely and differentiate into almost all cell types of the central nervous system. *Induced pluripotent stem cells* are adult somatic cells, the gene expression of which is artificially modified in order to make them pluripotent and produce unlimited autologous neurons for transplantation in neurodegenerative medicine. *Mesenchymal stem cells* have a high potential for nervous regeneration because of their ability to synthesize neurotrophic and proangiogenic factors and to overcome the blood-brain barrier that is essential for the proper delivery of neurotherapeutic agents into the central nervous system (Hasan et al. 2017). As such, mesenchymal stem cells promote the survival and regeneration of neurons, the growth of axons, and angiogenesis (Khan et al. 2018; Mukhamedshina et al. 2018). *Neural stem cells* are multipotent stem cells in brain tissue that are more specialized than embryonic ones. Although they have a decreased potential for self-renewal and usually differentiate into specific cell types of the brain tissue (e.g., oligodendrocytes, neurons, astrocytes), neural stem cells are more stable and less tumorigenic compared to embryonic ones.

The main mechanism used by stem cells to trigger neuroregenerative process is based on *paracrine factors*. For example, mesenchymal stem cells promote *neural proliferation* and *angiogenesis* by secreting growth factors (e.g., brain derived neu-

[26] Peripheral nervous system consists of nerves that can regenerate after injury because of the supportive growth environment of Schwann cells (Bhangra et al. 2016). Stem cell therapy can also be employed for repairing peripheral nerve injuries in order to regenerate a lost tissue (Goncalves and Przyborski 2018).

[27] After injury, the tissues of the central nervous system produce glial scar that hinders neuroregeneration. Glial scar formation is a reactive process during which astrocytes abnormally increase after injury to the central nervous system.

[28] Compared to most drugs for the treatment of neurodegenerative diseases, which cannot target neuronal cell death and are not able to arrest neurodegenerative processes, stem cells can promote neural regeneration (e.g., endogenous neuronal growth, synaptic connection, neural proliferation, angiogenesis) and prevent neural cells from further degeneration (e.g., anti-apoptosis, anti-fibrosis, and anti-inflammatory effects) (Das et al. 2019).

rotrophic factors, vascular endothelial growth factors, and nerve growth factors) (Das et al. 2019). Likewise, neural stem cells secrete growth factors (e.g., neurotrophic factors) that trigger *axonal growth* (Lu et al. 2014), *re-myelination of axons* (Maeda et al. 2019), and the proliferation of stem cells of the *spinal cord* (Assinck et al. 2017). Furthermore, paracrine factors can have an *anti-inflammatory action*, as shown by mesenchymal stem cells, which secrete anti-inflammatory factors (e.g., *cytokines*, *prostaglandins*) that modulate the reactivity and the phenotype of astrocytes and microglia, so as to produce anti-inflammatory and anti-apoptotic effects (Mukhamedshina et al. 2018).

Preclinical research[29] has shown that stem cells can offer potential benefits to patients suffering from neurodegenerative diseases, ischemic brain injury (Burns et al. 2009), and spinal cord injury (Yamazaki et al. 2020). For instance, embryonic, induced pluripotent, and neural stem cells can form *dopaminergic neurons* that improve neural functions in Parkinson (Sivandzade and Cucullo 2021). Furthermore, stem cell therapies -notably neural and mesenchymal stem cells- can have positive effects on Alzheimer's disease brain by *enhancing neurogenesis*, *replacing lost neurons*, and *improving synaptic plasticity* (Ager et al. 2015; Sivandzade and Cucullo 2021). Mesenchymal stem cells offer a promising way to treat Huntington's disease because of their ability to decrease immune cell dysfunction, enhance compensatory neurogenesis, reduce apoptosis, activate mitochondrial function, and promote cell survival (Connor 2018). Stem cells are also employed for treating amyotrophic lateral sclerosis by replacing the damaged/dead motor neurons, regulating inflammation, and promoting the expression of neurotrophic factors (Sivandzade and Cucullo 2021). For the treatment of ischemic brain injury, neural stem cells are employed to favor endogenous neurogenesis, whereas exogenous stem cells are used to carry out neuroprotection, cell replacement, and neuroplasticity (Burns et al. 2009).

Several neurological diseases, such as *neurodegeneration*, *brain traumatic injury*, *brain ischemia*, are characterized by dramatic changes in the *nervous tissue* (Bonneh-Barkay and Wiley 2009; Bonilla and Zurita 2021). Neurodegenerative diseases are characterized by *problems in protein folding and the formation of protein aggregates*, an *altered composition and organization of the* brain *ECM*, and changed *mechanical forces* (Bonneh-Barkay and Wiley 2009). Moreover, the development of a *proinflammatory environment* is a common feature of neurodegenerative diseases, brain traumatic injury, and brain ischemia (Bonilla and Zurita 2021; Liu et al. 2021). In all these cases, the organization of *damaged neural tissue* influences *stem cell fate*. Let us therefore examine how the latter is affected by the former.

