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Review

The potential of human fetal mesenchymal stem cells for off-the-shelf bone tissue engineering application

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ABSTRACT

Mesenchymal stem cells (MSCs) have become one of the most promising cell sources for bone tissue engineering (BTE) applications. In this review, we first highlight recent progress in the understanding of MSC biology, their in vivo niche, multi-faceted contribution to fracture healing and bone re-modelling, and their role in BTE. A literature review from clinicaltrials.gov and Pubmed on clinical usage of MSC for both orthopedic and non-orthopedic indications suggests that translational use of MSC for BTE indications is likely to bear fruit in the ensuing decade. Last, we disscuss the profound influence of ontological and antomical origins of MSC on their proliferation and osteogenesis and demonstrated human fetal MSC (hfMSC) as a superior cellular candidate for off-the-shelf BTE applications. This relates to their superior proliferation capacity, more robust osteogenic potential and lower immunogenecity, as compared to MSC from perinatal and postnatal sources. Furthermore, we discuss our experience in developing a hfMSC based BTE strategy with the integrated use of bioreactor-based dynamic priming within macroporous scaffolds, now ready for evaluation in clinical trials. In conclusion, hfMSC is likely the most promising cell source for allogeneic based BTE application, with proven advantages compared to other MSC based ones.

1. Introduction

Despite the capacity of the human skeletal system to rejuvenate itself, non-union bony fractures remain a major clinical challenge, requiring the use of bone grafts to achieve defect healing [1]. Accordingly, bone has become the second most transplanted tissue in the world, with more than 1.5 million grafts in United States annually [2]. Currently-used bone grafts such as autografts, allografts and synthetic grafts are unable to fulfill the increasing clinical demand for effective bone grafts, because of their inherent drawbacks, such as the limited availability and donor site morbidity of autografts [3,4], the reduced healing potential and risk of pathogen transmission with allografts [5], and the inferior healing rate and lack of remodeling capacity associated with use of synthetic grafts [1]. In order to overcome the deficiencies of current bone grafts and fulfill the unmet clinical need, tissue engineering strategies have been pursued to develop tissue engineered bone grafts (TEBG) with both off-the-shelf availability and potent bone repairing capacity.

2. Cell-based bone tissue engineering approach

Since the description of Tissue Engineering by Langer and Vacanti in 1993 [6], a number of bone tissue engineering (BTE) strategies have been explored, which can be broadly categorized into cell-based or growth factor (GF)-based approaches, based on

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the manner in which osteogenic cells are introduced to the repair site (Table 1). Cell-based approaches deliver exogenous osteogenic cells to the defect site, relying on growth factors secreted by donor cells to boost the defect healing process; in contrast, GF based approaches supplement exogenous growth factors which mobilize endogenous osteogenic cells to the injury site to promote bone regeneration [7].

There are several cogent arguments in support of a cell-based approach for BTE (Table 1). Firstly, while GF based approaches afford immediate availability and simplicity in design, their efficacy is largely dependent upon the potency of the endogenous pool of osteogenic cells, which may be diminished in conditions such as severe trauma, poorly-controlled diabetes, chronic tobacco use, irradiation, aging, osteoporosis or other metabolic derangements [8,9]. Conversely, cell-based approaches work independently of endogenous osteogenic cells, and can therefore achieve better clinical outcome in patients with a diminished pool of osteogenic progenitors [9]. Secondly, GF based approaches are limited by the short half-life of GFs in vivo as well as the technical difficulty associated with supplying different GFs at optimal dosages in tandem with the physiological requirements at different stages of bone regeneration [10–12]. This is not an issue with the use of exogenous osteogenic cells introduced through cell-based approaches, which secrete a wide spectrum of GF at physiological doses at the physiological temporal-spatial micro-gradients required in the bone healing process [13]. Last but not least, the additional challenges with GF based approaches include their prohibitive cost, uncertain dosage due to the large variation of GF efficacy between animals and humans (up to 100 fold), and safety concerns, epitomized by the fatal outcomes with Medtronic's Infuse Bone Graft (recombinant Bone Morphogenetic Protein 2) in 2008 [14]. In contrast, cell-based approaches may be inherently safer due to their physiological regulatory capacities.

