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Viability and functionality of mesenchymal stromal cells loaded on collagen microspheres and incorporated into plasma clots for orthopaedic application: Effect of storage conditions

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ABSTRACT

Background: There is evidence showing that human mesenchymal stromal cells (MSC) seeded on collagen microspheres (CM) and incorporated into platelet rich plasma (PRP) clots induce bone formation. For clinical trials it is very important to establish standardization of storage and shipment conditions to ensure the viability and functionality of cellular products. We investigate the effect of storage temperature and time on the viability and functionality of human MSC seeded on CM and included into PRP clots for using in the further clinical application for bone regeneration.
Methods: MSC/CM/PRP clots were stored at room temperature (RT), 4 °C and 37 °C for 12 h, 24 h and 48 h. At each period of time, MSC were evaluated for their viability and functionality.
Results: MSC from MSC/CM/PRP clots maintained at RT and 37 °C for 24 h showed a high viability (90%) and maintained their capacity of proliferation, migration and osteogenic differentiation. In contrast, MSC/CM/PRP maintained to 4 °C showed a significant reduction in their viability and migration capacity. MSC from MSC/CM/PRP clots maintained at RT for 24 h induce osteogenesis in the subcutaneous tissues of mice, after four months of transplantation.
Discussion: Our results show that MSC incorporated into CM/PRP clots and maintained at RT can be utilized in bone regeneration protocols during the first 24 h after their processing.

Introduction

Mesenchymal stromal cells (MSCs) are multipotent cells with the capacity to differentiate into different cell lineages such as osteoblasts, chondrocytes and adipocytes [1,2]. Based on their capacity to induce tissue regeneration, they constitute a potential therapeutic strategy in regenerative medicine. In fact, MSC have been used in a variety of clinical protocols for tissue regeneration including bone and cartilage regeneration [3–6]. Although MSC is one of the cells most used in preclinical and clinical protocols [7–9], the great variability in clinical outcomes limits its usage in patients. This has been attributed to the variability of protocols and reagents used as well as the storage and transportation methods [5,10]. For clinical trials it is very important to establish standardization of storage and shipment conditions to ensure the viability and functionality of the cellular products.

Whereas MSC characterization is performed in almost all protocols, there is very little information about the functionality of these cells in the finished product. This is important because cells may be altered in their functionality between their processing at the laboratory and their implant in patients, especially when it is necessary to move to remote locations. For these reasons, it is necessary to ensure the survival of MSC during transport from the laboratory to the hospital.

It is known that *in vitro* storage reduces the viability and functionality of MSC. Likewise, it is known that MSC administered in suspension have a very low survival in tissues [3,11]. Optimal scaffolds would provide a surface that allows not only the adhesion, but also the proliferation and migration of MSC into the tissue [12–14]. Recently, we showed that a complex of collagen microspheres (CM) and platelets rich plasma clots (PRP) constitute optimal scaffolds for using MSC in clinical trials to induce bone

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regeneration [15]. In the present study, we evaluated the effect of storage temperature and time on the viability and functionality of MSC from MSC/CM/PRP clots maintained at different temperatures and time of storages.

Material and methods

Reagents

Conjugated murine monoclonal antibodies anti-human CD105 were obtained from Biolegend (USA). Alpha-MEM culture medium, MCDB and TrypLE™ Express were obtained from Invitrogen. Chang Medium was obtained from Irvine Scientific. Collagen microspheres (CM) were obtained from Cosmo Bio Co (Japan).

Animals

Female C57BL/6 mice (8–12 weeks old) were obtained from the IVIC Laboratory Animal Center, and maintained on a standard laboratory diet and housed in a controlled environment. All animal experimentation was performed in accordance with institutional guidelines.

Mesenchymal stromal cells

MSC used in this study were from bone marrow of three patients which were part of a clinical protocol for bone regeneration [15]. All patients gave their informed consent to participate in the study. The study protocol was approved by the Bioethics Committee of Hospital Universitario de Caracas. MSC were kept frozen until their use in this study. The MSC were cultured with alpha-MEM-Chang medium (Irvine Scientific,USA) supplemented with 20% autologous serum (regular medium) and used when they reached 70–80% confluence. MSC were analyzed for the expression of CD105 by flow cytometry. Cellular processing was performed at the Cellular Therapy Unit of our institution, under GMP conditions.

Multipotent differentiation of MSC

The multipotential capacity of MSC was examined by culturing these cells in secondary cultures using osteogenic, chondrogenic and adipogenic differentiation media [1,2], and they were stained with alizarin, alcian blue and oil red.

