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Research Report

Natural Growth Factor: Platelet Rich Plasma Stimulates Proliferation of Fibroblast Cell Culture

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ABSTRACT

Platelet rich plasma(PRP) is a source of many natural growth factor that can modulate wound healing. PRP has become a valuable adjunct in dentistry. Fibroblast cell in ligament periodontal play on important role in periodontal regeneration. The study was performed to investigate the influence PRP 10%–100% on the proliferation of fibroblast cell culture in vitro. A fibroblast culture was estabilished from baby hamster kidney (BHK-21) were tested on proliferation was measured with MTT assay after induced PRP 10–100%. We showed that PRP stimulate the proliferation of fibroblast, PRP 80% as the optimal choice to a good enough proliferative stimulus. PRP has proven to be effective at improving surgical results in periodontal surgery. PRP also show promise in periodontal regenerative medicine.

Key words: *PRP, natural growth factor, proliferation of fibroblast*

INTRODUCTION

In recent years,scientific research and technology has provided a new perspective on understanding healing process to wards regenerative medicine and tissue engineering. Growth factor play a crucial role in the healing process. The application of natural growth factor: Platelet Rich Plasma (PRP) has been safely used and documented in many fields including: dentistry, orthopedics, neurosurgery, cosmetic and maxillofacialsurgery. Based on this principle platelets are introduced to stimulate a supra-physiologic release of growth factor in an attempt to jump start healing in chronic injuries. Platelet also release many bioactive proteins responsible for attracting macrophages, mescenchymal stem cells and osteoblast.[1,2]

Tissue repair normally begins with clot formation and platelet degranulation leading to release of various cytokines and coagulation factors, which modulate inflammatory response.To date, more than 30 different cytokines have been found in platelets including PDGFs,TGFs,EGF and IGF.[3] The PRP can be prepare by separating from fresh anticoagulated blood by simple centrifugation, which concentrates platelets up to six times the baseline count in whole blood.[4] The PRP play important role in repairing wounds by providing growth factors and extracellular matrix proteins that attract new cell.

Platelet rich plasma has become a valuable adjunct in dentistry such as periodontitis therapy. Periodontitis is an inflammatory disease which manifest clinically as loss of supporting periodontal tissues including fibroblast periodontal ligament, gingival fibroblast and osteoblast. These changes often lead to an aesthetically and functionally compromised dentition. Conventional open flap debridement falls short of regenerating tissues destroyed by the disease.[5] Clinicians and scientists in dentistry are investigate the use of PRP as a way to enhance the body's natural wound-healing mechanisms and towards attaining complete periodontal regeneration. However,controversies exist in the literature regarding concentration of PRP stimulating fibroblast proliferation. The aim of this article are to investigate the influence of platelet rich plasma 10%–100% on the proliferation of fibroblast cell culture in vitro, to determine optimum PRP concentrate for fibroblast proliferation.

MATERIAL AND METHODS

Platelet Rich Plasma Preparation

Platelet rich plasma was prepared from venous blood obtained from healthy volunteers after obtaining their signed informed consent. Blood samples were collected in

9 ml tubes with trisodium citrate anticoagulant. The blood was first separated into two layers by centrifugation at 272 g for 7 min. The upper layer was collected and centrifuged at 1.288 g for 7 min. PRP was collected from the resulting 1–1,5 ml sediment.[6]

PREPARATION OF FIBROBLAST CELL CULTURE

Cell BHK-21 were cultur under Dulbecco's modified Eagle's medium (DMEM, Cambrex, Walkersville) with 4mM L-gluthamine, 0.05 units of penicilin per 0.05 ug of streptomycin served as the basal medium, which was supplemented with 10% fetal Bovine serum. When cells reached confluent, cell culture were seeded in well plates.

