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ORIGINAL ARTICLE

Evaluation of bone regenerative capacity in rats claverial bone defect using platelet rich fibrin with and without beta tri calcium phosphate bone graft material

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KEYWORDS

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Abstract *Aim:* To compare bone regeneration in noncritical rat calvarial bone defects filled with platelet-rich fibrin (PRF), alone or combined with beta-tricalcium phosphate (β-TCP), using micro-computed tomographic (MCT) evaluation.

Animals and methods: Two calvarial bone defects were created in each of 45 male Sprague–Dawley rats (age: 20–22 weeks, weight: 350–450 g), using a dental trephine with an external diameter of 3 mm. The 90 defects were randomly allocated among three groups, each containing 30 unilateral defects in a total of 30 rats. Defects in the control group were allowed to heal spontaneously. Defects in the PRF group received PRF alone. Defects in the PRF/β-TCP group received PRF mixed with β-TCP in a 50\50 percentage. Nine animals (three per group) were killed after 1, 2, 3, 4, and 6 postoperative weeks, and 18 calvarial defects from each period were analyzed for new bone formation and bone mineral density using MCT. Results were compared by a one-way Analysis of Variance with the POST HOC Least Significant Difference test.

Results: The volume and mineral density of bone formed in the control group were significantly different from those of the other two groups. Greater bone regeneration was observed in defects receiving PRF with β-TCP compared to defects receiving PRF alone in the first 2 weeks ($P < 0.001$). However, differences in the volume and density of newly formed bone between the PRF and PRF/β-TCP groups were not significant at 3, 4, and 6 postoperative weeks ($P > 0.005$).

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Conclusion: The addition of β-TCP to PRF significantly improved bone regeneration in the first 2 weeks after surgery. Although the differences between results with and without the addition of β-TCP to PRF were statistically insignificant from weeks 3 to 6, it was nevertheless apparent that the group receiving the combination showed better results. We suggest a synergistic mechanism for this effect.

1. Introduction

Various growth factors are increasingly being used – either alone or together with bone graft materials – in dental and medical applications, especially in maxillofacial surgery for bone augmentation and regeneration. Among these factors are platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) (Yilmaz et al., 2014). PRP has been found to improve bone regeneration due to its high concentration of bioactive proteins (Zimmermann et al., 2001; Zhang et al., 2012). However, studies of the effects of PRP on new bone formation in experimental and human subjects have found inconsistent results (Wiltfang et al., 2003; Gerard et al., 2006, 2007; Kazakos et al., 2011; Zhang et al., 2012). Some authors have attributed this inconsistency to the extremely short-term effects of grafted PRP, due to the rapidly decreasing concentration of bioactive proteins (Schmitz and Hollinger, 2001; Marx, 2004).

Choukroun's PRF is a recently developed second-generation platelet concentrate, which is prepared through a simple method that does not require the addition of chemicals to blood samples before centrifugation. The end product is an autologous fibrin matrix containing platelets and leukocyte growth factors, which are obtained from inside the fibrin clot, which may explain the slow release of bioactive proteins (growth factors) from the PRF (Dohan et al., 2006; Dohan Ehrenfest et al., 2009; Zhang et al., 2012). The platelet (thrombocyte) count in PRF is three-to-seven times greater than its normal concentration in blood. Growth factors obtained from PRF include platelet-derived growth factor (PDGF), transforming growth factor (TGF), and insulin-like growth factor (Marx, 2001; Dohan Ehrenfest et al., 2009).

Several clinical studies and case reports have referred to PRF as a promising biomaterial that can be used alone or in combination with other bone graft materials to accelerate bone regeneration (Lee et al., 2012; Mazor et al., 2009; Magremanne et al., 2009; Choukroun et al., 2006). However, studies of the potential synergistic effects of PRF when used in combination with different bone graft materials have found inconsistent results, possibly due to the different bioactive properties of graft materials (Choukroun et al., 2006; Zhang et al., 2012).

Histologic, radiographic, and mechanical analytical methods are available for evaluating the volume and quality of newly formed regenerated bone. For example, advances in radiographic analysis, such as micro-computed tomography (MCT), allow for the three-dimensional reconstruction and measurements of the bone volume (BV) and bone mineral density (BD) of newly formed bone in the study defect (Spicer et al., 2012). Animal models of bone defect healing have been developed in many species, including mice, rats, rabbits, dogs, pigs, sheep, and goats. Much research has focused on rodent models, however, because of their reproducibility and economic considerations (Spicer et al., 2012; Pearce et al., 2007). The generally accepted critical size for calvarial defects is 8 mm, although smaller defects have been used, with a pattern of two defects per animal. This approach allows fewer animals to be killed (Kim et al., 2010; Spicer et al., 2012).

To the best of our knowledge, there have been no clear reports of the application of PRF combined with beta-tricalcium phosphate (β-TCP) in intramembranous bone, the main type of cranio-maxillo-facial bone. Therefore, the aim of the present study was to use histological and MCT evaluations to compare new bone regeneration in noncritical rat calvarial bone defects filled with either PRF alone or a PRF/β-TCP combination.

2. Materials and methods

This study was approved by the College of Dentistry Research Center, King Saud University, Saudi Arabia, duly governed by its "Ethical Consideration for Animals" document, in conformity with National Institute of Health guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985). Forty-five 20- to 22-week-old male Sprague–Dawley rats (weight: 350–450 g) were used. Rats were individually housed in metallic cages with ease of access to water and food in the Laboratory Animal Center of King Khalid University Hospital, King Saud University, Saudi Arabia, under veterinary supervision.

Rats were anesthetized by intramuscular injection of xylazine (5 mg/kg; Lloyd Laboratories, Shenandoah, IA, USA) and ketamine (20 mg/kg; Sigma–Aldrich, St. Louis, MO, USA). The surgical area was shaved from the bridge of the snout between the eyes to the caudal end of the skull/calvarium. After shaving, an alcohol swab was used to remove hair trimmings, and the area was disinfected with a 10% povidone–iodine solution (Riyadh Pharma, Riyadh, Saudi Arabia) the surgical procedure is illustrated in Fig. 1. The subcutaneous area of the surgical field was injected with half a cartridge of 1.8 mL of 2% lidocaine (Parhawk Laboratories, Inc., Lenexa, KS, USA). An approximately 1.5-cm incision was created, using a #15 blade, down to the periosteum over the scalp from the nasal bone to the caudal of the middle sagittal crest or bregma. The periosteum was sharply incised down the sagittal midline with the same scalpel. Finally, the periosteum was elevated from the cranial bone laterally with a mucoperiosteal elevator.

A 4-mL blood sample was obtained from the orbital sinus of each rat. The blood sample was collected in a plain tube, which was immediately centrifuged at 3000 rpm for 12 min to prepare the PRF.

While blood samples were being centrifuged, two noncritically sized (3-mm diameter) bone defects were created, with