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The Potential of Cell-Homing Differentiation of Dental Pulp Into Various Tissues Through Applied Platelet-Rich Plasma

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ABSTRACT
Pulpal necrosis is a problem in the teeth root that might be caused by caries, trauma, or deformities during the development. Should it not appropriate handling, it will affect first loose teeth or being non-vital teeth. The platelet-rich plasma (PRP) is a potential biomaterial for inducing dental pulp cell homing differentiation to various pulp cells by which it is a plethora of growth factors needed for the cell-homing differentiation such as PDGF, TGF-β, IGF, and BMP. The tissue regenerative of dental pulp such as revascularization, bone regenerative, and neuro-regenerative is three-important issues in restoring damaged dental pulp. Therefore, this review attempts to describe the potential of PRP relates to those issues.

Keywords: *Cell-homing, Dental pulp, Platelet-rich plasma, Growth factors, Differentiation*

1. INTRODUCTION

The dental pulp is a unique part of the tooth compares with others, located at the centre of the tooth and surrounded by hard intact tissue, namely enamel and dentine [1]. Anatomical form likes a canal, and it contains blood vessels, nerves, and connective tissue. Those nerves and blood vessels pass through the pulp canal from apical foramen: a small hole located at the root tip. During the growth phase, the immature pulp is a more massive pulp canal than a mature pulp because of the high blood supply thereof. The growth of the pulp canal by increasing the ages is confirmed by narrowing the canal through root apical. Surprisingly that presenting of dental pulp stem cells (DPSCs): self-renewal ability cells, in the dental pulp, contributes to a condition that has high proliferation and multipotent differentiation likes the other sources of stem cells such as bone marrow, umbilical cord, and adipose tissue. The existing DPSCs in dental pulp, therefore, contribute to regenerative tissue in a tooth structure.

Pulpal necrosis is a problem in the teeth root, which could be caused by caries, trauma, or deformities during the development of permanent teeth [2]; then, it can affect teeth too early loss and non-vital condition; as a result, problem in aesthetic, teeth functions, speech, and so forth. Clinically, the typical procedure to overcome the necrosis in the pulp is treated by an apexification procedure using either calcium hydroxide ($Ca(OH)_2$) or immediate mineral trioxide aggregate (MTA) plug. However, this procedure reported that it does not promote further root development and vulnerable to short-term success [3]. Therefore, nowadays, regenerative endodontic is an alternative way in the biologically based procedure that used to allow continued root formation and apical closure by mesenchymal stem cells as homing cells in the pulp.

Principally in regenerative endodontic procedures (REPs), based on the stem cell sources that involve in this purpose, it is divided into two categories by which cell homing based and cell-based regenerative. The cell homing based is endogenous cells undertake tissue regeneration or repair that originated from its dental pulp, whereas the cell-based regenerative requires the exogenous stem cells to achieve dental pulp regeneration. DPSCs as the cell homing in the pulp, its cells are the superior mesenchymal cells compares with other stem cells which enable to differentiate into varies mesenchymal derived cells such as osteoblast, adipocytes, fibroblasts, cementoblasts, chondrocytes, or odontoblast by which depends on growth factor present around these cells [4]. Traditionally, REPs purpose of inducing these cells to differentiate by using the growth factors from induced hemorrhage: apical bleeding, in the root canal to form a blood clot and

scaffold. However, the growth factor concentrations are limited and unpredictable. Moreover, erythrocytes in the blood clot suffer necrosis and affect to this treatment.

A self-renewal cell of dental stem cells in teeth involves in development and maintenance of adult dental tissues by which depends on the dentine niche of growth factors or bioactive molecules. Platelet from blood contains abundant of these bioactive molecules to induce DPSCs differentiation called growth factors. Hence, root canal hemorrhage in REPs treatment purposes to attract as much as possible a number growth factors released by activating platelets from whole blood, and the blood clot products from this mechanism could be as natural scaffold that acts as a framework for cell-adhesion site in cell regeneration.

