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Significance of Biotic Factors in Mesenchymal Stem Cell Fate in Regenerative Medicine

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Abstract: Stem and progenitor cell research is a complex and exciting field which promises curative discoveries in numerous areas including cancer, diabetes, and regenerative medicine. Use of biotic factors or growth factors has played an essential role in the development of stem cell research. These biologically active components have been administered into stem cells either to improve or maintain the stem cell proliferation, or to encourage controlled differentiation into more defined cell types. Small molecules such as 6-Bromoindirubin-3′-oxime (BIO), cardiogenol-C, etc can help stem cell research by controlling or influencing the regulatory changes in a controlled manner and to help understand the mechanisms during stem cell differentiation. Extra cellular matrix (ECM) is another significant biotic factor, which mediates cell and tissue behavior by influencing cell-matrix interactions. Thus, in this review we would like to emphasize significance of various growth factors in stem cell research.

Keywords: 6-Bromoindirubin-3′-oxime (BIO), cardiogenol-C, hyaluronic acid, purmorphamine, small synthetic molecules, vascular endothelial growth factor.

1. INTRODUCTION

Stem cell, defined as, a cell which has the capability to self-adapt as well as to differentiate into distinct cellular subtypes [1, 2]. Although stem cells are classified into four main categories, embryonic stem cells are most important as they are able to differentiate into cells representing all three developing germ layers, as well as all the extra embryonic cell types [3, 4]. Adult stem cells have more constrained lineage potential and they are naturally able to repopulate cells residing in their particular cell niche within the tissues. Cancer stem cells are the third category, and recent studies have demonstrated that few cancers contain specific cell subtypes which are responsible for cell explosion within a tumor [5, 6]. Growing these cancerous stem cells separately allows them to differentiate into all types of cells found within the tumor. Induced pluripotent stem cells (iPSCs) are the most recent and important category. These cells are able to procure from somatic cells by reprogramming either by gene modifications or by exposing cells to various factors which have been shown to revert them back to stem cell like cells [7], which can further differentiated into all three germ layers in a similar fashion to embryonic stem cells (Fig. **1**).

Application of bioactive molecules or growth factors play an essential function in the advancement of stem cell research and therapy. These biologically active compounds have administered into stem cells either to improve or to maintain stem cell proliferation, and to encourage controlled differentiation into more defined cell types [8, 9]. Present review provides a detailed study on the use of biologically active molecules, which interact with stem cells and mediate their behavior. Since cells are vulnerable to extreme concentrations of growth factors, determining appropriate concentration of each growth factor is an essential step in controlled differentiation steps. Along with stem cell differentiation studies, this article also focus on the therapeutic benefits of biologically active molecules in construction of three dimensional (3D) tissues/organs, which are supportive in regenerative medicine.

The general trend in regenerative medicine is to use biologically active components that play an active role in 3D tissue regeneration. Therefore, understanding the interactions among biologically active molecules with their surroundings, including cell/tissue fluids allows the selection of best growth factor which enables an enhanced performance [10, 11].

Types of bioactive compounds: The lists of biologically active components that control the stem cell fate are explained in following Fig. (**2**).

2. GROWTH FACTORS

Researchers are exploring the effects of growth factors on stem cell differentiation leaping a one step closer towards understanding differentiation of stem cells into more specialized cells that make up specific tissues such as bone, cardiac tissue, etc [12]. Also these growth factors are responsible for stem cell differentiation into three germ layers namely ectoderm, mesoderm and endoderm. It is proved that, ectoderm give rise to brain and skin; mesoderm give rise to heart,

