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ORIGINAL ARTICLE

Plasma rich in growth factors stimulates proliferation, migration, and gene expression associated with bone formation in human dental follicle cells

Hitoe Okada [a], Kosuke Takahashi [a,b], Naomi Ogura [a,b], Risa Tomoki [a], Ko Ito [a,b], Toshirou Kondoh [a,b*]

[a] *Department of Maxillofacial Surgery, Nihon University School of Dentistry at Matsudo, Matsudo, Japan*
[b] *Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo, Matsudo, Japan*

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Abstract *Background/purpose:* Plasma rich in growth factors (PRGFs), which is prepared from autologous blood from patients, has been reported with regards to bone regeneration for dental implants. Human dental follicle cells (hDFCs) have the capacity to commit to multiple cell types such as the osteoblastic lineage. The aim of this study is to evaluate the effects of PRGFs for mineralization in hDFCs.
Materials and methods: PRGFs was prepared from whole blood centrifuged at 460*g* for 8 minutes. hDFCs isolated from the dental follicle with collagenase/dispase were cultured with growth medium or osteogenic induction medium (OIM) containing PRGFs or fetal bovine serum. Concentrations of the growth factors were examined using an enzyme-linked immunosorbent assay kit. A cell migration assay was used for two-dimensional movement. Gene expressions were examined with real-time polymerase chain reaction using a DyNAmo SYBR Green quantitative polymerase chain reaction kit.
Results: The platelet concentration in PRGF Fraction 2 was 2.14-fold higher than in whole blood. White blood cells were not detected in PRGFs. Transforming growth factor-β levels were higher than insulin-like growth factor-1, platelet-derived growth factor-AB and -BB, and vascular endothelial growth factors in PRGF Fraction 2. Proliferation and migration by hDFCs increased in OIM supplemented with PRGFs in a dose-dependent manner and were higher in hDFCs cultured in OIM plus 10% PRGFs compared with OIM plus 10% fetal bovine serum. PRGFs

* Corresponding author. Department of Maxillofacial Surgery, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba 271-8587, Japan.
E-mail address: kondo.toshiro@nihon-u.ac.jp (T. Kondoh).

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upregulated the gene expression of *type I collagen*, *osteomodulin*, *alkaline phosphatase*, *bone morphogenic protein-4*, and *transforming growth factor-β* in hDFCs.
Conclusion: PRGFs may promote bone regeneration due to it including high levels of growth factors.

Introduction

Platelet concentration products, which are autologous constituents of inductive factors obtained from blood, contain high concentrations of platelets with various growth factors.[1] Platelet-rich plasma (PRP) has been used in bone augmentation for dental implants.[2,3] However, PRP formulations have different biological activities, depending on the various protocols used to obtain them.[4,5] The system of plasma rich in growth factors (PRGFs) is a method for concentrating platelets[6,7] and is advantageous as it requires only one centrifugation step and is leukocyte-free, thus avoiding high levels of proinflammatory cytokines.[6] PRGFs allows delivery to the site of injury of a cocktail of proteins and growth factors that promotes wound healing and regeneration of tissue and bone.[8,9] Numerous studies have been published describing the benefits of PRGFs *in vivo*. However, the majority of studies about the use of PRGFs only describe the final outcome at the tissue level and do not investigate the mechanism of PRGFs. Therefore, investigation of the role of PRGFs in bone and/or tissue regeneration should involve a study of the biological properties and molecular functions using a cell culture system.

The dental follicle, an ectomesenchymal tissue that surrounds the developing tooth germ, contains stem cells and lineage committed progenitor cells or osteoblast/cementoblast precursor cells.[10] Human dental follicle cells (hDFCs) also have the capacity to commit to multiple cell types, not only to cells of the osteoblastic lineage, but also to cells of adipogenic and neurogenic lineages.[11,12] We previously reported that hDFCs can differentiate into osteogenic lineage cells in osteogenic induction medium (OIM) without dexamethasone,[13] which has various biological effects such as anti-inflammatory properties. In addition, hDFCs express stem cell markers and growth factor receptors, and highly express LIM homeobox 8, which is associated with development of the palatal mesenchyme and tooth germ.[14] In addition, hDFCs are easily accessible for cell culture and have a higher proliferation capacity.[15] According to these findings, we suggested that hDFCs may be useful for therapy and in basic research of the maxillofacial region bone.

In this study, we measured the concentration of growth factors in PRGFs and examined the effect of the soluble factors in PRGFs on proliferation and gene expression in hDFCs treated with PRGF supernatant compared with fetal bovine serum (FBS). We evaluated the efficacy of PRGFs as a substitute for engineered bone tissue in the maxillofacial region.

Materials and methods

Preparation of different blood products

For blood product preparation, whole blood from four young healthy donors (two men and two women, average age 29.5 years) was collected from the external jugular vein after informed consent was obtained. Whole blood was immediately placed into 5-mL sterile extraction tubes containing 0.5 mL of 3.8% sodium citrate as an anticoagulant. The whole blood was divided into two aliquots.

The first aliquot was used to obtain PRGFs that was prepared from whole blood, which was centrifuged in accordance with Anitua's protocol.[6–8] Briefly, tubes were centrifuged at 460*g* for 8 minutes. The plasma fraction (1 mL over the buffy coat) was collected as Fraction 2 (F2), whereas Fraction 1 (F1) was the layer above F2. PRGF F2 was incubated with 10% calcium chloride solution at 37°C for 1 hour to trigger platelet activation and growth factor release. Activated PRGF F2 was centrifuged at 3000*g* for 15 minutes, and then the supernatant was isolated (PRGF F2 supernatant).

The second aliquot was used to obtain serum. Whole blood was left for 30 minutes at room temperature and then centrifuged at 1000*g* for 15 minutes.

The numbers of blood cells in PRGFs and serum were immediately measured, and then the samples were stored at −80°C until use.

PRGF extraction was performed according to the guidelines established by the Ethics Committee of Nihon University School of Dentistry at Matsudo (Recognition number: EC14-13-029-1).

Number of blood cells

The numbers of platelets and white blood cells (WBCs) were counted using XE-2100 (Sysmex, Hyogo, Japan).

Enzyme-linked immunosorbent assay

Concentrations of growth factors including insulin-like growth factor (IGF)-1, transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF)-AB and -BB, and vascular endothelial growth factor (VEGF) were measured using enzyme-linked immunosorbent assay kits (Quantikine enzyme-linked immunosorbent assay kits; R&D Systems, Mckinley, MN, USA) according to the manufacturer's instructions.