Neurodegenerative diseases are characterized by the accumulation of proteins in the intracellular and extracellular space[30]. Recent studies have shown that protein deposition in the extracellular space affects stem cell fate determination. The exact role of Amyloid-beta (Aβ) peptides is still not fully understood, as they can have

[29] Most of current studies on stem cell therapy for the treatment of neurodegenerative diseases are preclinical and it has not yet evaluated the tolerance and efficacy in clinical trials (Sivandzade and Cucullo 2021).

[30] Alzheimer's disease is characterized by the accumulation of amyloid-beta peptides (Serrano-Pozo et al. 2011), whereas Parkinson's disease (Gundersen 2010), amyotrophic lateral sclerosis (Blockhuis et al. 2013) and Huntington's disease (Arrasate and Finkbeiner 2012) by Lewy Bodies.

either *negative* or *positive* effects on stem cells (Lee et al. 2013). On the one hand, Aβ peptides seem to *impair neurogenesis* of human neural stem cells[31], and *reduce the number of mitochondria* of neural stem cells, thus decreasing ATP levels and favoring oxidative stress (Santos et al. 2021). On the other hand, Aβ peptides (e.g., Aβ42 peptides) could *favor neurogenesis* of neural stem cells by *promoting the migratory potential* of neural stem cells toward inflamed lesions in animal brains (López-Toledano and Shelanski 2004).

Age-related neurodegenerative diseases (e.g., Alzheimer's and Parkinson's diseases) are characterized by changes in the structure and organization of the brain ECM[32], such as the expression of proteoglycans and association with protein aggregation, amyloidosis, and microglial activation (Bonneh-Barkay and Wiley 2009). These changes involve the co-deposition of ECM components (e.g., heparan sulfate proteoglycans and chondroitin sulfate proteoglycans) that lead to the loss of protective perineuronal nets and increased susceptibility to cell death. Dying neurons can induce inflammation, degradation of the ECM and induce a stronger inflammatory response (Bonneh-Barkay and Wiley 2009; Sonbol 2018). The alterations of brain ECM *prevent* stem cells from *differentiating correctly*. For example, the presence of altered amyloid precursor could hinder successful stem cell therapy for Alzheimer's disease, because they induce stem cells to differentiate into glial cells rather than into neurons (Sugaya and Vaidya 2018).

Neurodegenerative disorders are characterized by a *decrease in ECM stiffness*[33] and a disruption of brain ECM mechanics (Hall et al. 2021), which determines *altered mechanical forces*[34]*and aberrant cues* acting on neural and stem cell. A number of works indicate that softer substrates[35] promote neural differentiation (Keung et al. 2012) and oligodendrocyte progenitor cells differentiation (Lourenço et al. 2016), whereas stiffer substrates lead to glial differentiation (Pogoda and Janmey 2018). This suggests that brain ECM stiffness in neurodegenerative diseases could promote stem cell differentiation or also stem cell migration; yet, it is not currently well known how the brain ECM stiffness of neurodegenerative diseases affects stem cell

[31] Aβ peptides activate GSK-3β signalling pathways, which decrease β-catenin levels and impair neurogenesis of human neural stem cells (Lee et al. 2013).

[32] Brain ECM consists of three main parts -the *basement membrane*, the *perineuronal net*, and the *neural interstitial matrix*- and includes cells that are placed in proximity with a limited stromal space. Compared to the ECM of other tissues, the brain one lacks some components that are commonly found in other organs (e.g., fibronectin and collagen) and exhibits different types of proteoglycans that are localized to intercellular spaces between neurons and glia (Bonneh-Barkay and Wiley 2009).

[33] Both in Alzheimer's and Parkinson's diseases, there is a change in the stiffness or elasticity of the ECM: in Alzheimer's it is a change in ECM, whereas in Parkinson's a change in the stiffness/elasticity of substantia nigra (Barnes et al. 2017). In multiple sclerosis, the central nervous system basement membrane become discontinuous and levels of fibrillar collagen increase, leading to perivascular fibrosis (Barnes et al. 2017).

[34] The stiffness of brain ECM affects the mechanical signals that are sent to focal adhesion protein complexes, which regulate neural differentiation through the phosphorylation of focal adhesion kinase (FAD) and also cytoskeleton rearrangement through the Rho/ROCK, Src family kinases, and ERK1/2 signaling pathways (Mammoto et al. 2012).

[35] Softer substrates are less than 1000 Pa, whereas stiffer substrates are between 1000 and 10,000 Pa.

fate determination and further studies are required to support this hypothesis (Barnes et al. 2017; Hall et al. 2021).

