INTEGRA® DERMAL MATRIX BIOENGINEERED WITH PLATELET RICH PLASMA (PRP) AND MESENCHYMAL STROMAL CELLS TO SERVE AS NICHE FOR SKIN REGENERATION

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Abstract

Our precedent studies suggest that the use of Platelet-Rich Plasma (PRP), as a supplement for growth medium represented an optimal substitute for animal serum as well as a source of multiple growth factors, was able to satisfactorily support cell viability, cell proliferation and influence stemness gene expression in MSCs. Moreover, dermal matrix Integra® appeared to be a suitable substrate for MSCs colonization and growth. For this reason, we wanted to extend our experimentation with the aim of evaluating whether the combination of artificial dermal matrix Integra® together with platelet rich plasma could favor stem cell differentiation and to investigate possible molecular events involved, for a possible use in clinical applications. The analysis by confocal microscopy, has confirmed the data detected by FLIM, that the cells seeded on the matrix in the presence of PRP showed a better organized actin cytoskeleton with stress fibers adhering to the substrate. In particular, in these conditions is assisted to a considerable reduction of the expression of stem cell markers such as oct4, suggesting a possible role of the dermal matrix Integra® in cell differentiation. Furthermore, the stem cells MSC began to express collagen type-1, clearly evident in the peripheral region of the cells, suggesting the tendency of these cells to acquire a fibroblast-like phenotype in these experimental conditions. Finally, the cells plated on the dermal matrix were analyzed to evaluate the expression of epithelial markers such pan-cytokeratin. No reactivity was detected both in the absence and in the presence of PRP. The data obtained in this study indicate that Integra® in association with growth factors derived from platelet gel seems to be a microenvironment conducive to growth, as well as the formation of a three-dimensional organization directed towards an apparent differentiation in the sense stromal.

Keywords: *confocal microscopy; bioengineered; collagen type-1; pan-cytokeratin; stem cell markers; microenvironment;*

INTRODUCTION

Mesenchymal stem cells, or mesenchymal progenitor cells were first identified as a subpopulation of bone marrow cells (Friedenstein et al., 1974). Later researches showed that MSCs can be isolated from other types of tissues as well, including adipose tissue, placenta, periosteum, trabecular bone and femur (Cowan et al., 2004, Oreffo et al., 1998, Miao et al., 2006, Chai et al., 2012). MSCs can be characterized based on their fibroblast-like morphology and ability to differentiate into various cell types (Friedenstein et al., 1970). MSCs are also capable of suppressing allogeneic responses and appear to be non-immunogenic (Keating et al., 2006). Therefore, MSCs are increasingly used in regenerative medicine as a source of cells for restoring worn-out or damaged tissues such as cartilage, cardiac muscle or bone, in combination with platelet rich plasma and synthetics matrices. The platelet gel (platelet rich plasma - PRP) is traditionally used as a source of platelet growth factors (Caloprisco et al., 2010). The autologous PRP is considered as a suitable strategy to promote the healing of wounds or ulcers in different location, using it directly on injuries (Borzini et al., 2006). In fact, are also used in oral and maxillofacial autologous platelet blood components as a source of growth factors to induce regenerative stimulus on bone (Caloprisco et al., 2004). Also, it was evaluated the regenerative effect induced by platelet gel in ulcerative chronic lesions of skin (Caloprisco et al., 2004, de Leon JM et al., 2011). The tissue engineering, which is proposed to build in vitro biological tissue intended for replacement of parts of the human body damaged or affected by pathologies, is intended to represent a further tool available to the physician for the treatment of a variety of human diseases. These represents the substrate for cell development spatially default, thus constituting a culture system in a dynamic environment (Lutolf et al., 2009; Nichols et al., 2009; Peerani and Zandstra, 2010; Dickinson et al., 2011; DiMaggio et al., 2011). Recently, is been satisfactorily tested synthetic matrix as a skin substitute in acute or chronic ulcers. The product used in this study Ematrix is made from a combination of platelet gel and a dermal matrix commercial: Integra®. The aim of this study was to investigate whether the combination of artificial dermal matrix Integra® together with platelet rich plasma could favor stem cell differentiation and to investigate possible molecular events involved, for a possible use in clinical applications.