However, induced growth factors from whole blood have a weakness in osteogenesis, which presenting leucocyte: cells of the immune system can inhibit bone formation by increasing pro-inflammatory cytokines and affect bone regeneration [5]. Nevertheless, in the other case, the leucocyte has a positive outcome in tendinopathy treatments [6]. On the other hand, utilizing autologous platelet-rich plasma (PRP) is plausible in attempting to reduce leucocyte contains considering the growth factors as bioactive molecules in regenerative tissue. Accordingly, this review will describe the potential of PRP in inducing dental pulp regeneration by employing DPSCs which including angiogenesis, osteogenesis, and neuroregeneration.

2. PLATELET-RICH PLASMA

Platelet-rich plasma (PRP) is autologous or allogeneic of the blood-derived product as a small volume of plasma containing higher platelet concentration than based line. It can release various bioactive molecules such as growth factors, microRNA, cytokines, exosomes, and other proteins that involve in regeneration or repair of different tissue types [7]. In consequence, PRP has been used widely in dermatology, oral/maxillofacial surgery, aesthetic surgery, orthopedic, and sports medicine through activation using calcium chloride ($CaCl_2$) or thrombin.

The potential of usage PRP in medical treatments has grown many protocols and kits that support to harvest platelet from whole blood. Those produce various sort of blood-derived products which have successfully applied in therapeutic purposes, namely pure-platelet rich plasma (P-PRP), leucocyte-platelet rich plasma (L-PRP), pure-platelet rich fibrin (P-PRF), and leucocyte-platelet rich fibrin (L-PRF), which in the harvesting process, PRP uses anti-coagulant in whole blood in separating plasma with red blood cells, whilst no anti-coagulant for PRF.

Briefly, the conventional way of PRP and PRF protocol, first centrifugation of whole blood will separate blood in three layers: plasma, buffy coat, and red blood cell. The collecting plasma part and applying the subsequent centrifugation will produce P-PRP at the bottom one-third part of the plasma. The collecting plasma and buffy coat layer will provide L-PRP after the second centrifugation. Besides, in the same way, it also happens to P-PRF and L-PRF. On the other hand, developing commercial kits today give an easy way for the operator to harvest platelet from whole blood.

Related to the efficacy of PRP as an autologous biomaterial, a very safe therapeutic option, the PRP has been used widely in several dentistry treatments offered by its plethora-released growth factors such as in maxillofacial surgery, dental guide bone regeneration, dental guided tissue regeneration, and periodontal. The PRP also shows antibacterial properties against *P. gingivalis*; thus, this material suggested can become an adjunct to periodontal treatments.

3. REVASCULARIZATION OF THE DENTAL PULP

Successfully revascularization of dental pulp indicates REPs flourishing. This procedure is not merely enough at surgical endodontic, apexification with calcium hydroxide or mineral trioxide aggregate (MTA) plug. Beyond this, root canal disinfecting, matrix providing from a blood clot to make sure the cells could grow, and coronal access sealing are necessary things for blood vessel formation [8]. Some reports informed that revascularization occurred associates with increasing the root length and thickness [8-10]. PRP as aforementioned enables to release abundant of growth factors promote DPSCs differentiation and proliferation. Especially in revascularization and angiogenesis in dental pulp, involving complex molecular mechanisms that requires diverse a few agonist growth factors such as vascular endothelial growth factor (VEGF), fibroblastic growth factor (FGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF) to stimulate revascularization and angiogenesis [1].

The variable number of growth factors and red blood cell necrosis on the apical bleeding method is a limitation on the success revascularization. Hence, PRP can be optional to overcome this shortage. Jadhav et al. reported that presenting the misconception about the number of stem cells in mature pulp determines revascularization to be successful, which only immature teeth: to whom less than 16-year-old patients will be a success for revascularization. This case report explained that applying PRP on a 40-year old patient was the successful outcome of revascularization [11]. On the other hand, Murray *et al.*, in his meta-analysis of clinical efficacy of 222 cases of immature teeth, noticed that both PRP and PRF are more frequently to induce apical closure than blood-clot revascularization [12]. It means that the highest growth factor that contains PRP to baseline enables attracted adjacent root mesenchymal stem cells to differentiate and

proliferation. Thus, the fibrin formed by activated platelets can be being a natural-mesh like for cell-to-cell migration and deposition in apical regeneration.