Neuroinflammation is a hallmark of brain injury[36], ischemic brain[37], and neurodegenerative diseases[38] and influences the survival, differentiation, and migration of stem cells by modulating cell-to-cell signaling pathways such as Notch and Wnt (Russo et al. 2011). Depending on how microglia, astrocytes, and macrophages are activated during inflammation, they can trigger or inhibit neurogenesis (Russo et al. 2011; Kizil et al. 2015). Some mediators of immune cells (e.g., cytokines, chemokines, nitric oxide) negatively regulate neurogenesis, reduce the proliferation of neural stem cells and hamper their maturation and migration (Kizil et al. 2015), whereas activated microglia[39] can trigger neurogenesis and oligodendrogenesis (Russo et al. 2011).

To conclude, the use of endogenous and exogenous stem cells for neuroregeneration depends on the *altered neural microenvironment* of neurological diseases. In neurodegenerative diseases, the formation of protein aggregates inside and outside neural cells, the change in the composition and structure of the brain ECM, and the decrease in the ECM stiffness affect the signaling processes modulating stem cell differentiation, proliferation and migration. Likewise, the inflammatory microenvironment, associated to neurodegenerative diseases, brain injury and ischemic brain, can both trigger and inhibit neurogenesis and stem cell maturation, proliferation, and migration.

5 Stem cell microenvironment as a network of *asymmetric mutual dependent constraints*

The previous sections have underlined that stem cell fate is closely linked to their microenvironment. However, what stem cell niche is and how it is causally related to stem cells are quite problematic in current biological research. Accordingly, the aim of this section is to evaluate the causal relationship between stem cells and their microenvironment and clarify the notion of stem cell microenvironment on the basis of our case-studies. We will therefore provide a characterization of stemness that is consistent with that of stem cell microenvironment.

[36] Brain injury determines not only a mechanical breakdown of brain tissue and necrotic death, but also a cascade of cellular events (e.g., oxidative stress, mitochondrial dysfunction, and blood-brain barrier disruption) that contribute to a pro-inflammatory microenvironment that ultimately leads to the infiltration of immune cells (microglia) into the damaged brain parenchyma (Bonilla and Zurita 2021).

[37] Ischemic brain is characterized by high rates of apoptosis and necroptosis, the release of damaged-associated molecular patterns and matrix metalloproteinases, leading to inflammatory responses such as astrocyte and microglia activation, the release of cytokines and chemokines, and infiltration of leukocytes and neutrophils (Liu et al. 2021).

[38] Neurodegenerative diseases are characterized by the secretion of proinflammatory cytokines (e.g., tumor necrosis factor-α, interleukin-1β, and interleukin-6) that trigger neuroinflammation (Russo et al. 2011).

[39] Microglia are activated by molecules such as interleukin-4 and interferon-γ (Russo et al. 2011). Microglia (T cells) secrete molecules (e.g., BDNF) that positively regulate neurogenesis (Kizil et al. 2015).

It is acknowledged that stem cell fate and behavior depend on the *integration* of a number of biochemical (e.g., soluble factors or cell-surface signaling molecules) and biophysical (e.g., the mechanical forces produced by matrix stiffness or the physical properties of the substrate) cues sent from the microenvironment (Kumar et al. 2017). Nevertheless, current biological research tends to focus on how *single components* of microenvironment (e.g., soluble factors, cells, ECM) generate *specific* cues (see, for example, Gattazzo et al. 2014; Sobacchi et al. 2017), rather than considering their integration as the result of a *system of relations* that collectively interact with stem cells by modulating them[40]. This way of conceptualizing stem cell microenvironment seems to be inadequate and potentially misleading, because it gives the impression that microenvironment constituents modulate stem cells fate *separately* and *individually*. In fact, the causal relationship between stem cells and their microenvironment can better be grasped by adopting a *relational* and *dynamical ontology* that considers biological levels and processes as mutually *related*, leading to macroscopic behaviors that are *emergent* and *time dependent* (Bertolaso 2016).

In such a framework, there is neither an upward (from stem cells to tissue microenvironment) nor a downward (from tissue microenvironment to stem cells) causation, but rather a *bidirectional* model of causation in which stem cells *constrain* the behavior (i.e., the mechanisms) of the other components of the niche and vice versa (see Bertolaso 2016; Bertolaso and Velázquez 2022). Here, two clarifications are necessary. First, the notion of *constraint* appears fundamental to understand the stem cells-microenvironment relationship and designates any entity (E_1) that limits the *degree of freedom* of another entity (E_2) at a specific time, thus establishing a specific set of behavior and actions that E_2 can perform (Umerez and Mossio 2013). Secondly, stem cells and their niche mutually constrain in an *asymmetric* way, inasmuch as the type of constraint action of stem cells on tissue microenvironment is *qualitatively* different from that of the niche on stem cells. This model of causation has been synthesized by the concept of *reciprocal asymmetric causation* (Fig. 1), where entities constrain one another in a "process of co-determination [that] involves different dimensions of causality and ensures the integration of functional macrostates at different scales" (Bertolaso 2016, p. 100).

