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Original Research Article

In vitro bone sialoprotein-I expression in combined gingival stromal cells and platelet rich fibrin during osteogenic differentiation

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Abstract

Purpose: *To analyze the expression of bone sialoprotein - I (BSP - I) after the addition of platelet rich fibrin (PRF) in gingival somatic cell (GSC) culture medium during osteogenic differentiation in vitro.*
Methods: *GSCs were extracted from healthy, 1-month-old, male Wistar rats (Rattus Novergicus), weighing 250 - 300 g, and which had been randomly selected (n=4). These cells were cultured for 14 days and passaged every 4 days. Five subcultures of GSCs were cultured in three plates (M24) (N = 54; n = 6) for 7, 14 and 21 days in three preconditioned culture media (group I: plain culture media; group II: preconditioned osteogenic culture media, and group III: preconditioned osteogenic culture media with platelet rich fibrin). The expression of BSP-I was immunocytochemically (ICC) examined with monoclonal antibodies. Homogeneity and normality tests ($p > 0.05$) were then performed followed by an analysis of variance (ANOVA, $p < 0.05$).*
Results: *The highest expression of BSP-I was found in group III (Day 21, 13.00 ± 2.000), while the lowest expression of BSP-I was found in group I (Day 7, 7.33 ± 1.155). There were significant differences between the groups ($p = 0.000$, $p < 0.05$).*
Conclusion: *PRF stimulates and significantly enhances the expression of BSP-I in GSC culture during osteogenic differentiation. Thus, PRF can be used to accelerate regeneration of alveolar bone defects.*

Keywords: *Alveolar bone, Bone Sialoprotein-I, Gingival Somatic cells, Osteogenic ability, Platelet rich fibrin*

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INTRODUCTION

Gingivitis and periodontitis represent the most common diseases affecting the oral cavity. Severe periodontitis constitutes the sixth most prevalent disease (11.2 %) with around 743 million people affected worldwide and experienced annual increases between 1990 and

2010 of 57.3 % [1,2]. Periodontal disease constitutes the second most prevalent dental health problem in Indonesia after caries and malocclusion. In 2013, Basic National Health Research Department (RISKESDAS) reported the prevalence of periodontal disease as having reached 96.58 %, thereby representing the second most common oral and dental condition in Indonesia [2-4]. Numerous previous studies relating to dentistry have analyzed the relationship between bacterial activity and inflammation resulting from periodontal disease which lead to problematic alveolar bone defects [5]. To overcome these defects, numerous techniques such as hydroxyapatite [6,7] or Carbonate-apatite bone graft [8] have been developed to stimulate and enhance the regeneration of alveolar bone.

At the time of writing, increasing attention is being paid to biomaterial and cell material interaction [9]. Consequently, it was proposed to combine Gingival Stromal Progenitor Cells (GSCs) and Platelet Rich Fibrin (PRF) in order to stimulate and increase the regeneration of alveolar bone defect. By developing a combination of GSCs and PRF, it is possible to achieve the complete regeneration of alveolar bone defect [7,8]. GSCs osteogenic differentiation can be detected by Bone Sialoprotein-I (BSP-I) in its later stages [9-11]. However, information about the effect of the addition of PRF on the expression of BSP-I in GSC culture medium during osteogenic differentiation in vitro remains limited. The main aim of this study was to analyze the expression of BSP-I after the addition of PRF to GSC culture medium during osteogenic differentiation *in vitro*.

Ethical clearance

This experimental study was approved by the Department of Dental Medicine, Airlangga University (approval no. 289/HRECC.FODM/ XII/2017), and carried out in accordance with International Guidelines on Animal Model Study for Scientific Laboratory use [12].

Research design

The study was analytic and true experimental in nature with post-test only control group design. A simple random sampling method and a Hosmer-Lemeshow's sample size formula were used to determine the sample. GSC was extracted from the mandible free gingiva of four male, one-month-old Wistar rats weighing between 250 and 300 grams. Before the gingivectomy was performed, all the animals were euthanized with anesthesia (ketamine and xylazine 60 mg/body weight). The extracted GSCs were frequently sub-cultured for 3-5 days in accordance with Nugraha *et al* recommendations regarding SPCs isolation and culture method [13]. The GSCs (passage 3 - 5) were expanded in three 24-well culture plate (N = 56; six samples in each group) for 7, 14 and 21 days in three different culture media.

Platelet-rich fibrin extraction

Wistar strain rats were sacrificed using rodent anesthesia (60 mg /BW) in order to obtain PRF. The blood of these subjects was aspirated and extracted through their hearts. About 3-6 ml of blood was extracted with a 10ml syringe before being placed in a plain blood tube and swing centrifuged at 3,000 rpm/min for ten minutes) (Thermofisher, US). The PRF was then placed in a M24 culture plate of the treatment group [7,14].

Osteogenic differentiation ability of GSCs combined with PRF

There were three groups used in this study consisting of group I: GSC cultured in α-Modified Eagle Medium (αMEM) only; group II: GSC cultured in preconditioning osteogenic culture media (100 µg / ml sodium pyruvate, 0.2 mM ascorbic acid - 2 phosphate, dexamethasone 10 -7 M, 2 mM Lx -glutamine) (Sigma Aldrich, US) and group III: GSC combined with PRF cultured in preconditioning osteogenic culture media. All GSC culture media were changed every 3-4 days. The capacity for GSC osteogenic differentiation was examined on days 7, 14 and 21 [8].