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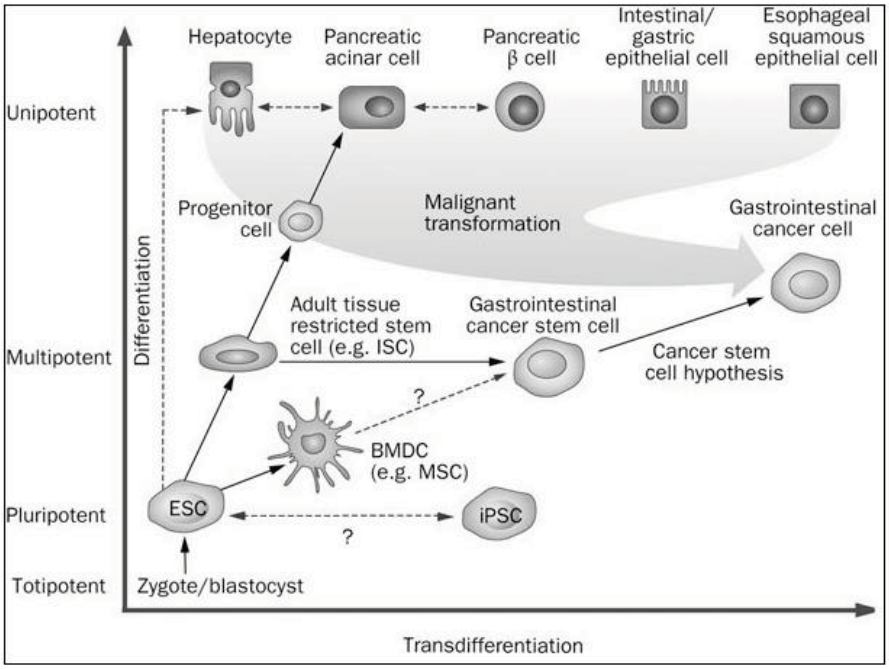

Fig. (1). Schematic illustration of the potency and differentiation status of the different stem cell types [7].

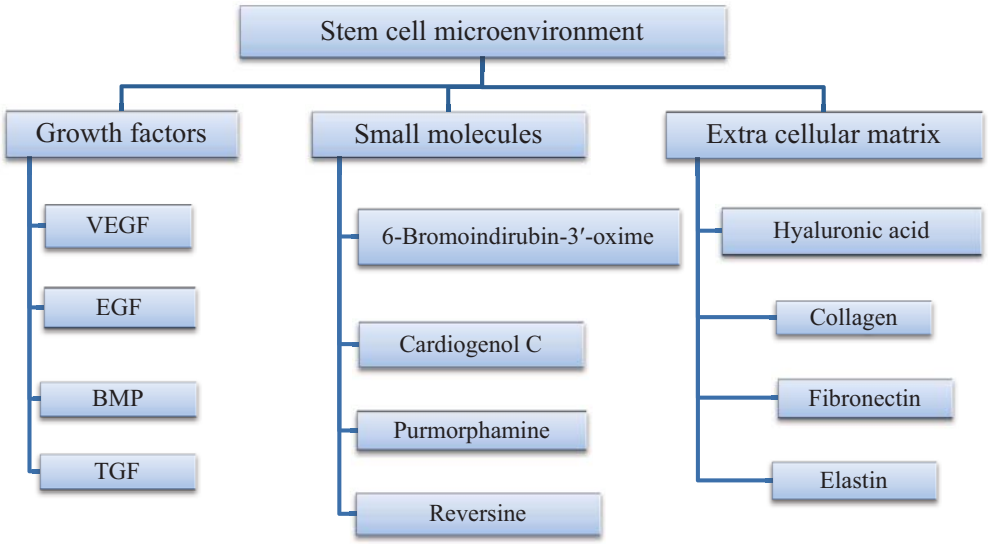

Fig. (2). List of bioactive compounds that determine the stem cell fate during their growth and differentiation.

bone, cartilage and muscles; and endoderm give rise to parts of gastro intestinal tract [11]. Here we will be discussing the role of various growth factors on the stem cell fate.

2.1. Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is an endothelial cell specific mitogen known to induce angiogenesis [13, 17]. Well known *in vitro* activity of VEGF is the skill to endorse growth of vascular endothelial cells derived from arteries, veins, and other lymphatics [14, 15]. It is made clear by the researchers that, VEGF also promotes angiogenesis in 3D *in vitro* models (implants/scaffolds), inducing microvascular endothelial cells to proliferate into collagen gels and form blood capillary like structures [16]. VEGF is also responsible for distinct angiogenic response in a variety of *in vivo* models like chorioallantoic membrane of chicken, cornea of rabbit, and matrigel plug in mice [17, 18]. In addition VEGF also affect the chemotaxis and increased production of B cells [19]. Subsequently, VEGF reported to have hematopoietic effect (promotes RBC growth), inducing colonies by mature subsets of granulocyte macrophage progenitor cells [20]. During bone tissue engineering, bone development and angiogenesis are very closely associated. Therapeutic angiogenesis is a novel approach that promotes new blood vessel formation by delivering angiogenic growth factors like VEGF, which mediate the oxygen and nutrient supply to actively dividing osteoprogenitor cells (osteoblasts) [21, 22].

