MESENCHIMAL STEM CELLS AND PLATELET RICH PLASMA-ADSORBED MATRIX FOR DERMAL REGENERATION. IN VITRO STUDY FOR AN INNOVATIVE PROTOCOL OF REGENERATIVE MEDICINE.

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ABSTRACT

Recent studies suggest that therapeutic application of stem cells may be useful for tissue regeneration in acute and chronic cutaneous ulcers. One intriguing approach to this challenge is the use of such cells in combination with biomaterials as well as bioactive molecules and growth factors capable of improving the integration into the engraftment site and stimulate the cell-mediated healing process. In this study we have performed an in vitro study with the aim of evaluating whether platelet rich plasma (PRP) could favor stem cell survival and proliferation, and whether its addition to a dermal matrix(Integra) was able to stimulate migration, adhesion, and differentiation of human bone- marrow- (BMSC) and adipose tissue- (ASC) derived stem cells. It was found that PRP, in the absence of serum, caused a proliferative effect in a dose-dependent manner, as judged by ^{3}[H]- thymidine incorporation assay and MTS test, suggesting that platelet derived growth factors can act as a substitute of cell growth medium. Cell proliferation was accompanied by increasing expression of the stem cell marker Oct-4. Interestingly, when cultured on the matrix, the cells displayed a marked tendency to colonize the pores and firmly adhere to the meshes, indicating that Integra scaffold could be a suitable material as a template for dermal regeneration. Indeed, by two photon microscopy and fluorescence lifetime imaging (FLIM), it was possible to observe that the cells were twisted around the meshes, with an elongated and flattened morphology, being hardly distinguishable from the substrate. The adherent stem cells also exhibited a well organized actin cytoskeleton, implying a possible role for Integra matrix in the promotion of cell differentiaton. The adsorbtion of PRP highly potentiated stem cell colonization into the matrix. In conclusion, the present data add evidence in favor of cell-based therapy for the healing of cutaneous ulcers, indicating that the use of biomaterials scaffolds together with platelet-derived grouth factors largely enhance the potential regenerative efficacy of stem cells.

Key words: *biomaterials; growth factors; mesenchymal stem cells; multiphoton microscopy; platelet-rich plasma; regenerative medicine*

INTRODUCTION

In the last decades, the overall increase of chronic degenerative diseases associated with aging demographics has stimulated the development of effective strategies for repairing or regenerating the injured tissues. In such direction, cell therapy using either autologous or allogeneic stem cells, has been considered till now one of the most promising tools to achieve such task. Human mesenchymal stem cells (hMSCs) derived from adult tissues are considered a leading candidate in regenerative medicine based on their unique features which may be extremely useful when translated in clinical applications (Bianco and Robey 2001). In fact, these cells display an almost ubiquitous distribution, being found not only in bone marrow, but also in different tissues, including skeletal muscle, adipose tissue, bone and liver (Zuk et al., 2002; Da Silva Meirelles et al., 2008; Nincheri et al., 2009); they show a great plasticity and ability to trans-differentiate into other cell types (Bailey et al., 2006; Rosenthal, 2003), and a high capability to migrate to the sites of injury (Satija et al., 2007). In search for novel options in the field of regenerative medicine, in the present study we investigated the effects of PRP and an artificial dermal matrix (Integra®), previously shown to provide a wound template for vascular and fibrocyte in-growth (Nguyen and Dickson 2006), on viability and proliferation of human adult bone marrow-derived mesenchymal stem cells. We also combined PRP and hMSCs within the same Integra® support with the aim to obtain a multifunctional platform with the eventual goal to potentiate the regenerative capabilities of the single treatments and improve the global restoration of the tissue functions.

MATERIALS AND METHODS

PRP preparation

PRP was obtained by plateletpheresis from the whole blood of adult healthy volunteers after receiving an informed consent and centrifuged at 3000 rpm for 10 min; the platelets were next

leucodepleted, irradiated and counted automatically using a hematology analyzer. Platelet concentration in PRP was calculated as 2.10^6/µl ±10%. PRP was activated with a solution of 10% calcium digluconate and thrombin (1:10).

PRP adsorption on Integra® matrix

PRP was dropped on the dermal layer of Integra® matrix, stirred and left in place until gel formation; the final platelet concentration within the matrix was of 1,2. 10^6/µl of PRP. Integra® (INTEGRA® Bilayer Matrix Wound Dressing - Integra Lifesciences Corp., Plainsboro, NJ) is a bilayer membrane system used as dermal regeneration template for skin replacement, routinely utilized in plastic surgery (Machens et al., 2000). The dermal layer is made up of a porous matrix of bovine tendon collagen (92%) and chondroitin-6-sulfate (8%) with a mean pore diameter ranging from 30 to 120 µm, and a global porosity of 98%. The epidermal substitute layer is made up of synthetic polysiloxane polymer.

RESULTS

Isolation, culture and immune-characterization of hMSCs

hMSCs were successfully culture-expanded. Morphologically homogeneous populations of fibroblast-like cells with more than 90% confluence were observed after 14 days of culture; after the first passage, the cells grew exponentially, requiring weekly passages: primary culture cells (14 days) were trypsinized and replated, reaching a cellular expansion up to a 109–1010 factor in 5 months. We next confirmed that the isolated and culture hMSCs displayed the necessary properties to be classified as true hMSCs. The flow cytometry analysis showed, in fact, that hMSC cultures appeared uniformly positive for CD29 (β1 integrin), CD44 (receptor for hyaluronic acid), CD166 (activated leucocyte cell adhesion molecule-1, ALCAM-1), CD90 (Thy-1), CD73 (ecto-5′ - nucleotidase), HLA-ABC and CD105 (type III TGF β 1–3 receptor or endoglin). HLA-DP QR was expressed in less than 2% of the population. There was no detectable contamination of hematopoietic cells: in fact, markers of the hematopoietic lineage, such as the lipopolysaccharide receptor CD14, CD34 and the leucocyte common antigen CD45, were not detectable.

Osteogenic and adipogenic differentiation of hMSCs were readily inducible as described previously (30), indicating that these cells were indeed of mesenchymal derivation. Among typical mesenchymal genes, hMSCs expressed detectable levels of bone sialoprotein, osteocalcin, PPAR-γ , type I collagen, and alkaline phosphatase transcripts.

hMSCs viability and proliferation

hMSCs were cultured for 72 h in the presence of PRP at different dilutions. High concentrations of PRP (1:1, 1:5 dilutions) caused massive cell detachment, whereas the lower ones (1:50, 1:100 dilutions) proved to be the most effective concentrations in maintaining hMSCs viability as assessed by MTS assay . Consistently, by light microscopy, the cellular density, also significantly enhanced after treatment of the cells with 1:50 and 1:100 PRP dilutions for 72 h when compared with the corresponding control in serum free- and growth medium .

DISCUSSION

First, we showed that PRP, used 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 hMSCs. Moreover, Integra® appeared to be a suitable substrate for hMSCs colonization, as judged by two-photon microscopy combined with fluorescence lifetime imaging (FLIM) and confocal analysis. The cells were then seeded on Integra® + PRP for 24 and 48 h. Notably, in these conditions, the seeded cells exhibited a greater attitude to colonize the scaffold, showed improved cell adhesion and spreading as compared with those cultured on Integra® alone, and acquired a fibroblast-like phenotype indicating that the bioengineered scaffold provided an appropriate environment for cellular growth and differentiation.

In conclusion, these results, even though preliminary, provide clues for the design of new therapeutic strategies for skin regeneration, consisting in the combination of mesenchymal stem cells with engineered biomaterials.

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