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Review Article

The potential of enriched mesenchymal stem cells with neural crest cell phenotypes as a cell source for regenerative dentistry

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Summary Effective regenerative treatments for periodontal tissue defects have recently been demonstrated using mesenchymal stromal/stem cells (MSCs). Furthermore, current bioengineering techniques have enabled *de novo* fabrication of tooth-perio dental units in mice. These cutting-edge technologies are expected to address unmet needs within regenerative dentistry. However, to achieve efficient and stable treatment outcomes, preparation of an appropriate stem cell source is essential. Many researchers are investigating the use of adult stem cells for regenerative dentistry; bone marrow-derived MSCs (BM-MSCs) are particularly promising and presently used clinically. However, current BM-MSC isolation techniques result in a heterogeneous, non-reproducible cell population because of a lack of identified distinct BM-MSC surface markers. Recently, specific subsets of cell surface markers for BM-MSCs have been reported in mice (PDGFRα^+ and Sca-1$^+$) and humans (LNGFR$^+$, THY-1$^+$ and VCAM-1$^+$), facilitating the isolation of unique enriched BM-MSCs (so-called ''purified MSCs''). Notably, the enriched BM-MSC

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population contains neural crest-derived cells, which can differentiate into cells of neural crest- and mesenchymal lineages. In this review, characteristics of the enriched BM-MSCs are outlined with a focus on their potential application within future regenerative dentistry.

Contents

1. Introduction

Following tooth loss, damage to periodontal tissues or jaw bones caused by dental caries, periodontal disease, or tumor extraction, it is difficult for humans to self-regenerate the lost tissues [1]. Current dental therapies therefore use artificial materials and prostheses to restore lost dental structures. However, limitations of these therapies include incomplete recovery of functional tissues and esthetic issues [2]. Therefore, regenerative therapies are receiving increased attention within the dental field [3]. For example, patients with reduced alveolar bone height often undergo alveolar bone augmentation before a dental implant can be placed. Presently, autograft, allograft, xenograft, and bone grafting materials are used for alveolar bone augmentation; however, it is difficult to maintain the grafted bone height and volume for a long period after the augmentation treatment [4], even following autograft bone augmentation [5,6], which is considered the gold-standard procedure.

Several studies have shown that fetal dental epithelium and dental mesenchyme can be suitable cell sources for bioengineered teeth [7,8] that erupt at the transplant site of mouse alveolar bone defects [9]. A more recent technological approach for bioengineered teeth involves fabricating a tooth structure using periodontal tissues, including alveolar bone [10]. Therefore, stem cell-based therapy is expected to be a powerful tool in regenerative dentistry, with many researchers engaging in stem cell biology and tissue engineering research to establish cell-based approaches to regenerative dentistry [11].

There are basically two types of stem cells in bone marrow: hematopoietic stem cells (HSCs) [12–15] and mesenchymal stromal/stem cells (MSCs) [16,17]. Bone marrow-derived MSCs (BM-MSCs) are plastic-adherent and proliferative cells that are able to differentiate into osteoblasts, adipocytes and chondrocytes [17]. Human BM-MSCs (hBM-MSCs) are now clinically applied world-wide because of their proliferative ability and multi-potent differentiation potential.

One potential problem of using hBM-MSCs is that their therapeutic success rate varies among patients, partly because BM-MSCs as a population are heterogeneous and contain progenitors of osteoblasts, adipocytes, chondrocytes, and other mononuclear cells [18,19]. According to the 2006 statement of the International Society for Cellular Therapy [20], hBM-MSCs are minimally defined by their (1) capacity to attach to tissue culture-treated plastic dishes, (2) specific surface antigen expression [$CD105^+$, $CD73^+$, CD90 (known as THY-1)$^+$, $CD45^-$, $CD34^-$, $CD14^-$ or $CD11b^-$, $CD79a^-$ or $CD19^-$, and $HLA\text{-}DR^-$], and (3) multipotent differentiation potential. However, these criteria are not definitive, and the statement mentioned that the criteria would require modification as new facts and data become available [20]. A lack of specific cell surface markers to define BM-MSCs has hampered progress in understanding the molecular basis of their clonal potential. In contrast, specific markers for HSCs and HSC-derived hematopoietic lineage cells are well-established [21,22]; therefore these cells can be successfully isolated and analyzed using flow cytometry [12,23]. Based on the resulting understanding of HSC characteristics, remarkable and reproducible progress in transplant therapy using HSCs has been achieved [24].

To provide standardized BM-MSC-based treatment outcomes, it is therefore important to pursue and define specific BM-MSC markers. Recently, combinations of cell surface markers for BM-MSCs, including $PDGFR\alpha^+$ and $Sca\text{-}1^+$ (PαS) in mice [25] and $LNGFR^+$, $THY\text{-}1^+$, and $VCAM\text{-}1^+$ (LTV) in humans [26], have been identified. These marker combinations enable the prospective isolation of highly

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