2.2. Epidermal Growth Factors (EGF)

Members of the epidermal growth factor **(**EGF) family were well known for their ability to stimulate cell proliferation. Also plays vital role in many developmental processes including promoting mitogenesis, differentiation of mesenchymal stem cells and epithelial cells [23]. Generally, EGF released by a cell might be picked up by itself, stimulating its own growth, or by neighboring cells/tissues, stimulating their proliferattion [24, 25]. On the other hand, EGF extensively elevate the proliferation of bone marrow endothelial cells, which are very significant in dicreasing the tumor development and improving vascularization process [26, 27]. Further studies also confim that, EGF could directly spur the growth of stem cells in irradiated bone marrow cells as reported 20 fold higher engraftment when compared to the control group grown without EGF supplements.

It is concluded that EGF family has its significant impact on almost all aspects of stem cell biology like cell proliferation, differentiation, migration and modes of cell delivery. EGF family of ligands appears to be generalized expanders and survival adjuvants while not affecting MSC's differentiation. Moreover, presenting EGF in a tethered form has been studied with respect to sustained signaling, making it one of the growth factors of foremost importance [28].

2.3. Bone Morphogenic Proteins (BMP)

Bone morphogenic proteins are group of peptides belong to transforming growth factor beta (TGFβ) super family. BMPs were generally involved in guiding the physiological activities like cell multiplication, differentiation, and death [29]. Also, BMPs participate in adult tissue repair in which regulation of stem cell performance is prominent (Table **1**).

Table 1. Types of BMPs and their significance in human physiology.

Class of BMPs	Function	Reference
BMP1	Metallo-protease play significant role in cartilage synthesis	[35]
BMP2	Disulfide linked homodimer play key role in osteoblast differentiation	[36]
BMP3	Promotes bone synthesis	[37]
BMP4	Regulates the development of epidermis and play a role in fracture healing	[38]
BMP5	Significant in cartilage development	[39]
BMP6	Maintain the joint integrity in adults	[40]

Experimental studies on BMPs have reported their existence into more than 20 subtypes, few of them have a discrete function while others have overlapping functions depending on their interaction with different receptors and tissues in which they are differentially expressed [30]. For example, in embryonic stem cells (ESCs), BMP signals emerges to be necessary for their self renewal but this step is dependent on its ability to chunk neuronal differentiation in addition to its ability to promote non-neuronal differentiation [31, 32]. While MSCs differentiation, BMP signals promote osteoblast differentiation through Bmpr1b receptor [33]. In case of intestinal stem cells (ISCs), BMPs inhibits stem cell expression and differentiation; whereas in hematopoietic stem cells (HSCs), BMPs restricts stem cell numbers by minimizing the niche size [34]. A decisive and relative review on BMPs in different growth conditions and with different stem cell types is necessary for a balanced view towards BMPs role in the regulation of stem cell fate, and thus will offer essential components into understanding the complex signaling pathways of stem cell self renewal and differentiation.

2.4. Transforming Growth Factors (TGF)

Transforming growth factors and its super family, including activins and BMPs have been widely applied in the growth and maintenance of different organs, in which stem cells play significant roles [41, 42]. It is clear from the literature that, signals from TGF family have shown to influence the gene expression profiling of both embryonic and adult stem cells. Especially, Nodal signals are prone to have important role in preservation of ESC characteristics [43].

Fig. (**4**) depicts the correlation of TGF signals in the specification of germ layers between *in vivo* and *in vitro* ESC differentiation systems. Ectoderm is differentiated from human and mouse ESCs in the absence of TGF signals, while primitive streak differentiation is persuaded by activin/Nodal and BMPs. Whereas, mesodermal differentiation occurs in the presence of BMPs and medium range activin/Nodal signals [44]. In this regard, it is clear that TGF family members are very significant during embryogenesis and somatic cell differentiation [45]. Better understanding of TGF role in embryonic and somatic cell functionalities will support both basic and applied areas of stem cell research.

