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Concentration-dependent effect of platelet-rich plasma on keratinocyte and fibroblast wound healing

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Abstract

Background aims. Platelet-rich plasma (PRP) has been found to contain a high concentration of growth factors that are present during the process of healing. Studies conducted found that application of PRP accelerates wound healing. In this study, we characterized the skin cell suspension harvested using the co-isolation technique and evaluated the effects of PRP (10% and 20%, v/v) on co-cultured keratinocytes and fibroblasts in terms of wound healing. *Methods.* Human keratinocytes and fibroblasts were harvested via co-isolation technique and separated via differential trypsinization. These cells were then indirectly co-cultured in medium supplemented with 10% or 20% PRP for 3 days without medium change for analysis of wound-healing potential. The wound-healing potential of keratinocytes and fibroblasts was evaluated in terms of growth property, migratory property, extracellular matrix gene expression and soluble factor secretion. *Results.* The co-isolation technique yielded a skin cell population dominated by fibroblasts and keratinocytes, with a small amount of melanocytes. Comparison between the 10% and 20% PRP cultures showed that the 10% PRP culture exhibited higher keratinocyte apparent specific growth rate, and secretion of hepatocyte growth factor, monocyte chemoattractant protein-1, epithelial-derived neutrophil-activating protein 78 and vascular endothelial growth factor A, whereas the 20% PRP culture has significantly higher collagen type 1 and collagen type 3 expressions and produced more granulocyte-macrophage colony-stimulating factor. *Conclusions.* PRP concentration modulates keratinocyte and fibroblast wound healing potential, whereby the 10% PRP promotes wound remodeling, whereas the 20% PRP enhances inflammation and collagen deposition.

Key Words: *fibroblasts, keratinocytes, platelet-rich plasma, skin, tissue engineering, wound healing*

Introduction

Wound healing is a dynamic process, involving phases of inflammation, proliferation and remodeling that overlap in space and time [1]. Early wound closure can reduce the infection risk and patient morbidity. However, many factors can disrupt the wound-healing process, including chronic diseases and advanced age. Disruption in the normal healing process leads to the development of chronic wound, which is more difficult to treat and has a higher risk of hypertrophic scar formation upon healing. The conventional split skin graft is normally used to close large wounds. Advancements in regenerative medicine and tissue engineering have led to the development of tissue-engineered skin substitutes. However, currently available tissue-engineered skin substitutes still have limitations, such as prolonged culturing process for autologous skin substitutes and the risk of immune rejection for allogeneic skin substitutes.

Platelet-rich plasma (PRP) is a portion of blood plasma with platelet concentration greater than baseline [2,3]. It is a rich source of cytokines, chemokines, growth factors and matrix proteins [4,5]. Application of certain growth factors, such as platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor-β and epidermal growth factor, have been shown to exert beneficial effects on healing [6,7].

PRP is usually prepared from autologous blood and has been used to treat both acute and chronic wounds [8,9]. PRP can form gels with the addition of thrombin and/or calcium chloride. PRP gel aids hemostasis and acts as a depot for growth factors. In addition, PRP gel can also act as a scaffold for the

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delivery of cells to accelerate healing. A systematic review and meta-analysis carried out by Carter *et al.* concluded that application of PRP expedited the healing of wounds, especially chronic wounds [10]. In addition, the study found that the application of PRP lowered the risk of infection in acute wounds.

PRP has normally been applied clinically as platelet gel, with the addition of calcium chloride and/or thrombin to stimulate the polymerization of fibrinogen content within the PRP. This step is critical in maintaining the applied PRP on the wounds and to ensure the homogenous distribution of applied PRP. The majority of the *in vitro* studies conducted on PRP focused on the effects of activated PRP, rather than platelet gel, on wound healing [11–13]. Thus, to understand mechanism of action of platelet gel, we added 10% and 20% PRP (v/v), which forms gel spontaneously without the addition of calcium chloride or thrombin to the keratinocyte-fibroblast co-culture. The wound healing modulatory effects of platelet gel were evaluated in terms of growth property, migratory property, extracellular matrix gene expression and soluble factor secretion. In addition, we characterized the skin cell suspension harvested via the co-isolation technique by evaluating the cell yield, viability and population balance.

Methods

Skin sample

With the approval from the Universiti Kebangsaan Malaysia Research and Ethical Community (approval project code FF-2013-254), 6 redundant skin samples were obtained from consented patients who underwent abdominoplasty.

PRP preparation

Whole blood was collected from consented donors by venipuncture into vacutainer containing 3.2% sodium citrate (Greiner Bio-One, Frickenhausen, Germany). Collected bloods were centrifuged at 100*g* for 15 min to separate PRP and red cells. Harvested PRP was concentrated by discarding 2 mL of plasma after the second centrifugation at 600*g* for 5 min.

Cell isolation and culture

Skin cells were isolated as described previously [14–16]. Briefly, the skin sample was trimmed of hair and fat before cut into small pieces of 1–2 mm^2 and digested with collagenase type I (Worthington, Lakewood, NJ, USA) and trypsin- ethylenediaminetetraacetic acid (TE; Mediatech, Herndon, VA, USA), sequentially, to isolate the cells. The isolated cells were cultured in medium Epilife: F12: Ham's Dulbecco's modified Eagle's medium (DMEM) (2:1:1; Gibco, Invitrogen, Grand Island, NY, USA) supplemented with 5% fetal bovine serum (FBS; PAA Laboratories, Pasching, Austria).

Co-cultured keratinocytes and fibroblasts were separated via differential trypsinization once the cells achieved 80% confluency by exposing the culture to TE for 5 min. Detached fibroblasts were cultured using Ham's F12:DMEM (1:1) medium with 10% FBS, and the keratinocytes were cultured with Epilife medium.

In all the experiments, P2 keratinocytes and fibroblasts were co-cultured using cell culture insert (BD Biosciences, San Jose, CA, USA), in which the keratinocytes were seeded on cell culture insert (surface area of 4.2 cm^2), and the fibroblasts were seeded on the companion plate (surface area of 9.6 cm^2; Figure 1). The number keratinocytes and fibroblasts seeded on respective culture surface for growth property analysis was 5×10^4 cells and for flow cytometry, reverse transcriptase polymerase chain reaction (RT-PCR) and multiplex enzyme-linked immunosorbent assay (ELISA), were 1.5×10^5 cells. For scratch wound assay, 5×10^5 keratinocytes, and 3×10^5 fibroblasts were seeded on respective layers. The plates were incubated overnight at 37°C and 5% CO_2 to permit cell adhesion before fresh medium Epilife:Ham's F12:DMEM (2:1:1) supplemented with 5% FBS, 10% PRP or 20% PRP were added. The addition of 10% and 20% PRP in this experimental setting resulted in the formation of thin platelet gel on the culture surface without the addition of calcium chloride or thrombin. Thereafter, the plates were incubated for 3 days without changing the medium.

Cell yield and viability

The trypan blue exclusion assay was performed to determine cell yield and viability upon isolation. Briefly, 50 μL of cell suspension was mixed with 50 μL of trypan blue solution (Sigma-Aldrich, St. Louis, MO, USA). Then the cells were counted using hemocytometer. Cell yield and viability were calculated using the following formulas:

$$\text{Cell yield} = (\text{total viable cell}/0.9) \times 10^3 \times D \times V$$

$$\text{Viability} = (\text{total viable cell}/\text{total cell}) \times 100\%,$$

where D = dilution factor = 2, V = total volume of cell suspension, 0.9 μL = volume in hemocytometer chamber, and 10^3 = conversion factor from microliter to milliliter.