Fibroblast Proliferation Assay

Fibroblast cell BHK-21 were seeded in 84 well plates in 0,5mL of the MEM medium with 2% FBS. When cells reached 30% confluent growth, the medium was washed off and cells were incubated for 24 hours in the MEM medium without serum. The medium refreshed before adding PRP. Twelve different growth condition were tested in the MEM medium with PRP 10%; 20%; 30%; 40%, 50%, 60%, 70%, 80%, 90%, 100%, positive control, negative control. The effect of different concentrations of PRP on cell proliferation was also studied. After 4 to 5 days of incubation, the proliferation of fibroblast measured with an MTT assay (Sigma-Aldrich Chemie). This is a colorimetric assay that measures the chemical reduction of MTT (3-4[4,5-Dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide) into formazan, which is directly proportional to number of viable cells in the tested culture. Cultures were incubated 2-3 hours in a culture medium with 0,5 mg/ml of MTT. The resulting formazan was then eluted with acidified isopropanol (0,04N HCL in isopropanol) and the optical density was measured at 570 nm. Proliferation was measured in seven replication. Optical densities were expressed as the means +- standard deviation. Differences between means were assesed by Anova, value of $p < 0,05$ was considered to be statistically significant

RESULT

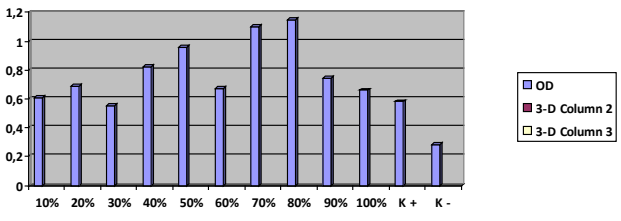

Figure 1. Proliferation of fibroblast cell culture in the presence 10%–100% PRP

All component of PRP 10%–100% significantly stimulated the growth of fibroblast when compared to the negative control (figure 1, $p < 0,05$). Fibroblast cell growth was enhanced in a dose dependent manner, but 90% and 100% PRP proliferation decreased. PRP 80% showed the strongest effect.

DISCUSSION

The wound healing process is a complex mechanism characterized by four distinct, phases: hemostasis, inflammation, proliferation and remodelling. All these events are coordinated by cell interactions and soluble GFs released by various cell type. Platelet Rich Plasma is a biological source of various GFs which stimulate the proliferation of gingival fibroblast, osteoblast, periodontal ligament fibroblast, stromal stem cell, endothelial cell and enhance healing of ulcers. In our study we showed that PRP stimulates the proliferation fibroblast cell culture (BHK21) in vitro, because PRPs contain approximately 7,9 times as many platelets as whole blood and it's activation was associated with release a large amounts of PDGF-AB and TGF-beta 1. PRP is a rich source growth factors including EGF,TGF-beta, IGF-1, Ang-2 and PDGF. PRP may suppress cytokine release,limit inflammation and thereby promote tissue regeneration. TGF-beta in PRP stimulated undifferentiated mesenchymal cell proliferation, regulates mitogenic effect. PDGF stimulates chemotaxis and mitogenesis in fibroblast cell. [5]

We showed that 80% PRP are the strongest effect to stimulate proliferation cell, which is inconsistent with finding report by Jeras et al [5] ,They showed that 20% PRP have strongest effect in human dermal fibroblast, because they could not measure higher concentration with MTT. Weibrich et al observed an advantageous with platelet concentrations, they state that higher concentrations might have a paradoxically inhibitory effect. Schanabel evaluated collagen content, PRP at 100% concentration stimulated the greatest collagen type 1, collagen type III without increasing expression of the proinflammatory matrix metalloproteinases. PRP led to significantly increased level of growth factors and suppressed inflammation by promoting secretion of LXA4.

The use of natural and autologous growth factor reduces the risk of transmissible infection and alergic reaction. In our study, we did not observe any cytotoxic effect in high concentration of PRP. PRP is no plausible mechanism by which growth factor result in neoplastic development, because growth factor act on cell surface receptors, do not enter the cell and do not cause DNA mutation.

Bieback[6] show that PRP have significantly higher proliferative effect on mescenscymal stem cell (MSC) providing 1320–1400 fold expansion in 11 days compared

Table 1. Proliferation of fibroblast cell culture in the presence 10%–100% PRP

PRP	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	K+	K–
OD	0,61	0,688	0,55	0,82	0,96	O,67	1,1	1,15	0,74	0,66	0,58	0,28

with only 254-fold expansion with Fetal Calf Serum. The fact that PRP as alternative supplements trigger for MSC expansion in clinical setting, since the large number of MSC required for tissue engineering. This finding could be useful in preparing PRP in advance and for storage in a –70° C for multiple apllications.

In conclusion, we show that PRP enhance proliferation of fibroblast cell culture. We promote 80% PRP as the optimal choice to a good enough proliferative stimulus. Therefore, we are currently adressing this issue in molecular and functional experiments.

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