MATERIALS AND METHODS

Cell cultures

In the present study was been used mesenchymal stem cells from bone marrow (MSCs) taken from the iliac crest of healthy donors by aspiration bone marrow. The isolated cells were plated in a culture medium Iscove's MDM (Life-technologies) supplemented with L-glutamine, Hepes (25 mM), gentamicin (50 g / ml) and 10% fetal bovine serum (FBS). The cells were incubated at 37 ° C in a humidified atmosphere containing 5% CO2. After 14 days the cells reached

confluence, were detached with trypsin-EDTA and 0.025% 00:02% and re-suspended in complete medium.

Confocal immunofluorescence

The cells seeded on the matrix Integra®, for 24 and 48 hours, in the absence and presence of PRP, previously incubated with MytoTracker CMXRos (Molecular Probes), were fixed in 0.5% paraformaldehyde in PBS for 10 minutes at room temperature. Subsequently the samples were permeabilized with 0.5% Triton-X for 5 minutes, rinsed in PBS and incubated in blocking buffer (PBS with 1% BSA) for 20 minutes. Finally, the samples were incubated with phalloidin conjugated to Alexa Fluor488 (1:40; Molecular Probes) for 20 minutes at room temperature with the aim of highlighting the filaments of F-actin. After several washes in PBS and water, the matrices with the labeled cells were observed by confocal microscopy.
In parallel experiments, the cells seeded on the matrix MSC Integra® for 24 and 48 hours, in the presence or in the absence of PRP, were fixed in paraformaldehyde, were labeled with phalloidin conjugated with Alexa Fluor488 and subsequently incubated with the following primary antibodies: Anti-Human oct-4 (1:10; RD systems, Inc.); rabbit anti-collagen type-1 (1:50; Santa Cruz), anti-Human pan cytokeratin (1:50; Leica Biosystems). The immune reaction was revealed using anti-mouse secondary antibodies conjugated with Alexa Fluor488 (1: 200; Molecular Probes) and anti rabbit conjugated with Alexa Fluor568 (1: 200; Molecular Probes). Nuclei were contrasted with propidium iodide (Molecular Probes).

RESULTS

Analysis confocal laser scanning microscope

In this study was investigated whether the combination of the matrix Integra® with PRP could facilitate a possible differentiation of stem cells MSC. For this purpose the MSCs seeded on dermal matrix added to PRP, were fixed and stained with phalloidin conjugated to green fluorophore Alexa 488 in order to highlight the actin cytoskeleton. The analysis by confocal microscopy, has confirmed the data detected by FLIM, the cells seeded on the matrix in the presence of PRP appeared after 48 h, compared to those not treated with PRP, the more numerous and elongated, also showed a better organized actin cytoskeleton with stress fibers adhering to the substrate, suggesting a possible role of the dermal matrix Integra® in cell differentiation. In particular, in these conditions is assisted to a considerable reduction of the expression of stem cell markers such as oct4, at 24 hours and, in particular to 48 hours, compared to the control (cells grown in the plates at the same time), (Fig. 1B and 1C). Furthermore, the stem cells MSC began to express collagen type-1, clearly evident in the peripheral region of the cells at 48 hours, suggesting the tendency of these cells to acquire a fibroblast-like phenotype in these experimental conditions (Fig. 1D). Finally, the cells plated on the dermal matrix were analyzed to evaluate the expression of epithelial markers such pan-cytokeratin. No reactivity was detected both in the absence and in the presence of PRP (Fig.1 F)

The data obtained in this study indicate that Integra® in association with growth factors derived from platelet gel plasma seems to be a microenvironment conducive to growth and cell survival,

as well as the formation of a three-dimensional organization directed towards an apparent differentiation in stromal sense.