4. BONE REGENERATION

An autologous blood product, platelet-rich plasma composed of concentrated platelet, can release some growth factors having a beneficial effect on osteogenesis like insulin-like growth factor (IGF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). The fibrin structure of activated PRP, as aforementioned, gives beneficial on cell migration and proliferation. However, some reports explicitly noticed that leucocyte contain in the biomaterials or around cell-bone regenerative may deliver increasing pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) which are either inhibits extracellular matrix formation or causes extracellular matrix degradation [5].

The cell homing of DPSCs succeeded isolate from extracted human third molar and shown their ability to osteogenic differentiation, and calcium phosphate formation [2]. Sadheginia *et al.* in a study about the potential of fibrin glue and PRP on the proliferation and osteogenic differentiation of human dental pulp stem cells (hDPSCs) informed that both these materials increased the mineralization and osteoblastic differentiation of harvested cells and bone marker expressions [13]. Another studied on PRP effect on several dental stem cells, there showed that treatment with 2% PRP resulted in the highest level of proliferation and ALP activity; however, its ALP activity decreased with a higher concentration of PRP [4]. Thus, the ability of DPSCs to the multilineage differentiation and applying agonist growth factors from autologous PRP could enhance the restorative of damaged-dental pulp particularly in osteogenesis inductions.

5. NEUROREGENERATIVE OF THE DENTAL PULP

The highest multilineage potential of DPSCs is plausible promising to restore the teeth caused by the traumatic or non-vital condition, but there was limited information about the successful neuroregenerative of the dental pulp. Nevertheless, some *in vitro* studies have informed the prosperity of DPSCs trans differentiating into neuron-like cells by particular conditioned media [15]. Ghoorha *et al.* reported that DPSCs cultures had success in differentiating into neurons at early passage [16]. Another reported by Nosrat *et al. in vitro* culture system, there was an interaction between the dental pulp cells and trigeminal neurons in which as functional connectivity with the central nervous system. The dental pulp cells produce several neurotrophic factors, NGF, BDNF, and GNDF mRNAs are found in the dental pulp at the time of the onset of dental pulp innervation, in a culture that promotes survival and a specific and elaborate neurite outgrowth pattern from trigeminal neurons [17].

The neuron cells are a complicated cell to grow compared to others; it depends on the environment and the specific condition [18,19]. Platelet concentrate as a plethora of several growth factors and cytokines involved in tissue repair. Stole *et al.* had confirmed that PRP and PPP could induce spiral ganglion neuron in vitro and could be an alternative for pharmacology intervention; thereby, those contain a balanced and natural composition of trophic factors [20]. According to some released growth factors from PRP as aforementioned, some growth factors work together and interact efficiently for wound healing such as TGF-β, PDGF, VEGF, IGF, and bFGF are series of growth factors as being crucial for cell proliferation and differentiation, stimulating of angiogenesis and scar control in the process of regenerating peripheral nerve structures. For instance, PDGF might stimulate trophic activity on a neuron, induce Schwann cell proliferation; differentiation; and myelin formation, and TGF-β regulates mitogenic effects of other growth factors (nerve growth factor, or brain-derived neurotrophic factor) [21]. To put together, as a reservoir of growth factors and cytokines that related to conditioned media for cell proliferation and differentiation, particularly for the cell-homing of DPSCs, brought the PRP potent as a treatment material in REPs.

6. CONCLUSION

The lack of cell-homing differentiation at the conventional procedures of dental pulp necrosis handling, challenging to dentistry practitioners to find the new materials to raise the cell-homing potential in pulp repairing. PRP, an autologous material, is a safe material that has an abundant of growth factors and cytokines being potential as an alternative biomaterial in where its efficacy in making a worthy-conditional environment for re-vascularization, bone re-regeneration, and neuro re-regenerations of the cell homing of the dental pulp.

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