Review Paper: Embryonic Stem Cell and Osteogenic Differentiation

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ABSTRACT

Bone tissue engineering has been one of the most promising areas of research, providing a potential clinical application to cure bone defects. Recently, various stem cells, including embryonic stem cells (ESCs), bone marrow-derived mesenchymal stem cells (BM-MSCs), umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs), adipose tissue-derived stem cells (ADSCs), muscle-derived stem cells (MDSCs), and dental pulp stem cells (DPSCs) have received extensive attention in the field of bone tissue engineering due to their distinct biological capability to differentiate into osteogenic lineages. Application of these stem cells to bone tissue engineering requires their in vitro differentiation into bone forming cells, osteoblasts. For this purpose, efficient in vitro differentiation towards osteogenic lineage requires the development of well-defined and proficient protocols. This protocol would reduce the likelihood of spontaneous differentiation into divergent lineages and increase the available cell source for application to bone tissue engineering therapies. This review article critically examines the various experimental strategies used to direct the differentiation of ESC, BM-MSC, UCB-MSC, ADSC, MDSC, and DPSC towards osteogenic lineages and their potential applications in tissue engineering, particularly in the regeneration of bone.

1. Introduction

Millions of patients worldwide suffer from bone diseases or defects such as osteosacoma, osteoporosis, and bone fractures. To date, regeneration of bone tissue has been an important issue for biological repair in the field of regenerative medicine.

Recently, bone tissue engineering, an interdisciplinary field at the intersection of engineering, biology, and medicine, has emerged as one of the most promising ap-

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proaches to develop biological bone substitutes that restore, maintain, or improve bone tissue function. Regarding the aging population and shortage of donor tissues, various stem cells are considered as potential sources for bone tissue engineering [1] (Figure 1). Recently, manipulation of culture conditions has been researched [2] for directing the differentiation of various stem cells such as embryonic stem cells (ESCs), bone marrow (BM)-derived or umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs), adipose tissue-derived stem cells (ADSCs), muscle-derived stem cells (MDSCs), and dental pulp stem cells (DPSCs) to form bone-forming cells.

In addition, these stem cells have been used to generate three dimensional (3D) biological bone constructs in a suitable biomaterial and cultivation system. In this review, we summarize recent culture strategies for osteogenic differentiation of ESCs, BM-MSCs, UCB-MSCs, ADSCs, MDSCs, and DPSCs and their application to bone tissue engineering [3].

2. Discussion

The first derivation of a pluripotent mouse ESC line from mouse embryo was reported in 1981 [4]. ESCs can be derived from the inner cell mass of the blastocyst. These cells possess unlimited self-renewal activity and turn into any cell lineages [5]. These ESCs characteristics allow them to be among the most useful cell sources for tissue engineering and regenerative medicine. Lineage specific differentiation of ESCs can be directed under specific culture conditions and by manipulating the microenvironment [6].

Recently, considerable attention has been devoted to direct ESC differentiation into osteogenic lineage. This research has highlighted the potential use of ESCs in the field of bone tissue engineering [7]. During in vivo embryogenesis, undifferentiated ESCs initiate early differentiation into 3 primary germ layers: ectoderm, mesoderm, and endoderm through gastrulation at an early stage of development [8]. Osteogenic lineage cells with bone forming capacity are derived from the somatic mesoderm or the ectomesenchymal cells of the neural crest. These are considered to be differentiated from the mesodermal progenitor cells or mesenchymal progenitor cells [9].

Hence, the induction and enhancement of in vitro mesoderm formation has been the objective of numerous studies for deriving sequential differentiation of meso-

