FACTORS GOVERNING THE DIFFERENTIATION OF STEM CELLS IN TISSUE ENGINEERING - A REVIEW

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Introduction

Tissue engineering is an interdisciplinary field which applies the principles of biology and engineering to the development of functional substitutes for damaged tissue [1]. For constructing these substitutes, two basic components are needed, namely a biomaterial component, simulating the extracellular matrix of anchorage-dependent cells, and a cell component, which adhere, migrate, proliferate and differentiate on the biomaterial and exert desired functions. Recently, stem cells emerged as a promising cell component with several advantages over the differentiated cells, such as a high proliferation capacity, lower senescence, paracrine production of various growth and immunomodulatory factors and ability to be differentiated into various cell types (for a review, see [2]).

Types of Stem Cells

Stem cells can be divided into four main groups, namely embryonic stem cells, foetal stem cells, adult stem cells, and induced pluripotent stem cells (iPSCs). Embryonic stem cells (ESCs) isolated from blastocyst are pluripotent, i.e. able to differentiate into all cell types except placental cells, but their use in humans is restricted by ethical and legal issues. Stem cells with markers of pluripotency can be obtained from extrafoetal tissues, i.e. from placenta, amnion, chorion or umbilical cord. Among adult stem cells, bone marrow mesenchymal stem cells (bmMSCs) and adipose tissue-derived stem cells (ADSCs) became the most popular due to their relatively good accessibility and availability in relatively high quantities. These cells are only multipotent, i.e. able to differentiate to a limited number of cell types, but they can be routinely used in autologous form. The iPSCs, created by genetic reprogramming of differentiated somatic cells, are associated with a high risk of tumorigenicity due to the use of viral vectors (for a review, see [2]). From this point of view, the adult stem cells are the most promising stem cell type for tissue engineering.

Factors Controlling the Differentiation of Stem Cells

Stem cells can be differentiated into desired cell types by biochemical and mechanical signals. During tissue engineering, which is carried out mainly under *in vitro* conditions, the main source of biochemical signals is cell culture medium. Mechanical stimulation is provided by dynamic cell culture systems. Cell adhesion substrate can be a source of both biochemical and mechanical signals.

Composition of the cell culture medium. Each direction of cell differentiation requires the presence of specific growth factors and other biomolecules in the culture medium. For example, differentiation towards vascular smooth muscle cells (VSMCs) occurs when the medium is supplemented with transforming growth factor-beta 1 (TGF-β1) and bone morphogenetic protein-4 (BMP-4) [3], and differentiation towards endothelial cells (ECs) requires the presence of vascular endothelial growth factor (VEGF) [2]. Osteogenic medium contains β-glycerophosphate, dexamethasone, and vitamins C and D3 [3,4], and the medium for differentiation towards keratinocytes is supplemented with epidermal growth factor or keratinocyte growth factor [2].

Mechanical stimulation of cells. Each direction of stem cell differentiation also requires specific type of mechanical stimulation. For example, cyclic strain is needed for differentiation towards VSMCs, while laminar shear stress promotes the differentiation towards ECs. Osteogenic cell differentiation is stimulated by vibrational stress, and the differentiation towards keratinocytes by pressure or uniaxial strain stress. Mechanical stimulation can be, at least partly, substituted by electrical or magnetic stimulation [2].

Properties of the cell cultivation substrate. The differentiation of stem cells can also be influenced by the composition, architecture, physicochemical and mechanical properties of the cultivation substrate, such as its wettability, functionalization with various biologically active substances, two- or three-dimensional architecture, and particularly stiffness. For example, very soft substrates, having mechanical characteristics similar to those of brain tissue, direct the differentiation of stem cells towards neurons. On stiffer substrates, mimicking the muscle tissue, the stem cells became myogenic, and on the stiffest matrices, the stem cells differentiate towards osteoblasts (for a review, see [2]).

Differentiation of stem cells into difficult-to-reach cell types

The differentiation of adult stem cells, particularly of those of mesenchymal origin (bmMCS, ADSCs), towards more specialized cells, such as endothelial cells or keratinocytes, is considered difficult and is scarcely achievable by the factors mentioned above. The reason is the polarization (i.e. functional specialization) of the basal and apical cytoplasmic membrane or the need of transdiferentiation from mesodermal to ectodermal cells. In this case, the cell reprogramming becomes necessary. Modern approaches offer this reprogramming without genetic manipulation, namely by using synthetic messenger RNAs (mRNAs) or self-replicating RNA, which encode markers characteristic for pluripotent stem cells, such as Oct4, Klf4, c-Myc and Sox2, and do not integrate into the host genome [5].

Conclusions

Stem cells are promising cell component for advanced tissue engineering and can be differentiated in a variety of cell types using biochemical and mechanical signals arising from the appropriate composition of cell culture media, cell adhesion substrate and mechanical stimulation. When the differentiation by physiological signals is difficult and incomplete, generation of pluripotent cells by non-genome integrating methods will facilitate possible clinical application of these cells.

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