The conceptualization of the stem cells-microenvironment relationship as a network of reflexive asymmetric constraints requires examining the *mutual relationship* between stem cells and *extracellular matrix* (Fig. 1). Although ECM can take many forms, *basement membrane* and *interstitial matrix* are the most important types: the former, which underlies epithelial tissues, is a specialized and flat-laminar ECM consisting of interconnected molecules (e.g., collagen IV, laminin, and proteoglycans); the latter is a combination of fibrous materials (i.e., collagen and non-collagenous proteins), water, and proteoglycans (Walma and Yamada 2020). Basement membranes and interstitial spaces are the *scaffold* of the intercellular space and act as *selective constraints* upon *dynamic spatial relations* of cells (Bich et al. 2019), because they *establish* and *select* the spatial patterns and positions of stem cells, thus modulating

[40] In more philosophical terms, we could say that biological practice tends to provide mereological accounts of microenvironment by focusing on how the (*local*) *mechanisms* performed by microenvironment components affect cellular behaviour.

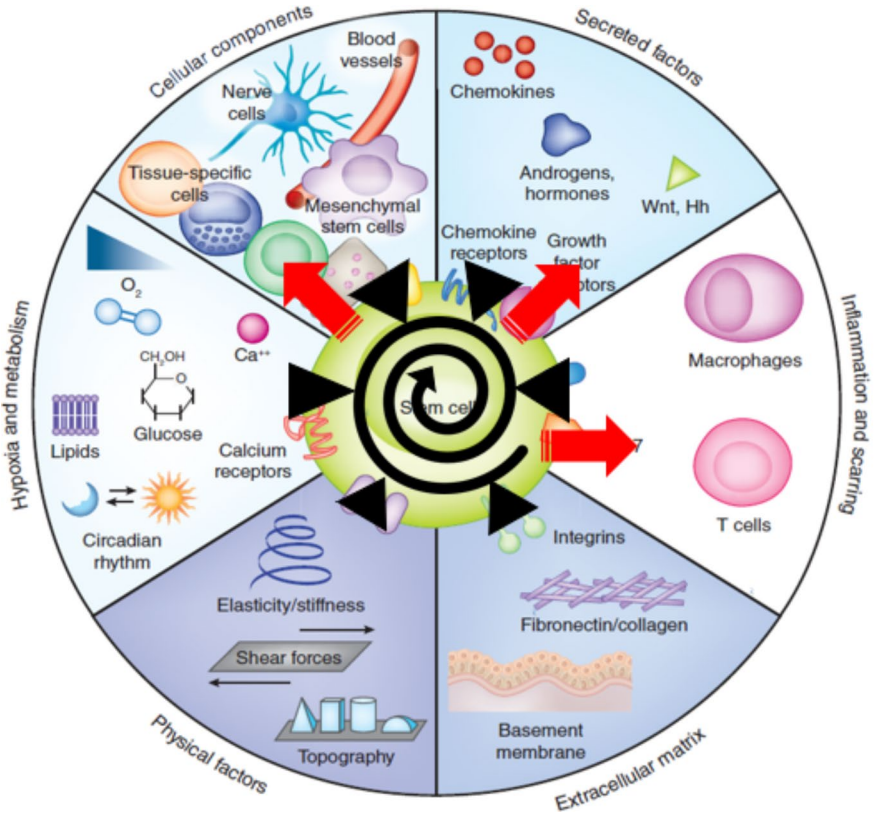

Fig. 1 A reciprocal and asymmetric model of causation between stem cells (at the center) and microenvironment (the area surrounding stem cells). Red straight arrows illustrate the constraining action from stem cells to microenvironment. Black arrows and spiral represent the constraining action from microenvironment to stem cells. It is worth noting that the different visual representation of red and black arrows corresponds to two reciprocal and asymmetric (i.e., qualitatively different) forms of causation. (Figure readapted from Lane et al. 2014)

cell-cell interactions, cell behavior and fate. It is precisely for this reason that ECM and supramolecular structures are an *enabling condition* for the *relational* nature of cells, including the stem ones, in tissue microenvironment[41]: they not only make it possible that stem cells *interact* with the other components of the niche, but also that they are *functionally integrated* with them[42]. Let us therefore explore the causal relationship between ECM and (stem) cells in physiological conditions and then how this relationship changes in pathophysiological conditions according to our case-studies.