The TGF super family has its role in multilineage differentiation of mesenchymal progenitor cells from adult tissues such as bone marrow and umbilical cord blood. Thus making them functional to investigate mechanisms that regulate the tissue development and regeneration, such as cartilage development [46, 47]. From the literature it is proved that TGFβ promotes the intracellular cascades, especially JNK (c-Jun N-terminal kinases), p38, ERK-1 (extracellular signal regulated kinases-1) and MAP kinases which promote the cartilage gene expression [46].

3. SMALL MOLECULES IN STEM CELL RESEARCH

Conventionally, stem cell cultures are grown on serum containing and serum free media. Inconsistent results between experiments in above cases are due to variation in nutrient concentration between media batches [48]. These issues can be effectively answered by 'small molecules' that can really help stem cell cultures and they are not very hard to make. In near future, they may become a part of standard media components (Table **2**).

3.1. 6-Bromoindirubin-3′-Oxime (BIO)

6-Bromoindirubin-3′-oxime (BIO) is glycogen synthase kinase 3 (GSK-3) inhibitor which activates the WNT signaling pathway and also acts as regulator for several other cell

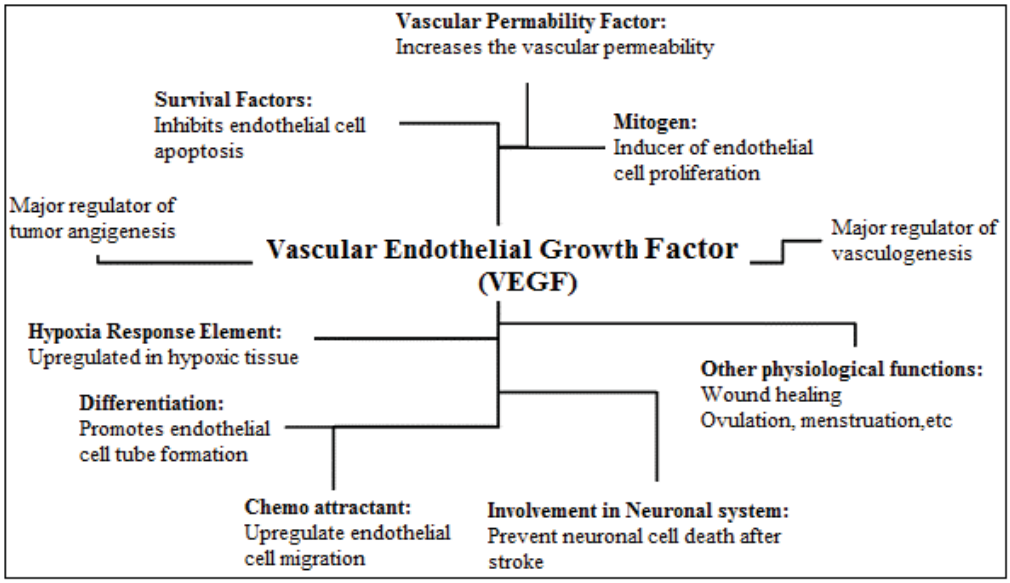

Fig. (3). Diverse functions of vascular endothelial growth factors (VEGF).