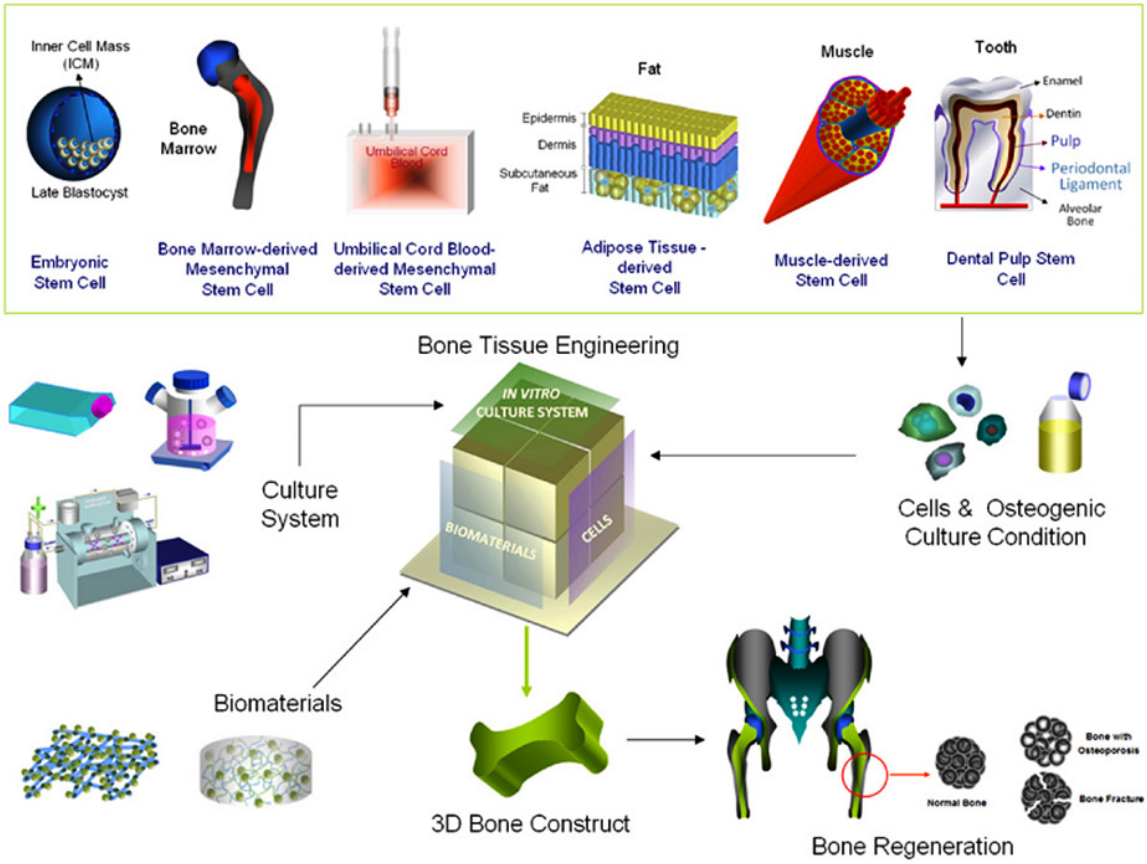

Figure 1. Stem cells-based bone tissue engineering.

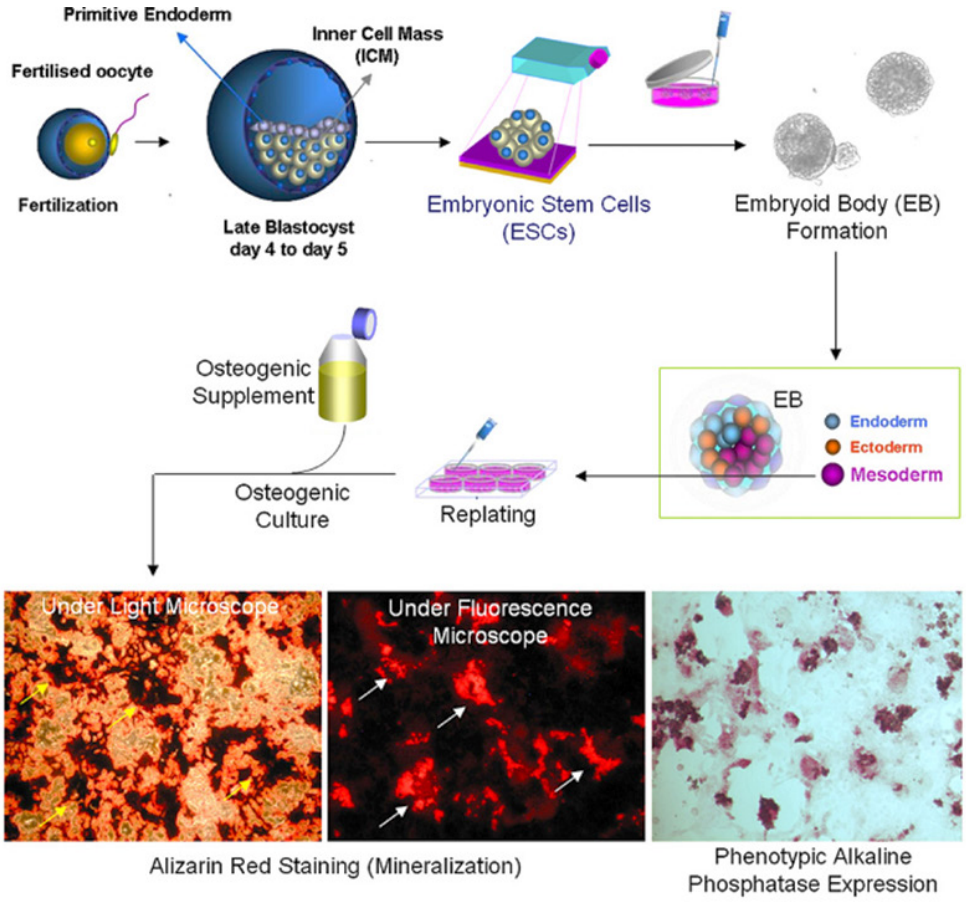

Figure 2. Culture strategy for osteogenic differentiation of ESCs.