In physiological conditions, ECM is characterized by a balance between the different ECM components (i.e., type 1 collagen, fibronectin, proteoglycans and gly-

[41] This consideration applies in general to the relationship between cells and tissues.

[42] Here, by 'physiological integration', we mean that the cellular behavior of stem cells (e.g., metabolism, life cycle, motility, communication) depends on the overall physiological behavior and homeostasis of their microenvironment.

cosaminoglycan chain, adipocytes, metalloproteinases, fibroblasts, and inflammatory cells) that provide the fibers of the interstitial matrix with an optimal *elasticity* and maintain an adequate *thickness* of basement membrane. Fiber elasticity ensures *tensional homeostasis*, which is the ability of cells, tissues, and organs to maintain a homeostatic (set point) level of mechanical stress[43] (Stamenović and Smith 2020). As such, not only is ECM able to resist to a variety of tensile stresses, but it also exerts *mechanical forces* that are transduced into chemical signals that modulate cellular behavior by acting upon membrane receptors. More specifically, *cell adhesion complexes*, consisting of integrins and cytoplasmic proteins (e.g., talin, α-actinin), transduce mechanical stimuli by regulating kinases (e.g., focal adhesion kinase, tyrosine protein kinase CSK) and generating biochemical cascade signals that activate regulatory pathways involved in cell growth, differentiation, and division (Faulk et al. 2014). The thickness of the basement membrane affects *epithelial cell-cell adhesion* and *apical-basal polarity*, which maintain cellular cohesiveness, support epithelial structures, and establish intracellular signaling pathways controlling cell growth, survival, and migration (Chatterjee et al. 2016). In addition to mechanical cues and support function, ECM controls cellular proliferation and differentiation through the spatial organization of *gradients* of *diffusible factors* (e.g., growth factors and cytokines) and their release in the presence of appropriate cell-mediated forces or proteolytic degradation (Rozario and DeSimone 2010).

In turn, cells constrain ECM behavior by controlling *ECM homeostasis* through *negative feedback* mechanisms that "sense changes within the ECM and restore values back to normal" (Humphrey et al. 2014, p. 805). For example, fibroblasts adhere to the ECM and generate tensile stresses to keep tensional homeostasis (Humphrey et al. 2014). Furthermore, cells play a fundamental role in *ECM turnover* inasmuch as their mechanical properties and degree of pre-stress affect mechanical loading that modulates cellular production and the removal of ECM constituents through proteolysis (Humphrey et al. 2014).

In pathophysiological conditions, both the composition and the 3D structure of ECM are badly impaired, usually characterized by the appearance of *inflammatory cells*, a *fibrotic scar*, and *changes in ECM composition*, which alter the *stiffness* of the ECM and the *thickness* of the basement membrane, thus affecting *tensional homeostasis*, *ECM mechanical forces*, *cell-cell adhesion*, and *apical-basal polarity* (Fig. 2). All these features are clearly exemplified by our case-studies, inasmuch as myocardial infarcted and damaged neural tissues exhibit an *inflammatory microenvironment*, which is characterized by an overproduction of myofibroblasts (cardiac infarcted microenvironment) and astrocytes and microglia (damaged neural tissue). In all these cases, the transformation of fibroblasts into myofibroblasts introduces changes in the composition (e.g., an overproduction of glycoproteins or proteoglycans, or the accumulation of proteins) and spatial organization of the ECM, thus altering the mechanical cues that control stem cells fate and behavior (Fig. 2). Furthermore, stiff ECM shrinks the thickness of the basement membrane, thus destabilizing *cell-to-*

[43] ECM stiffness is linearly related to ECM stress, so that "the cellular control of ECM stress is equivalent to controlling ECM stiffness" (Humphrey et al. 2014, p. 805).