Experimental design for evaluating MSC from MSC/CM/PRP clots storaged at different conditions

Early passages (3–5) of MSC were harvested and incubated in osteogenic medium for three days (Fig. 1A), and after they were harvested and seeded at 1×10^5 cells on CM and incubated for 24 h to promote adhesion to the microspheres (Fig. 1B). The MSC/CM preparation was mixed with PRP, and clot formation was obtained after adding 5% $CaCl_2$/thrombin (Fig. 1C). The MSC/CM/PRP clots were stored at different temperatures: RT, 37 °C and 4 °C for 12 h, 24 h and 48 h. After completing the incubation time, each clot was divided in order to assess the viability, migration, differentiation and cytological and histological

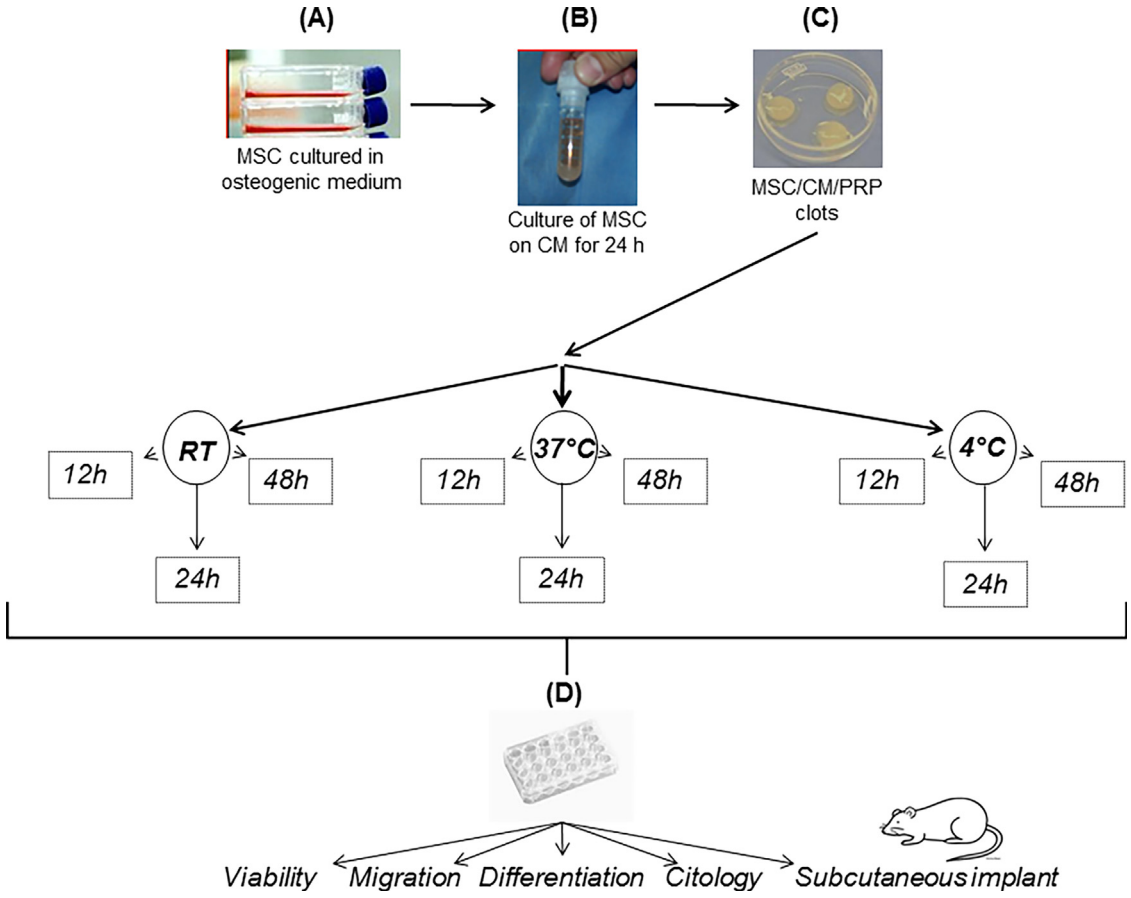

Fig. 1. Experimental design. MSC from BM (a) were cultured for 72 h in osteogenic medium (A) and seeded on CM (B). The MSC/CM preparation was included into PRP clots (C) and incubated at RT, 37 °C and 4 °C for 12, 24 and 48 h. After each period of time MSC/CM//PRP clots were placed in 24 wells plate and functional assays were performed for viability, migration and differentiation (D). MSC/CM/MSC clots maintained in culture for 21 days were subcutaneously implanted in mice.

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