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Effects of Plasma Rich in Growth Factors on Bone Formation in Rat Calvaria

Takashi Eda¹⁾, Kosuke Takahashi^{1,3)}, Satoshi Iwai^{1,3)}, Naomi Ogura^{1,3)}, Ko Ito^{1,3)}, Hiroyasu Tsukahara¹⁾,
Masaaki Suemitsu^{2,3)}, Noboru Kuboyama³⁾, Kayo Kuyama^{2,3)} and Toshiro Kondoh^{1,3)}

¹⁾ Department of Maxillofacial Surgery, Nihon University School of Dentistry at Matsudo, Matsudo, Japan

²⁾ Department of oral pathology, Nihon University School of Dentistry at Matsudo, Matsudo, Japan

³⁾ Research Institute of oral science, Nihon University School of Dentistry at Matsudo, Matsudo, Japan

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Abstract: Plasma rich in growth factors (PRGF) had been used in regenerative therapy such as soft tissue and newly bone formation. However, there has been little basic research into the efficacy of PRGF in bone regeneration. In this study, we evaluated the efficacy for new bone formation by transplantation of PRGF onto rat calvaria. The osteogenic potential was evaluated by histologic findings, immunohistochemistry and bone formation analyzed with micro-computed tomography (micro-CT). PRGF was prepared by centrifugation of rat whole blood (WB), and then was activated using 10% calcium chloride solution. The activated PRGF transplanted within a polytetrafluoroethylene (PTFE) tube was transplanted onto calvarial bone of rats. Histological observation demonstrated that PRGF group showed newly formed bone in a wide range. Immunohistochemistry showed Runx2, Osterix, Bone Alkaline Phosphatase (BAP) and Osteocalcin expressed in PRGF group during the early stage of bone formation. Micro-CT showed that PRGF group promoted an increase in bone volume which compared to control group. We concluded that PRGF has more capacity for bone regeneration, and PRGF may be useful in bone regeneration treatment.

Key words: Plasma rich in growth factors, Osteogenic potential, Bone regeneration, Platelet, Transplantation

Introduction

Platelet concentration products, which are autologous constituents of inductive factors obtained from blood, have been known high concentrations of platelets containing various growth factors¹⁾. Marx reported that the combined use of autogeneous bone with PRP was increased in radiographic and histomorphometric bone density²⁾. PRP has been used in bone augmentation for dental implants or trauma^{2,3)}. However, it has been shown that PRP formulations have different biological activities, depending on their various protocol because PRP preparation for different from Marx's protocol and machines⁶⁻⁸⁾.

Plasma rich in growth factors (PRGF) system is one of the methods for preparing platelet concentration^{9,10)}, has several advantages including the enhancement of bone regeneration and rapid soft tissue healing¹¹⁾. This system is advantageous as it requires one step of centrifugation and is leukocyte-free, thus avoiding higher levels of pro-inflammatory cytokines³⁾. In addition, PRGF contains high levels growth factors such as TGF- β and

VEGF, which are associated with bone regeneration^{9,12)}. However, there has been little basic research into the efficacy of PRGF in bone regeneration⁹⁾.

The aim of this study was to evaluate the efficacy for bone formation in transplantation of PRGF using histologic findings, immunohistochemistry and micro-CT analysis.

Materials and Methods

Preparations for PRGF

Twelve male Sprague-Dawley rats (15 weeks old) were obtained from Nihon SLC, Shizuoka, Japan and housed in isolation cages throughout the experimental period. The rats were fed a standardized diet (MF, Oriental Yeast Co., LTD.) and filtered tap water, and maintained under a 12h light/dark cycle at a temperature of 23 \pm 1 °C and relative humidity of 60 \pm 10 %. Following a one week preliminary period, the general well-being of the rats was checked and body weight was measured. Rats weighing 405-415 g in a healthy condition and with normal growth were used in this study. Rat whole blood (5 ml) was collected from the external jugular vein by syringe aspiration via direct venipuncture with a 21 gauge needle. Blood was immediately placed into 5 ml sterile extraction tubes containing 0.5 ml of 3.8

Corresponding to: Dr. Toshiro Kondoh, Department of Maxillofacial Surgery, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba 271-8587, Japan; Tel/Fax: +81-47-360-9394; E-mail: kondo.toshiro@nihon-u.ac.jp