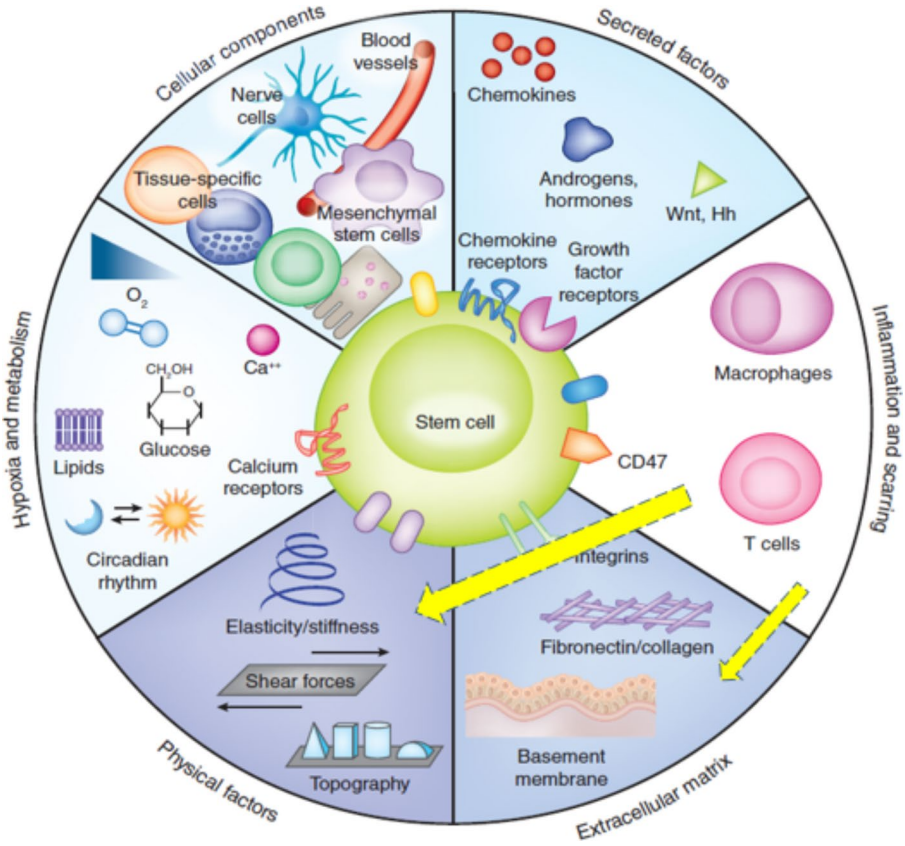

Fig. 2 The alterations of ECM and its biomechanical properties made by inflammation and scarring. Yellow dashed arrows illustrate the causal relationship between inflammatory cells, ECM, and physical factors. The modification of the biomechanical properties of ECM alters the mechanical cues controlling stem cell fate and behavior. (Figure readapted from Lane et al. 2014)

cell adhesion, cell-to-ECM attachment[44] and losing *apical-basal polarity*[45] (Frantz et al. 2010). A further interesting aspect is that stem cell fate is differently modulated depending on the type of microenvironment changes. Indeed, in infarcted cardiac microenvironment, the regenerative potential of stem cell is absent or short-termed, because pro-inflammatory cytokines and ECM stiffness hinder stem cell differentiation and proliferation. Contrariwise, neuropathologic microenvironment can both inhibit and trigger stem cell differentiation and proliferation because of key structural changes of neurodegenerative diseases and brain injuries such as the decrease in ECM stiffness or the activation of neural inflammatory cells.

[44] The alteration of cell-cell and cell-ECM adhesion molecules may promote cell migration and uncontrolled cell growth. In physiological conditions, when non-hematopoietic cells detach from ECM, they undergo a specific kind of apoptotic death (*anoikis*) that prevent them from migrating and invading other tissues. However, this mechanism fails in cancer, thus favoring invasion processes (Paoli et al. 2013).

[45] The loss of apical-basal polarity can favor basal extrusion and alterations in cell extrusion, which ultimately lead to tumorigenesis (Chatterjee et al. 2016).

Our case-studies also show that stem cells constrain and exert a causal power on the microenvironment by releasing *soluble factors* that promote neovascularization (Fig. 1). Stem cells also *modulate* the activity of innate and adaptive *immune cells* (Fig. 1) through the polarization of macrophages or an anti-inflammatory action, and they may *recruit* resident *stem* and *progenitor cells* to induce *cardiomyogenesis* and *neurogenesis*. Moreover, our case-studies suggest that stem cells contribute to the reconstitution and regeneration of ECM composition, structure, and physical properties only *in part*. Indeed, current stem cell therapies act on cellular and non-cellular (i.e., soluble factors) components of the ECM, but they do not act on the proteins and polysaccharides that compose it, nor on its stiffness and tensional homeostasis, nor on the reconstitution of the thickness basement membrane. As a result, the ECM scaffolds of cardiac and neural microenvironment remain damaged, preventing the functional organization of ECM from being fully restored and thus impairing the overall effectiveness of stem cells for the regeneration of infarcted microenvironment and damaged neural tissue.

A further aspect of ECM is its *temporal control* of stem cell dynamics. Depending on the functional state of a specific tissue (e.g., growth, injury, aging), ECM changes its stiffness, density, composition, 3D organization, or the state of activation of their proteins (Cimmino et al. 2018). These changes are transmitted to stem cells through a complex interplay of *biochemical* and *biophysical cues*, giving rise to coordinated cellular motility and differentiation. Collective migration of stem cells is a distinctive feature of tissue remodeling[46] and relies on cell-cell interactions[47] and cell-ECM interactions: the *geometry* and the stiffness of the ECM not only determine *stress gradients* and mechanical cues that *orient* cells in specific ways (e.g., simple laminar flows or vortices), but also *drive* their migration so as to minimize shear stresses and limit exchanges with potential neighbors (Ladoux and Mège 2017) and provide migrating cells with zones of uniform concentrations of cytokines and growth factors, allowing them to stop at their ultimate destination (Walma and Yamada 2020). Furthermore, for stem cells to differentiate, ECM is required to exert a *temporal control* over gene expression patterns of growth factors and matrix molecules in such a way that stem cells differentiate in specific regions at the correct time (Nair et al. 2012; Negrete and Oates 2021).

