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ORIGINAL ARTICLE

An improved protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow

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bone marrow;
isolation;
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Summary Mesenchymal stem cells (MSCs) from bone marrow are main cell source for tissue repair and engineering, and vehicles of cell-based gene therapy. Unlike other species, mouse bone marrow derived MSCs (BM-MSCs) are difficult to harvest and grow due to the low MSCs yield. We report here a standardised, reliable, and easy-to-perform protocol for isolation and culture of mouse BM-MSCs. There are five main features of this protocol. (1) After flushing bone marrow out of the marrow cavity, we cultured the cells with fat mass without filtering and washing them. Our method is simply keeping the MSCs in their initial niche with minimal disturbance. (2) Our culture medium is not supplemented with any additional growth factor. (3) Our method does not need to separate cells using flow cytometry or immunomagnetic sorting techniques. (4) Our method has been carefully tested in several mouse strains and the results are reproducible. (5) We have optimised this protocol, and list detailed potential problems and trouble-shooting tricks. Using our protocol, the isolated mouse BM-MSCs were strongly positive for CD44 and CD90, negative CD45 and CD31, and exhibited tri-lineage differentiation potentials. Compared with the commonly used protocol, our protocol had higher

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Biology of mesenchymal stem cells

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KEYWORDS
Mesenchymal stem cells;
Cell culture;
Scaffold;
Trauma and orthopaedic surgery

Summary Mesenchymal stem cells are present in many human tissues and serve as a readily available source of undifferentiated cells being capable to form specific tissues like bone, cartilage, fat, muscle and tendon. They represent an attractive and promising field in tissue regeneration and engineering for treatment applications in a wide range of trauma and orthopaedic conditions. This article covers the most important aspects of recent research data demonstrating the combination of physiological properties of mesenchymal stem cells (MSCs) and applications in the clinical setting.

Overview of proliferation, differentiation and tissue regeneration

Mesenchymal stem cells (MSCs) are non-hematopoetic, stromal cells that exhibit multilineage differentiation capacity being capable to give rise to diverse tissues, including bone, cartilage, adipose tissue, tendon and muscle (Fig. 1).

In the laboratory, MSCs can be isolated, expanded and be handled easily as they adhere to culture plastic containers. They can rapidly divide and form colonies. Expanded MSCs could be guided to differentiate along multiple phenotypic pathways through specific media containing growth factors or other substances like Dexamethasone, Indomethacin, Hydrocortisone and Transforming growth factor β (TGFβ).[35]

MSCs in the human body seem to be reservoirs of reparative cells without tissue specific characteristics. Different signals can direct them to mobilise and differentiate into cells of connective tissue lineages. Such signals might include damage in the tissues including trauma, fracture, inflammation, necrosis and tumors.[54] Chemotaxis[44] and the local microenvironment[53] can also play a role in the fate of MSCs.

MSCs reside in diverse host tissues. Originally Friedenstein et al. isolated MSCs from the bone marrow (BM) and stroma of spleen and thymus.[24,25] Subsequently BM aspirates was considered to be the most accessible and enriched source of MSCs.[70] Since then, MSCs have being isolated from various sites including cartilage, periostium, synovium, synovial fluid, muscle and tendons. Fetal tissue, placenta, umbilical blood and vasculature have been also reported to contain MSCs.[7,31,60]

Due to the fact that autologous BM procurement has limitations, adipose tissue could serve as a good source of MSCs as it exists in large quantities in our body.[20]

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Isolation and Characterization of Mouse Mesenchymal Stem Cells

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ABSTRACT

Objective. Mesenchymal stem cells (MSCs) have been studied in regenerative medicine because of their unique immunologic characteristics. However, before clinical application in humans, animal models are needed to confirm their safety and efficacy. To date, appropriate methods and sources to obtain mouse MSCs have not been identified. Therefore, we investigated MSCs isolated from 3 strains of mice and 3 sources for the development of MSCs in a mouse model.

