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Use of mesenchymal stem cells for cutaneous repair and skin substitute elaboration

Utilisation des cellules souches mésenchymateuses pour la réparation cutanée et l'élaboration de substituts de peau

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ABSTRACT

Human mesenchymal stem cells (MSCs) are a heterogeneous population of fibroblast-like cells, which are present in different locations, including bone marrow, adipose tissue, extra-foetal tissues, gingiva and dermis. MSCs, which present multipotency capacities, important expansive potential and immunotolerance properties, remain an attractive tool for tissue repair and regenerative medicine. Currently, several studies and clinical trials highlight the use of MSCs in cutaneous repair underlining that their effects are essentially due to the numerous factors that they release. MSCs are also used in skin substitute development. In this study, we will first discuss the different sources of MSCs actually available. We will then present results showing that bone marrow-derived MSCs prepared according to Good Manufacturing Practices and included in a dermal equivalent are able to promote appropriate epidermis growth and differentiation. These data demonstrate that bone marrow-derived MSCs represent a satisfactory alternative to dermal fibroblasts in order to develop skin substitute. In addition, MSCs could provide a useful alternative to sustain epidermis development and to promote wound healing.

Mots clés :
Cellules souches mésenchymateuses
Réparation tissulaire
(Myo)fibroblastes
Peau reconstruite

RÉSUMÉ

Les cellules souches mésenchymateuses (CSMs) représentent une population hétérogène de cellules ressemblant à des fibroblastes et qui sont présentent dans différentes localisations, notamment la moelle osseuse, le tissue adipeux, les tissus extra-fœtaux, la gencive et le derme. Les propriétés des CSMs (multipotence, prolifération *in vitro* et immunotolérance) sont intéressantes en vue de leur utilisation en réparation tissulaire et en médecine régénératrice. Aujourd'hui, différents travaux et études cliniques soulignent l'intérêt des CSMs pour aider la réparation cutanée, soulignant que leurs effets bénéfiques sont essentiellement dus aux nombreux facteurs que ces cellules sécrètent. Les CSMs sont aussi utilisées pour développer des substituts cutanés. Dans cette étude, nous discuterons d'abord les différentes sources de CSMs disponibles. Nous présenterons ensuite des résultats montrant que des CSMs issues de la moelle osseuse et préparées en accord avec les bonnes pratiques de production généralement définies, sont capables, lorsqu'elles sont utilisées pour construire un derme équivalent, de promouvoir de façon

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tout à fait satisfaisante, la croissance et la différenciation épidermique. Ces résultats démontrent que les CSMs issues de la moelle osseuse représentent une alternative intéressante à l'utilisation des fibroblastes dermiques. De plus, les CSMs contribuent au développement de l'épiderme et peuvent ainsi faciliter la réparation des plaies.

1. Introduction

1.1. Reminders on tissue repair mechanisms

Tissue damages and particularly skin wounds usually heal in a highly regulated sequence of overlapping processes that require coordinated activities of different cell types [1,2]. Wound healing phases include:

- a vascular and inflammatory phase during which vascular repair, fibrin clot formation and invasion of inflammatory cells into the wound with release of pro-inflammatory cytokines and growth factors are observed;
- a proliferative phase characterized by granulation tissue formation and re-epithelialization;
- a remodelling phase during which the extracellular matrix is reorganized with wound contraction followed by apoptotic death of inflammatory, vascular and fibroblastic cells.

In normal wound healing, platelets are a major source of pro-inflammatory cytokines and growth factors. Then, macrophages, endothelial and fibroblastic cells invade the wound space and the provisional fibrin extracellular matrix rich in plasma and inflammatory cell-derived cytokines and growth factors. The predominant factors are cytokines of the fibroblast growth factor (FGF) family, platelet-derived growth factor (PDGF), tumour necrosis factor (TNF)-α, epidermal growth factor (EGF), hepatocyte growth factor (HGF), transforming growth factor (TGF)-β1, and vascular endothelial growth factor (VEGF) [3,4]. Macrophages increase inflammatory responses and additionally secrete VEGF and FGF, which promote angiogenesis [5]. The formation of numerous new capillaries confers to the granulation of tissue, giving it a typical granular appearance. Neovascularization is essential for the synthesis, deposition, and organization of a new extracellular matrix. Moreover, FGF, TGF-β1, and PDGF cause fibroblast infiltration and activation into contractile myofibroblasts, which dominate the proliferation and remodelling phases of wound healing. Initially, fibroblastic cells use the fibrin matrix to migrate across the wound, subsequently adhering to fibronectin. Then, fibroblastic cells progressively transform in myofibroblasts [6], which deposit collagen (mainly collagen type III) into the wound bed, leading to the formation of a sophisticated extracellular matrix network to which they can adhere, permitting their migration, and allowing the final formation of the granulation tissue. The extracellular matrix not only regulates cellular functions via cell adhesion and migration, but also provides a storage and diffusion system for signalling molecules, ions, hormones, nutrients and waste products. When normal healing wounds are closed and re-epithelialized, cellularity within the granulation tissue decreases due in part to myofibroblast and endothelial cell apoptosis [7]. For wounds to heal successfully, all phases must occur in the proper sequence. Any factors interfering with one or several phases of this highly regulated process can cause impaired (chronic wounds) or overly (fibrosis or hypertrophic scarring) wound healing [8].

1.2. Definition of a MSC

Mesenchymal stem cells (MSCs) are described to be multipotent progenitor cells with ability to self-renewal and to differentiate into multiple cell lineages, such as adipocytes, chondrocytes, osteoblasts [9].

The term "mesenchymal stem cell" has been applied to these cultured cells because:

- they meet the criteria customarily accepted for stem cell definition, namely, a high capacity for self-renewal and the ability to differentiate into a number of different tissues;
- tissues into which they may develop are from mesenchymal origin [10].

These cells can also acquire a fibroblastic phenotype in classical two-dimensional culture [11]. Today, the term MSC has been revisited and defines adult "fibroblast-like" cells that can mature along multiple differential pathways according to their trophic activity [12,13]. Officially, MSCs have been defined by the International Society of Cellular Therapy as multipotent stromal cells on the basis of three main characteristics:

- their adhesion to a plastic support;
- their expression of a specific set of membrane molecules (CD73, CD90 and CD105), combined with a lack of expression of hematopoietic markers (CD14, CD34, and CD45) and human leukocyte antigen-DR;
- their ability to differentiate along osteoblastic, adipocytic and chondrocytic pathways [14].

2. Different sources of MSCs

Even if the first isolation of MSCs has been made in the bone marrow stroma, some researchers have now described MSCs in many others organs, such as adipose tissue, extra-foetal tissues, gingival tissue and dermal tissue (Fig. 1). In some specific situations (see below), these cells have been described to be involved during skin repair processes; in addition, the interest to use these cells to improve wound healing has been studied.

2.1. Bone marrow-derived MSCs

The bone marrow stroma is the first location where MSCs were described in 1966 by Friedenstein et al. [15]. Additional studies by Owen and Friedenstein [16] demonstrated the ability of these cells to differentiate into adipocyte and osteocyte lineages. These plastic-adherent cells, fibroblast-like cells are able to give rise to colonies of cells termed colony forming unit-fibroblasts. To isolate these cells, iliac crest aspiration is generally realized and MSCs are selected *in vitro*. This method is invasive and the number of MSCs present in the bone marrow swab is limited (from 0.001 to 0.01% of bone marrow nucleated cells). Conventional procedure to select bone marrow-derived MSCs is relied on the expansion of unselected bone marrow cells based on their capacity to adhere on the plastic surface of