The reciprocal and asymmetric relationship between stem cells and tissue microenvironment can also be observed in the bone marrow, where hematopoietic stem cells can be transplanted. However, compared to solid tissues, this latter exhibits a semi-solid organization in which the ECM exerts a less rigid (spatial and temporal) control on the behavior of hematopoietic stem cells, which allows them to migrate to free bone marrow niches more easily (Liesveld et al. 2020). This organization of the bone marrow tissue explains why (hematopoietic) stem cell transplantation is potentially more effective in the bone marrow for the treatment of leukemias, compared to that in the cardiac and nervous tissue: although the ECM of the bone marrow regulates hematopoietic stem cells proliferation and differentiation (e.g., through

[46] More generally, the motility of cells is collectively controlled during morphogenesis and carcinogenesis.

[47] The transmission of force from a cell to another is based on the coupling of cell-cell junctions and actomyosin.

ECM proteins, stiffness gradient between endosteal and perivascular niches, and biomechanical forces), they can find their way home to free bone marrow niches, thus finding suitable conditions to survive, proliferate, and differentiate, which give rise to a long-term effective hematopoiesis (Caocci et al. 2017).

A dynamical and relational ontology of the stem cells-microenvironment relationship based on a reciprocal asymmetric causation gives us some clues as to the notion of *stemness*. Laplane (2015, 2016) suggests that there are four possible ways of thinking stemness: two *intrinsic* and two *extrinsic*. Intrinsic view of stemness can be *categorical* and *dispositional*: in the former case, stemness is an intrinsic property of a stem cell *independent* of its environment; in the latter, stemness is an intrinsic property of a stem cell emerging only in the *right environment*. Extrinsic view of stemness support the idea that stemness derives from the specific *interaction* between microenvironment and cells (*relational* views) and that it is not a property of cells but rather of a *system* such as a tissue (*systemic* views).

On the basis of our case-studies, we argue, stemness cannot be a categorical property, because it depends on the interaction between the cell and the microenvironment. This leaves three options that are not mutually compatible if considered at the same epistemic level: *dispositional*, *relational, or systemic*[48]. On the one hand, the fact that stem cells are able to differentiate and proliferate in vitro with the biochemical and mechanical cues provided by an engineered scaffold suggests that stemness could be a physical *disposition inherent* to cells. On the other hand, our case-studies show that the regenerative potential of stem cells is triggered under *specific microenvironmental conditions*, so that in this sense it is *relational*. Indeed, this capacity becomes *effective* when it is *embedded* in a very specific context (i.e., stem cell microenvironment) that constrains the behavior and fate of stem cells and that enables them to interact with the other components of the microenvironment. Such context is effective as far as it embodies specific *topological features* that maintain a polarization of (cells and tissues) shapes and an orientation of the physico-chemical gradients. Accordingly, stemness could be understood as a *systemic property*.

Thus, although we cannot conclude whether stemness is an intrinsic property (i.e., dispositional), or rather an extrinsic one (i.e., relational and systemic)[49], our account adds two important points lacking in Laplane's one: (1) a more explicit description of the microenvironment with a specific focus on the ECM; and (2) the importance of asymmetrical reciprocity. This is very important because it clarifies what one can

[48] From an *epistemic* point of view, the concepts of 'dispositional property' and 'relational and systemic property' are incompatible, because the former refers to an intrinsic property of stem cells, whereas the latter to an extrinsic one (Laplane 2015, 2016). However, from an *ontological* point of view, a dispositional property can be compatible with a relational and systemic property, since the functions of biological entities "are defined primarily by the context they are embedded within, and hence by the web of relations they are part of" (Bertolaso and Ratti 2018, p.1).

[49] As also stressed by an anonymous reviewer, our reciprocal and asymmetrical account of the stem cells-microenvironment relationship could eventually suggest that relational and dispositional views are opposite sides of the same coin: in both cases, the stemness is a context-dependent property; however, the difference lies in that in the first case only a small and defined category of cells can behave as stem cells, whereas in the latter which cell will act as a stem cell is much more flexible.

expect from a tissue depending on the alterations, and what would be required for regeneration to be efficient[50].