Cell-based BTE approaches seek to generate effective bone grafts within a biological microenvironment suitable for bone regeneration through seeding and priming osteogenic cells onto a scaffold matrix. This bone regeneration potential of cell-based TEBG can be explained by the "protected bone regeneration" theory, in which the success of bone healing is determined by three prerequisites: (i) an adequate blood supply, (ii) a critical mass of osteogenic cells, and (iii) a protected healing space [15]. Bone has considerable regenerative capacity to repair smaller defects by vascularization of the defect area, and transportation of fluids (including nutrients and growth factors) and cells (including osteogenic cells) to the defect site to facilitate bone regeneration (Fig. 1A). However, in the case of critical sized defects (CSD), these biological processes are interrupted by the interjection of soft tissues filling up the defect space, and rapid in-growth of fibrous tissue, leading ultimately to non-union of the fracture (Fig. 1B). The institution of a TEBG into a CSD maintains the integrity of the defect space by bearing the mechanical load and allowing infiltration of newly developing blood vessels, a crucial factor in the fracture healing process. In addition, the saturation of osteogenic cells and highly mineralized ECM in TEBG retards the invasion of fibrous tissue into the defect. Finally, osteogenic cells loaded within a TEBG will not only directly contribute to new bone formation, but can contribute towards vascularization and recruitment of native osteogenic cells from surrounding environments through the release of vaso-active growth factors and cytokines. In tandem with the bone regeneration process, biodegradable scaffolds will eventually be replaced by newly-formed bone tissue, leading to the complete healing of CSD(Fig. 1C).

Table 1
Growth factor versus Cell-based approach.

	GF based approach	Cell-based approach
Osteogenic cell origin	Endogenous	Exogenous
Growth factor origin	Exogenous	Endogenous
Off-the-shelf availability	Yes	Autologous therapy: No Allogeneic therapy: Yes
Duration in vivo	Short half-life	Extended duration
Cost	+++	+
Healing efficacy	Highly dependent on endogenous stem cell pool Mono or multiple GF delivery	Independent of endogenous stem cell pool Wide spectrum of GF secreted sequentially
Safety	Uncertain dosage and safety concerns	Physiological doses & regulation

3. Comparison of cell sources for BTE

The various cell types investigated for BTE applications can be categorized according to their differentiation status into fresh bone marrow, undifferentiated stem cells and differentiated osteoblasts [9,16] (Table 2). An ideal cellular source for cell-based BTE approaches should be non-immunogenic, non-tumorigenic, possess off-the-shelf availability, and potent proliferative and osteogenic potential [17].

Over the past three decades, the use of fresh un-manipulated bone marrow (BM) for treatment of fracture non-union has been widely practiced [18–20], with the advantages of a simple harvesting procedure and the lack of immune rejection with the use of autologous cells [9]. However, its efficacy is largely determined by the quality and quantity of the osteogenic progenitors residing within the BM [21], which can be significantly compromised in elderly patients or patients with comorbid diseases, making it least applicable in situations where it is most needed [16]. Moreover, the use of allogeneic BM without immunosuppression for fracture repair is inappropriate due to immune rejection of non-HLA-matched donor cells [22].

Osteoblasts are the major bone forming cells in vivo, demonstrating more efficacious bone healing qualities compared to fresh BM [9,23,24]. However, the isolation of the osteoblasts is considerably more demanding and complicated than either fresh BM or mesenchymal stem cells (MSC). In addition, they have limited capacity for proliferation, and hence the generation of clinically adequate numbers of cells is a significant challenge [9,16,24].

A variety of stems cell types have been evaluated for BTE application, including induced Pluripotent Stem Cells (iPSC), Embryonic Stem Cells (ESC) and MSC. iPSC and ESC are pluripotent stem cells which can differentiate into cell types of all three germ layers, including that of the osteogenic lineage [25–28]. Moreover, iPSC and ESC can be expanded significantly in vitro, which is necessary for achieving clinically-relevant cell numbers for BTE applications. However, their clinical translation has been hindered by several intrinsic drawbacks. These include the potential immunogenicity of differentiated hESC [29–33], and the emerging evidence that autologous iPSC may be rejected immunologically [34]. Prolonged culture expansion of ESC has also led to the accumulation of chromosomal abberations [35,36]. Another major concern is the inherent nature of both iPSC and ESC to form teratomas in vivo [37], with several strategies, such as the selection of fully differentiated cells for transplantation, now under intensive investigation to avoid teratoma formation [38–40]. Furthermore, there are unresolved questions about the functional maturity of osteoblasts derived from pluripotent cell sources [41].

MSC, known as marrow stromal cells or colony-forming units – fibroblast (CFU-F) or more recently multipotent mesenchymal