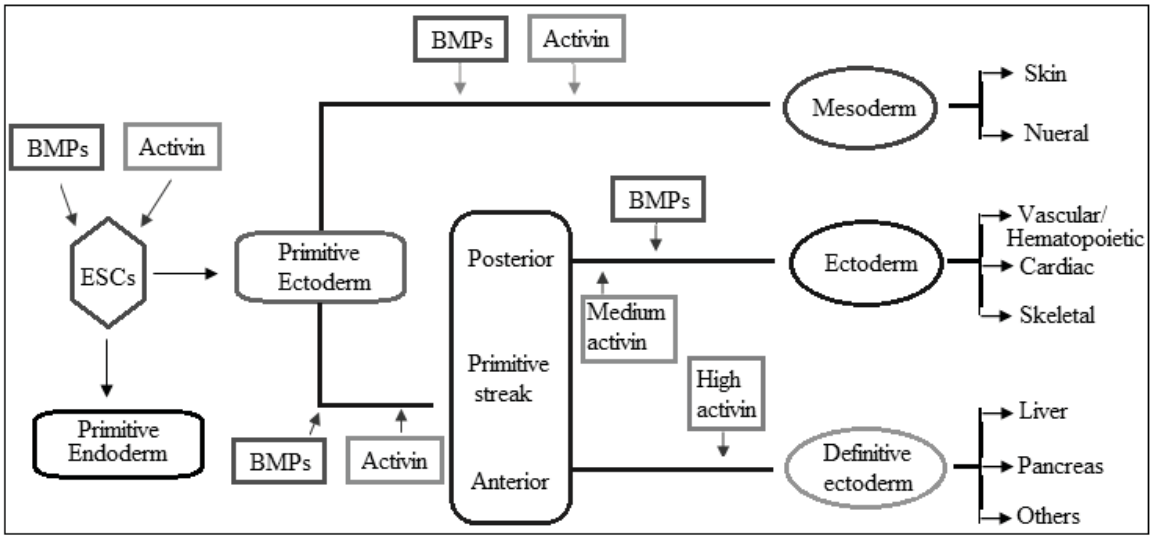

Fig. (4). Schematic of TGF members in stem cell multiplication and differentiation.

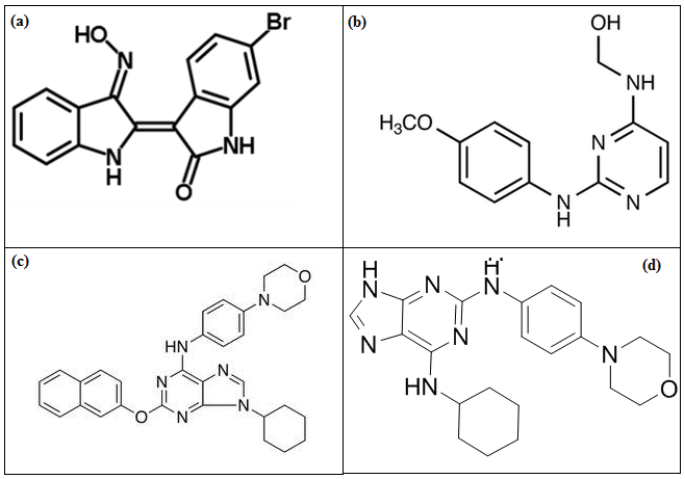

Fig. (5). Structure of (**a**) 6-bromoindirubin-3′-oxime; (**b**) Cardiogenol C; (**c**) Purmorphamine; (**d**) Reversine.

Table 2. List of small molecules affecting the renewal and differentiation of stem cells.

Cell Source	Small Molecules	Mode of Action	Reference
hESC, mESC	BIO (6-Bromoindirubin -3′-oxime)	Activates Wnt signalling which is followed by Oct3/4 expression; Maintains ESC self-renewal	[49]
hESC	Purmorphamine	Replaces Shh in ventral spinal generation	[50]
mESC	Retinol	Maintains feeder-independent self-renewal	[51]
mESC	Pluripotin/SC1	Promotes self-renewal	[52]
aNSC	Isoxazoles	Induces neuronal differentiation, blocks astrocyte differentiation	[53]
hESC	Pinacidil	Kinase inhibitor (ROCK2, PRK2 and others), enhances ESC survival	[54]
hESC	Thiazovivin	ROCK inhibitor, enhances ESC survival	[55]
hESC, hiPSC	Dorsomorphin (Compound C)	BMP inhibitor promotes neural differentiation in combination with SB-431542. TGF-β inhibitor, enhances neural differentiation	[56]

signaling pathways [57]. It is also competent of maintaining the pluripotency of embryonic stem cells both from humans and mice. BIO maintains the undifferentiated status of mammalian ESCs by activating series of key downstream factors, which finally activates transcriptional genes like NANOG and POU5F1 (OCT4). Thus, GSK-3 inhibitor can finally control the expression of vital ESC transcription factors [58]. In addition, GSK-3 inhibitor may severely damage the differentiation of ESCs into three germ layers by preventing the ubiquitination, and further degradation of catenin within the cytoplasm [59]. Though BIO is having significant effect on ESCs, due to complex multiple signaling pathways in BIO, very little is known at present about combination of BIO is effective in the derivation of embryonic stem cell colonies.