The very sense of stemness lies in the *network* of *mutual constraints* between stem cells and their microenvironment that ultimately explains (i) how and why stem cells can correctly proliferate, differentiate, and migrate in a specific niche and (ii) why scientists usually refer to the microenvironment role in terms of a modulatory capacity rather than a direct causal (i.e., mechanistic) influence[51]. This is reflected in the use of terms -quite often in the -ing form- such as 'affect', 'alter', 'promote', 'modulate', 'trigger', 'enable', 'restore', enhance' and 'favor' for qualifying the influence of microenvironment on stem cells. These kinds of evidences, together with other ones about the relevance of the temporal features of biological processes (Bertolaso 2016, cap 2; Loppini et al. 2020; Bertolaso and Longo forthcoming), open the way to a more *dynamic* analysis of the *topological* features that characterize the causal relevance of the context -or microenvironment- in space and time.

In this sense, a *relational* account of stem cells implies a *systemic* one, where 'system' is the spatio-temporal *coupling* between stem cells and their microenvironment. Thus, it is sensible to replace the expression 'stem cell therapy', which is cell-focused, with 'stem cell microenvironment therapy', which is tissue-centered.

6 Concluding remarks

In this paper, we have explored the causal relationship between stem cells and their microenvironment, and consistently with it, we have clarified the notions of stem cell microenvironment and stemness. We have argued that this relation is *reciprocal*, because microenvironment *constrains* stem cell fate (proliferation, differentiation, and migration) through the biochemical interaction of stem cells with cellular and non-cellular (soluble factors) elements of microenvironment, and through the biomechanical and biochemical interplay with ECM. In turn, stem cells *constrain* microenvironment functionality by modulating the behavior of cells (notably, immunomodulatory activity), releasing soluble factors that promote vascularization, and recruiting resident stem and progenitor cells.

At the same time, the reciprocal constraining action of stem cells and microenvironment is *asymmetrical* due to the asymmetrical causal relationship between ECM and stem cells. Indeed, ECM allows stem cells to be *spatially close* to other cells and to *get access to* soluble factors, thus permitting their biochemical interaction. Furthermore, ECM exerts a *spatial, temporal, and mechanical control* over stem cell fate that regulate the regenerative potential of stem cells. This explains why, when ECM is undermined in several pathological conditions, the regenerative potential of stem cells is limited, and stem cells are not able to restore the functional organization of ECM of healthy tissue.

[50] We warmly thank an anonymous referee for having pointed out this aspect.

[51] Bertolaso (2016) describes this difference in causal explanation by distinguishing between a 'by holding' and a 'by doing' causation.

As such, we have proposed that stemness is a dispositional, relational, or systemic property, insofar as the intrinsic nature of stem cells and their regenerative capacities lie in their *causal relationship* with their microenvironment, where 'microenvironment' designates a system of relations among the ECM, cellular, and non-cellular elements. For this reason, what is truly *regenerative* in the use of stem cells is their *interaction* with tissue microenvironment through the modulation of its functional components.

Despite the apparent recognition of the role of the microenvironmental context for the regenerative potential of stem cells, stem cell-based regenerative medicine reveals two main related shortcomings. Firstly, it treats stem cell fate determination as if it were just a matter of cues provided by an appropriate 3D (engineered) scaffold. Actually, this is only partly true, because the very regenerative potential of stem cells does not rely on the physical properties and mechanical cues of whatsoever engineered substrate, but rather on those of the in vivo ECM, which controls not only stem cells gene expression but also their biochemical and biophysical interactions with the cells and soluble factors of microenvironment (see Shimojo et al. 2020). In other words, although an artificial scaffold allows for stem cell fate determination, proliferation, and differentiation, regenerative properties ultimately depend on the constraints imposed by the in vivo ECM of the tissue in which they are engrafted.

Secondly, the primary target of stem cell-based therapies is not the reconstitution of the physical and mechanical properties of the ECM, but rather a modulation of cellular and non-cellular components and a partial reconstitution of some 3D features of the ECM. As a result, stem cell therapies come to a dead end: they pretend to regenerate a damaged tissue without restoring the physical and mechanical properties of the ECM, which is the ultimate source of the regenerative potential of stem cells. Thus, it is no wonder that the impaired ECM structure determines the failure, or at least the short-term success, of cardiovascular and neural regenerative medicine.

In the light of the above, some burning questions arise: how can the 3D structure and mechanical properties of the ECM be fully restored? Which devices can accompany and support stem cells in order to regenerate ECM and microenvironmental functionalities? Far from providing an answer to these difficult issues, we suggest that the theoretical tools provided by *mechanobiology* could be extremely valuable not only for understanding the mechanical properties and forces of the microenvironment, but also, and most importantly, for developing tissue engineering techniques for restoring, or at least improving, the functional organization and mechanical properties of the ECM and hence stem cell fate.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by GM and MB. The first draft of the manuscript was written by GM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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