The expression of BSP-I was examined by means of immunocytochemical staining using BSP-I monoclonal antibodies (cat. Sc – 21742) (Santa Cruz Biotechnology, US), a 3.3' diaminobenzidine (DAB) staining kit (Sigma Aldrich, US) and BSP-I monoclonal antibodies (cat. Sc – 21742) (Santa Cruz Biotechnology, US). The expression of BSP-I in all groups was analyzed using an electron microscope (H500L, Nikon Tokyo, Japan) at 200x magnification.

Statistical analysis

Data were analyzed by homogeneity and normality tests ($p > 0.05$) and subsequently with analysis of variance (ANOVA) ($p < 0.05$).

RESULTS

The expression of BSP-I was positive (brown color) in cultured GSC (Figure 1). Normal homogeneously distributed ($p > 0.05$) data was

obtained. In this study, the highest mean of BSP-I expression found in the Day 21 group III, while reduced BS- I expression was found in the Day 7 group I. BSP-I expression was found to be significantly different between the groups ($p < 0.05$, Table 1).

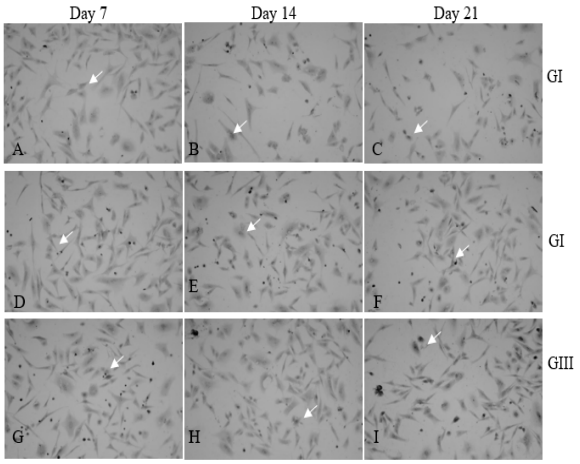

Figure 1: The positive expression of BSP-I (brown color) in the GSC (white arrow). BSP-I expression Group I (A, B, C); Group II (D, E, F); Group III (G, H, I) on Days 7, 14 and 21 (H500L, Nikon Tokyo, Japan) at 200x magnification. ***Note:*** GI = Group 1, GII = Group II, and GIII = Group III

Table 1: Data for the expression of BSP-I

Time	Group I	Group II	Group III
Day 7	7.33 ± 1.155	7.67 ± 1.528	11.33 ± 1.155
Day 14	8.67 ± 1.528	9.67 ± 3.215	11.67 ± 1.528
Day 21	9.00 ± 2.000	11.67 ± 1.528	13.00 ± 2.000

* p<0.05 (one-way ANOVA)

DISCUSSION

The highest BSP-I expression occurred in group III (Day 21). There were significantly contrasts across all groups ($p < 0.05$). The expression of BSP-I increased gradually from Day 2 to Day 7. These results were in line with those of Li et al and Tu *et al.* which posited that BSP-I expression constituted a late stage osteogenic differentiation marker [15,16]. The addition of PRF to GSC culture medium during osteogenic differentiation can stimulate BSP-I expression, a position adopted in the study by Duan *et al* that showed significantly increased BSP-I or Osteopontin expression in combined human GSC and PRF on Day 21 [10].

PRF contained rich and various growth factors such as Thrombospondin-1 which support cytokine migration, while facilitating and stimulating the osteogenic differentiation ability of GSPC. PRF enhances GPCC proliferation, differentiation and migration and the induction of bone formation by increasing BSP-I [17,18].

BSP-I is a glycoprotein most recently detected in osteoblast in 1986. Bone Sialoprotein is a multifunctional protein strongly expressed in bone that acts as a junction protein [19] only found at the intracellular level. BSP-I is a dynamic cytoskeleton regulator and gene expression. Extracellular BSP-I function interacts with certain surface cell receptors. The increased expression of BSP-I is involved in many processes such as alveolar bone remodeling and wound healing [20,21].

BSP-I is strongly expressed in inflammatory conditions in the Extracellular Matrix areas of mineralized tissue such as bone. BSP-I in bone tissue regulates cell-to-cell, cell-to- cell matrix interactions and cartilage-to-cell transition to produce compact bone in the repair process of fractures in osteoclast adhesion in bone matrix. miRNA regulates the secretion and expression of BSP-I such as miR181a / b / c / d. BSP-I is strongly expressed in the later stages of osteogenesis from Day 14 to Day 28 and widely secreted by SPCs that regulate osteogenic differentiation ability [16,22,23].

BSP-I plays an important role in bone remodeling, acting as an osteoclast regulator in the bone mineral matrix to maintain inorganic components of bone such as hydroxyapatite, Ca(PO4) (OH)2. Decreased expression of BSP-I will cause osteoporosis. BSP-I is also expressed in osteoclasts and osteoblasts responsible for bone remodeling in bone homeostasis [19,22,24].

CONCLUSION

GSCs cultured in PRF significantly increase BSP-I expression on Day 21. PRF increases and stimulates GSCs osteogenic differentiation ability as measured by BSP-I expression. Further research is required to analyze BSP-I expression during osteogenic differentiation of combined GSCs and PRF *in vivo.*

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was undertaken by the individual(s) named in this article who will bear all liabilities pertaining to claims relating to its contents. All the authors made substantial contributions to this study and/or manuscript and approved the final draft of the paper prior to its submission.

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