3.1.1. Cardiogenol C

Cardiogenol C, a novel small molecule from a class of 2, 4-diaminopyrimidine compounds, which could specially induce ESCs to differentiate into the cardiomyocytes [60]. It is reported that up to 90% of the Cardiogenol C treated cells positively expressed Mef2, GATA4, and Nkx2.5 transcriptional factors, which are involved in cardiogenesis [61]. Till to date, effect of Cardiogenol C on adult stem cell differentiation into cardiomyocytes is not reported. Also, it is still not clear how this molecule works on the group of proteins that it targets. Currently lot of research is going on to identify the molecular targets for cardiogenol C [62, 63]. These experimental studies may disclose the molecular mechanisms of cardiomyogenesis and the role of cardiogenol C in it, which ultimately assist the relevance of ESCs to repair the damaged myocardium in acute heart diseases.

3.1.2. Purmorphamine

Purmorphamine (2-(1-Naphthoxy)-6-(morpholinoanilino)-9-cyclohexylpurine) was the primary small molecule agonist prepared for the protein smoothening, a vital part of the hedgehog signaling pathway. Puromorphamine is also responsible for brain development and bone growth as well as other additional functions in the body [64, 65]. These puromorpamines are heterocyclic small molecules having morpholinoaniline substitution at the C6 position of the purine, with osteogenic induction activity were first developed by Wu *et al.* 2004 [66]. Puromorphamine has increased alkaline phosphatase (ALP) activity in case of mouse preosteoblasts cells and mouse embryonic mesoderm fibroblast. In addition, puromorphamine also up regulates the expression of bone transcription factors (BTFs), which influences the cell synthesis activity as revealed by an elevated total protein content [67]. This higher protein expression leads to higher bone like nodulation in the differentiating osteoblasts, suggesting the purmorphamine mechanism of action by two proposed methods: (i) stimulating the protein synthesis in osteoprogenitor cells and matrix mineralization by these cells, and (ii) improving the proliferation rate in osteoprogenitor cells, and therefore the nodulation by these cells.

3.1.3. Reversine

Reversine or N6 cyclohexyl-N2- [4-(4- morpholinyl) phenyl]-1H- purine-2, 6-diamine is a small molecule, which is a purine derivative. Reversine is the first synthetic, low molecular weight, easily permeable small molecule isolated, which influences the lineage devoted differentiation of multipotent progenitors [68] first isolated in 2003 by a group of scientist from Scripps Research Institute, La Jolla, USA [69]. Reversine molecules are also well known for their dedifferentiation potential of partially differentiated cells (myoblasts) into confluent multipotent progenitor cells. These cells can be further differentiate into mesenchymal lineages like osteoblasts (bone forming cells), adipocytes (fat cells) by supplying required growth supplements. Reversine also shown to have anticancer activity in the preclinical studies and clinical trials are under progress. These small molecules can potentially inhibit the cancer growth and induce cell death in various cancer cell lines including leukemia [70]. It is also quite impressive that, reversine is having more affinity towards cancer cells than healthy cells. These results made reversine a potential alternative in cancer chemotherapy.

Reversine is highly soluble in organic solvents like dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) with a solubility range between 10 to 20 mg/mL. Solubility

of reversine in buffers is always a difficult task and the better alternative way of dissolving reversine in buffer is initial dissolution in DMF followed by further dilution in buffer [68, 71]. Reversine is also heat and moisture sensitive compound (stored at 4^{o}C), which on decomposition yields hazardous byproducts, namely carbon monoxide, carbon dioxide, and nitrogen oxide.

4. EXTRACELLULAR MATRIX (ECM)

Extracellular matrix is the 3D micro environment, appreciated as a key regulator of cell and tissue behavior by mediating cell-matrix interactions, cytoskeletal structure, and integrin assisted signaling [72]. In general, ECM is made of proteins like hyaluronic acid, collagen, fibronectin and elastin which serve as store house of growth factors and cytokines [73]. The organization of ECM also influences the degree of cell attachment. Thus, cell/matrix interactions can be closely evaluated by examining individual components in detail.