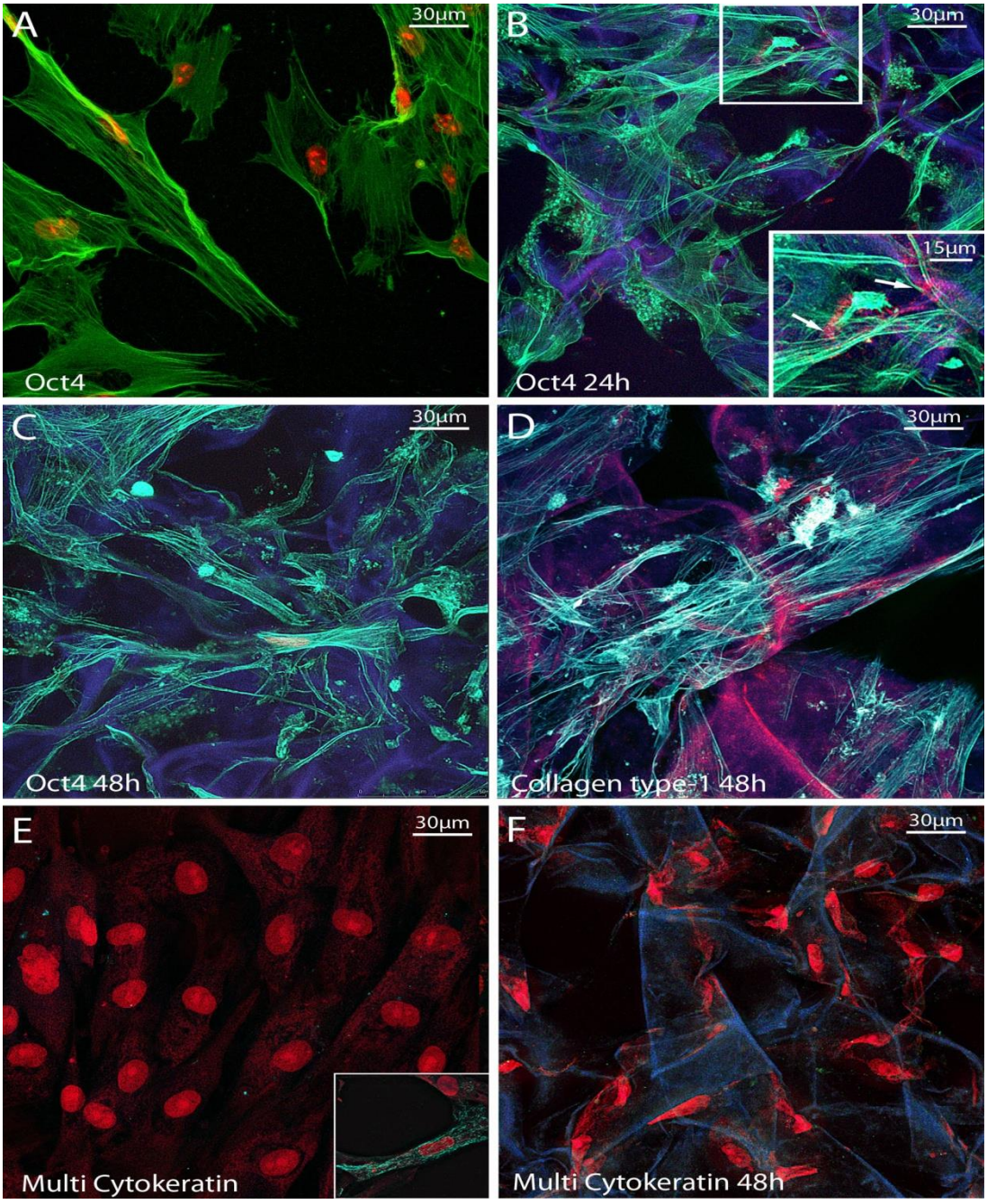

Fig.1. Images obtained by confocal microscopy. (A, E) MSC plated on the plates; (B, D, F) MSC seeded on the matrix Integra® at different times. Evaluation of the expression of oct4 (red), the collagen type-1 (purple) and the multi-cytokeratin (green). In the images A, D, the cell bodies are highlighted by marking of actin filaments (green),

nuclei (E, F) were contrasted with propidium iodide (red), while the mesh of the matrix Integra® are visible from autofluorescence (blue).

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