Materials and Methods. Male BALB/c, C3H and C57BL/6 mice were used to isolate MSCs from various tissues including bone marrow (BM), compact bone, and adipose tissue. The MSCs were maintained in StemXVivo medium. Immunophenotypes of the MSCs were analyzed by FACS and their growth potential estimated by the number of colony-forming unit fibroblasts.

Results. All MSCs that were isolated from BM, compact bone, and adipose tissue showed plastic-adherent, fibroblastic-like morphologic characteristics regardless of the mouse strain or cell source. However, culture of BM MSCs was less successful than the other tissue types. The FACS phenotype analysis revealed that the MSCs were positive for CD29, CD44, CD105, and Sca-1, but negative for CD34, TER-119, CD45, and CD11b. According to the results of the characterization, the adipose tissue MSCs showed higher growth potential than did other MSCs.

Conclusion. The results of this study showed that culture of adipose tissue and compact bone-MSCs was easier than BM MSCs. Based on the results of immunophenotype and growth potential, C57BL/6 AT-MSCs might be a suitable source to establish a mouse model of MSCs.

MESENCHYMAL STEM CELLS (MSCs), from various species and sources, can be enriched by isolation of cells with fibroblast-like morphology via their preferential attachment to tissue culture plastic.[1-9] The relative abundance of MSCs is routinely determined by counting the number of clonogenic precursors or the colony-forming unit-fibroblasts (CFU-F). Although the cells are generally assumed to be similar, some data suggest that species variations in the properties of these cells. MSCs have self-renewal properties and the capacity to differentiate into multiple lineages such as osteocytes, adipocytes, and chondrocytes.[3] This potential has rendered them a focus of study for cellular replacement therapy and tissue engineering. The MSCs are mainly regarded as hematopoietic support cells, as feeder layers for ex vivo expansion of hematopoietic stem cells (HSCs)[10,11] and as immunosuppressive effectors in vitro and in vivo.[4,8,12,13]

The mouse is a suitable experimental model system to study the cell biology and biochemical characteristics of MSCs. However, the standard method of plastic adherence has failed to yield a relatively pure MSC population from

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Mesenchymal stem cell tissue engineering: Techniques for isolation, expansion and application

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KEYWORDS
Mesenchymal stem cells;
Bone repair;
Culture;
Implantation

Summary Mesenchymal stem cells (MSCs) are undifferentiated multipotent cells which reside in various human tissues and have the potential to differentiate into osteoblasts, chondrocytes, adipocytes, fibroblasts and other tissues of mesenchymal origin. In the human body they could be regarded as readily available reservoirs of reparative cells capable to mobilize, proliferate and differentiate to the appropriate cell type in response to certain signals. These properties have triggered a variety of MSC-based therapies for pathologies including nonunions, osteogenesis imperfecta, cartilage damage and myocardial infarction. The outcome of these approaches is influenced by the methodologies and materials used during the cycle from the isolation of MSCs to their re-implantation. This review article focuses on the pathways that are followed from the isolation of MSCs, expansion and implantation.

Introduction

Tissue engineering with the use and manipulation of Mesenchymal stem cells (MSCs) is a novel treatment modality targeting applications in a great variety of pathologies. The advantages of this approach are numerous. They include a high quality repair with regeneration of the injured tissue but without fibrous tissue formation. The site morbidity is minimal compared to the currently used bone and cartilage autografts as a small number of cells is required with subsequent expansion ex-vivo. The risk for immunorejection and pathogen transmission appears to be very low. Furthermore, MSCs have high proliferation potential, can be handled and manipulated easily permitting differentiation prior implantation.

With the persistent objective of clinical applications, four main strategies have been used in tissue engineering. These consist of the use of i. Unfractionated fresh bone marrow cells, ii. Culture expanded MSCs, iii. Differentiated cells, and finally iv. Genetically modified cells that express key growth factors. [73] Excluding the first approach, all other strategies consist of a cycle of events that is initiated by the isolation of cells, culture until sufficient numbers are produced and finally re-implantation to the injured site.

The aim of this review article is to illustrate the different available methodologies that could be used in tissue engineering as well as to analyse their efficacy for a widespread clinical use.

MSCs

The human body houses several types of uncommitted progenitor cells capable of giving

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