4.1. Hyaluronic Acid

Hyaluronic acid (HA) is an anionic, non-sulfated glycosaminoglycan distributed throughout cartilage, epithelial, neuronal, and connective tissues. Due to its biocompatibility, HA may interact with surface receptors of stem cells and could influence their differentiation [74]. Several review articles previously elucidated HA's biological functions in medical treatments, wound healing, and in the drug delivery procedures [75]. Principally in tissue engineering, HA matrix is better known in fabrication of 3D scaffolds for hard tissues like cartilage and bone regeneration. In this case HA scaffolds can bind to proteins and cells through cell surface receptors such as CD44, ICAM-1 (Intercellular Adhesion Molecule-1) (or) CD54, and RHAMM (Hyaluronan-mediated motility receptor) [76]. In addition, we can engineer the back bone HA molecules by introducing functional groups for specific tissue engineering applications. Especially, while treating chronic wounds in patients with worse healing (diabetic patients), HA based scaffolds are ideal due to their ability to sustain hydrated environment conducive for cell permeation [77]. Thus, HA can be a principle bioactive matrix material during fabrication of tissue engineering scaffolds.

4.2. Collagen

Collagen is the most abundant proteins distributed in the ECM of skin, connective tissue, and bone, representing about 25% of the total dry weight of the individual [78, 79]. There are about 29 distinct collagen types characterized and all exhibits a classic triple helical structure. Collagen molecules are comprised of three α-chains assembled to give fibrous structures and collagen types I, II, III, V and XI are the best studied with physiological importance [80]. There are around twenty plus different α-chains reported based on their protein conformation, each produced by a unique gene. The combination of these chains, in sets of three gathered to gives rise to twenty nine different types of collagens, currently known [81, 82].

For cell based tissue engineering approaches we can use decellularized 3D collagen matrix or collagen blended with other bioactive components like glycosaminoglycans, chitin, elastin [83]. On the other hand, cross linking of inorganic components like hydroxyapatite and calcium phosphate with type-I collagen has improved the mechanical properties of scaffolds for bone tissue engineering [84-86]. In contrast, collagen scaffolds for cartilage regeneration tend to be more plastic and are ideally made of type-II collagen.

4.3. Fibronectin

Fibronectin is a disulfide linked dimeric glycoprotein exists in many cell types at a concentration of about 300 μg/mL in blood plasma [87]. Its interactions with fibrin, heparin, collagen, and cell surface receptors of the integrin family are accountable for cell adhesion, migration, and embryonic differentiation [88]. Further studies on fibronectin resulted in improved cell migration and spreading in comparison with the RGD (Arg-Gly-Asp) motif alone. It shows that fibronectin has supplementary roles through other synergistic binding factors, such as the amino acid sequence 'Pro-His-Ser-Arg-Asp' [89]. In addition, fibrous fibronectin biomaterials are prepared in the form of cables and mats, used in the repair of peripheral nerves or injured spinal cord [90].

4.4. Elastin

Elastin is a connective tissue, and a vital part that influences elasticity of the body parts, skin, cartilage, lungs and arteries [91]. Tropoelastin, another significant protein, a precursor protein of elastin, and elastin like peptides in their potential to self assemble under physiological conditions [92]. This is the basis for coacervation, which probably leads to alignment of tropoelastin molecules previous to intermolecular cross linking.

Ligaments contains high elastin, comprises up to 70% by dry weight, followed by arteries, 50%, and lungs, 30%. For tissue engineering applications, highly elastic and biocompatible scaffolds can be prepared by incorporating elastin with other proteinaceous materials [93]. Pure elastin biomaterials also used in autografts, allografts, syngraft, xenografts, decellularised extracellular matrix, and in purified elastin preparations [94, 95] and the best example for pure elastin autograft is coronary artery bypass surgery.

CONCLUSION

Prospects of cell based therapies depends on prevention of *in vivo* teratoma/tumor formation, in particular when using human stem cells (hSCs), such as induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). Fate of hSCs *in vivo* is tightly regulated by various physiological agents that can effectively inhibit anti-apoptotic factors, leading to selective and efficient proliferation and differentiation of stem cells. Upon conclusion, various biotic factors including growth factors, small molecules and ECM components that regulate the target pathway(s) for safe stem cell based therapies.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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