Effect of Injectable Platelet-Rich Fibrin (i-PRF) in Initial Treatment of Chronic Periodontitis

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The aim of the study

The aim of the study was to determine the effects of SRP supplemented with the local i-PRF application, in comparison with SRP alone, on periodontal disease course.

Study population

This study was a randomized controlled clinical trial with a split- mouth study design. Thirty systemically healthy chronic periodontitis (CP) patients, 13 male and 17 female, age 38.00±10.12 years, range 22 – 64 years were recruited from the Department of Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade. The study protocol was approved by the Ethics Committee of the School of Dental Medicine, University of Belgrade (36/11) and is in compliance with Helsinki Declaration. After being informed of the research methods, all the participants submitted written consent for the enrolment in the study. The study was registered at ClinicalTrials.gov as "NCT04178590".

The patients were enrolled in the study according to the following inclusion criteria: adults (age between 20 and 75 years), without systemic and/or other oral disease or ongoing drug therapy, which might have an impact on the clinical signs and symptoms of periodontitis, the presence of minimum 6 teeth per quadrant, at least two teeth with periodontal probing depth (PPD) ≥ 5mm and minimum clinical attachment level (CAL) 3 mm in each quadrant, and a horizontal bone loss, no involvement of furcation degree II or III of both quadrants of the mandible or maxilla, confirmed by full-mouth radiograph images.

Patients who underwent antibiotic therapy during the last 6 months or had initial and/or surgical periodontal treatment within last 12 months, patients with diabetes or in need of long-term use of anti-inflammatory and immunosuppressive medication, current or former smokers, as well as women who are pregnant or lactating, were excluded from the study.

Periodontal examination

Immediately before the first treatment, as well as at 1, 3 and 6 months, periodontal examinations were carried out by the same examiner. The examination was conducted on all teeth and tooth sites, except the third molars and the tooth sites associated with furcation involvements of II and III degree (Hamp et al.1975). To identify disease progression, certain variables were recorded from the mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual surfaces of each tooth: clinical attachment level (CAL), gingival margin level (GML), periodontal probing depth (PPD), bleeding on probing (BOP), and plaque index (PI). The examiner, a specialist in periodontology, performed and noted down all the recordings. The examiner had previously obtained the adequate level of competence and reproducibility skills according to the various clinical parameters and indices which were going to be utilized (Polson 1997). To avoid blood contamination, GCF collection was conducted one day after clinical examination, after non-surgical periodontal treatment.

Supragingival scaling and root planing and oral hygiene instructions

At the beginning of the study, the patients were thoroughly instructed on self-performed oral hygiene consisting of: use of the modified Bass brushing technique, a soft manual toothbrush, a regular toothpaste twice a day, and use of inter-dental brushes once a day. A full-mouth SRP was conducted using ultrasonic scaler and hand instruments (Hu-Friedy, Chicago, IL) under local anesthesia in one or two sessions during the period of 24 hours. The standard of oral hygiene was checked at each examination visit and further instructions were provided according to individual requirements. Three months following the completion of the baseline treatment, all the patients were recalled for professional supragingival plaque control and reinforcement of oral hygiene. Additionally, reinstrumentation was conducted by using the ultrasonic device in all the sites with a remaining PPD of $\geq$ 5mm.

Preparation of i-PRF

Blood samples were taken into two 10 ml tubes and prepared for i-PRF preparation. The whole blood without anticoagulant was then centrifuged at 700 rpm for 3 min (60×g) at room temperature

by a Duo Centrifuge (Process for PRF, Nice, France). The upper liquid layer was taken as i-PRF using a syringe.The i-PRF was applied in one quadrant (treatment group) of the chosen jaw (mandibula or maxilla), whereas the physiological saline was inserted in the opposite side (control group). Treatment allocation was decided by a toss of a coin. i-PRF was enabled to hold for a longer time by applying it into periodontal pockets through perforations at the point of interdental space on individually formed occlusal splints. After 15 minutes the splint was removed.

Gingival crevicular fluid (GCF) sampling

GCF was sampled at baseline and 1-, 3- and 6-month follow-ups, one day after clinical examination from two counter lateral periodontal pockets with PPD ≥ 5 mm, either in mandibula or maxilla. GCF was collected by absorbent paper strips (Periopaper, Amityville, NY, USA) after supragingival plaque had been removed, the tooth gently rinsed with water, air-dried and isolated with cotton rolls to avoid contamination from saliva. Four strips were inserted consecutively into the gingival sulcus up to 3 mm leaving them for 30s and pooled in two separate dried microcentrifuge tubes. The absorbed GCF volume was immediately estimated by using a calibrated instrument. Afterwards, the samples were stored at −70 °C pending the analyses.

Protein quantification with ELISA

Quantification of GCF biomarkers of periodontal disease (MMP -8, TNF- α and ALP) was performed using enzyme-linked immunosorbent assays (ELISA). GCF samples were defrosted by soaking the paper strips into 150 µl of 0,05% Tween 20 solution in sterile Phosphate-buffered saline (PBST) and eluted by 20 minutes of vortexing followed by 10 minutes of centrifugation at 10000 × g at room temperature. Immediately after centrifugation the supernatant was collected and used to carry out the assays. Protein quantification was performed in duplicate using following ELISA kits: Human Total MMP-8 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA); Human Tumor Necrosis Factor Alpha (TNF-α) and Human Alkaline Phosphatase (ALP) ELISA kits (Elabscience Biotechnology, Wuhan, China) according to manufacturer's recommendations. Optical density of each well was measured after color development at 450 and 570 nm wavelength using microplate reader (RT 2100C, Rayto Life and Analytical Sciences, Shenzhen, China). Concentrations of tested proteins were determined from generated standard curve.

Quantification of microorganisms

Bacterial DNA was isolated using a standard salting-out method. DNA extracts were stored at − 70 °C prior to quantitative PCR (qPCR) analysis. The amplification of target region for 16S rRNA was performed as previously described (Brajović, Popović, Puletić, Kostić, & Milašin, 2016), using SensiFAST SYBR® Hi-ROX Kit (Bioline Reagents Ltd, London, UK) and the following primers: Fw 5′-TCCTACGGGAGCACAGT′-3 and Rv 5′GGACTACCAGGGTATCTAATCCTGTT-3′. Prevotella melaninogenica (ATCC 25845) was used as reference strain for standard curve analysis, and the results were obtained as total gene copy number.