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dermal lineage cells such as osteoblasts, chondrocytes, cardiomyocytes, and so on [10]. In vitro differentiation of ESCs can be induced by removal of the feeder cell layer or the leukemia inhibitory factor (LIF) in the case of murine ESCs, which simultaneously causes the formation of 3D cell aggregates known as "embryoid bodies" (EBs) [11].

During in vitro EB formation, ESCs enter a similar differentiation pathway similar to in vivo early development [28]. These cell groups display regional specific differentiation into derivatives of the 3 germ layers: mesoderm, ectoderm, and endoderm [12]. The majority of current approaches for in vitro osteogenic differentiation employ EB formation, which results in spontaneous mesoderm formation as a prerequisite for terminal differentiation toward osteogenic lineages (Figure 2).

After EB formation, EBs or dissociated single cells from EBs are replaced onto tissue culture plates, and a number of molecules have been applied to induce in vitro osteogenic lineage differentiation from spontaneously formed mesoderm within EBs [13]. For instance, addition of supplements such as β-glycerophosphate, ascorbic acid, dexamethasone, retinoic acid, and 1,25-hydroxy vitamin D3 will result in increased differentiation of ESCs along the osteogenic pathway [14]. Furthermore, peptide or extracellular matrix (ECM) components such as leucine-rich amelogenin peptide and fibronectin induces osteogenic differentiation of mouse ESCs or human ESC-derived MSCs. This differentiation has been characterized by distinct osteogenic gene expression profiles, mineralization activity, and animal studies [15].

In other approaches, there has been a great deal of research dealing with the enhancement of mesoderm formation to achieve more precursor cells. This objective has been accomplished by co-culture with hepatic cells or by the use of conditioned medium from hepatic cells [16].

This principle operates on the fact that the visceral endoderm plays an important role in inducing mesoderm formation during in vivo gastrulation [17], and hepatic cells are known to be very similar with visceral endoderm in their biological function [1]. Furthermore, culturing ESCs in human hepatocarcinoma cell line (HepG2)-derived conditioned medium enhances meso-

derm formation, thus resulting in increased osteogenic differentiation in the presence of osteogenic supplements such as β-glycerophosphate, ascorbic acid, and dexamethasone [19]. Also, the limited control of lineage specific differentiation of ESCs within EBs, caused by the spontaneous formation of all 3 germ layers due to unknown mechanism(s) [20], might produce a limited yield of the cell type of interest. This demands simpler, more efficient, and convenient culture strategies by bypassing EB formation.

Recent reports have demonstrated that the direct differentiation of ESCs into osteogenic lineage cells was possible without EB formation by plating ESCs or HepG2 conditioned medium-treated ESCs directly onto tissue culture plates as a single cell suspension and by culturing them in the presence of β-glycerophosphate, ascorbic acid, and dexamethasone [20]. According to these findings, this may be a good culture strategy for applying functional ESC-derived osteogenic cells effectively to bone tissue engineering.

3. Conclusion

Based on extensive research on in vitro osteogenic differentiation of ESCs, they have shown great potential for bone tissue regeneration in combination with 3D polymeric scaffolds and appropriate culture systems.

Recently, a comparative study reported that the expression of osteogenic markers such as alkaline phosphatase and osteocalcin were enhanced highly in human ESC culture on 3D poly(lactide-co-glycolide) (PLGA) scaffolds in comparison with the same cells cultured on a 2D culture plate. In addition, nanofibrous structures, made of poly(l-lactic acid) (PLLA), influence favorably the osteogenic differentiation of ESCs both in 2D and 3D culture systems [21].

To date, various 3D scaffolds have been used for the formation of ESC-based 3D bone tissue-like constructs. For instance, self-assembling peptides made of commercially available peptides [22] were used to encapsulate ESC-derived EBs and the entrapped EBs within these hydrogels differentiated into osteoblast-like cells. At the final stages, these cells form mineralized bone-like tissues [24].

Another approach for ESC-based 3D bone tissue generation was to develop bone morphogenic protein (BMP)-inoculated 3D scaffolds, composed of PLGA and hydroxyapatite, as an ESC-derived osteoblasts delivery vehicle for generating bone-like tissue in vivo. Successful bone tissue formation by ESC-derived osteoblasts was achieved in studies involving subcutaneous implantation into immune deficient mice [25].

Besides 3D culture systems, bioreactor culture systems have also been recently applied to fabricate in vitro 3D bone-like tissue constructs by enhancing the mass transport of nutrients and providing a suspension culture environment [26], which facilitates 3D tissue formation. Finally, 3D bone-like tissue could be generated by culturing alginate-encapsulated mESCs or alginate-encapsulated mESCs on bioactive glass within a high aspect ratio vessel (HARV) bioreactor [27].

These findings have significant implications as well as potential applications for bone tissue engineering where ESCs can be used for the fabrication of tissue-engineered bone in vitro.

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Conflicts of Interests

The authors declared no conflict of interest.

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