Effect of two different parts of CGF on post-extractive alveolar ridge preservation: a preliminary histomorphometric analysis in a Split-Mouth design

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Tooth extraction produces alveolar bone resorption and soft tissue remodelling, so identification of adequate technique for alveolar ridge preservation after tooth extraction is fundamental for all specific cases. Among the several biomaterials, CGF can represent an ideal alternative considering its and its mechanical and biological properties. In this preliminary study we compared the effectiveness of the use of two different parts of CGF (WP-White Part and BC-Buffy Coat) versus natural healing (CTR) by a split-mouth randomized clinical design. Four healthy patients who needed extraction of three teeth were selected. Post-extractive alveolar sockets were filled randomly with CGF-WP, CGF-BC or nothing for CTR. After 60 days, before implant placement, a biopsy for each alveola was obtained for quantitative histomorphometric analysis. The data obtained showed that the use of CGF-WP could achieve good regenerative results, supporting the use of this part for the preservation of the post-extractive alveolar socket.

Tooth extraction produces alveolar bone resorption and soft tissue remodelling that can compromise dental implant treatment with damaging risk for anatomical structures. To date, there are different methods to maintain adequate alveolar ridge after tooth extraction, such as the use of grafting materials of human, animal or synthetic origin and growth factors with or without the use of bioresorbable or non-resorbable membranes (1-4).

It is known that material grafts (autografts, allografts, xenografts, or alloplastic grafts) are able to stimulate osteoblastic activity inducing bone formation, however these materials can negatively affect the healing process and the bone implant contact due to the permanence of nonvital residual particles that degrade slowly, and the risk of infection. Moreover, the high costs can also limit their use in clinical practice (5, 6).

Graft materials from a patient's own body components (autologous platelet concentrates) are among the new biologically active methods able to satisfy this requirement (7, 8). In fact, they contain a high concentration of growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-b1 (TGF-b1) and b2 (TGF-b2), epithelial growth factor (EGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF), which stimulates cell proliferation and up regulates angiogenesis enhancing the healing process and

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leading to better bone repair and regeneration (9, 10). To date, we know three main types of autologous platelet concentrates that can be classified as Platelet-Rich Plasma (PRP), Platelet-Rich Fibrin (PRF) and Concentrated Growth factors (CGF).

CGF is the latest generation of platelet concentrates, which was first developed by Sacco in 2006. It is a fibrin biomaterial rich in growth factors obtained by centrifugation of patient's venous blood at alternating speeds in short time. The different speeds permit to have a wider, denser, it is a fibrin rich organic matrix which contains growth factors, platelets, leukocytes and CD34+ stem cells which help in the process of regeneration and has immunological cells that are effective in regulating inflammation and minimizing the risk of infection and richer fibrin matrix, as reported by Rodella and collaborators (11, 12). About its efficacy, it is considered a biological inducing material which can improve the formation and the quality of the new-formed bone and facilitates the tissue healing (13-16).

Although various studies evaluating the effect of platelet concentrates such as PRP and PRF wound healing and alveolar preservation have been published (8, 17-20), there are few studies into the effects of CGF on post-extractive alveolar ridge preservation (21).

The purpose of this study was to assess the efficiency of two different part of CGF (WP-White Part and BC-Buffy Coat) in post-extractive alveolar ridge preservation by randomized split-mouth research design.

MATERIALS AND METHODS

Patient and Split-mouth research design

A prospective split-mouth design was applied to compare the efficacy of two different parts of CGF (WP-White Part and BC-Buffy Coat) in the post-extractive alveolar ridge preservation.

Patients aged over 18 years of age and scheduled for multiple tooth extraction for subsequent replacement with dental implants were recruited from private clinic. All patients were informed about the possible use of their data for clinical studies and provided written informed consent. The patient data were anonymized before analysis. The analysis was performed in accordance with the Declaration of Helsinki of (1975) and subsequent modifications.

For each patient, post-extractive alveola were not treated (natural healing) in the control site (CTR) whereas they were treated with two different parts of CGF (WP-White Part and BC-Buffy Coat) in the other two treatment sites. Exclusion criteria were inability or unwillingness to provide their informed consent; the presence of endocrine-metabolic disease or chronic, general or local disease; the presence of disease that may be affected by the surgery or by the intra-operative or post-operative medication; alveolar socket wall defects; heavy smoking habit ($\geq$10 cigarettes per day), due to its relationship with implant failure; patients with alcoholism and drug addiction; and treatment with bisphosphonates or antibiotics during the previous month.

CGF preparation

On the day of the surgery, 9 ml of venous blood sample were obtained from each patient in four sterile Vacuette tubes without anticoagulant additives using VACUETTE® Safety Blood Collection Set. Each sample was immediately centrifuged using a specific device (Medifuge MF200; Silfradent srl, Italy) in order to obtain the CGF, according to the manufacturer's instruction. After centrifugation, the upper layer (platelet-poor plasma, PPP) was removed with a sterile syringe, the middle layer (CGF) was collected with sterile tweezers and placed in a sterile petri dish, dividing it from the lower red blood cell (RBC) layer. Two parts of CGF were used: the upper white part (CGF-WP) and the lower Buffy Coat part (CGF-BC) according to previous data (12, 22). The different phases of CGF preparation are showed in the Fig. 1.

Surgical protocol

Before surgery, local anaesthesia (plexus block) was administered (articaine 4% with adrenaline 1:200000, Articaina Pirrel). Atraumatic tooth extractions were performed, and the post-extraction alveolar sockets were cleaned carefully with the physiological saline solution (0.9% of sodium chloride). Three alveolar sockets for each patient were differently treated and filled according to the split-mouth research design (Table I). Soft tissues were criss-cross sutured using 4/0 PGA suture (Omnia spa). No pharmacological therapy was prescribed. The patients

were advised to follow a soft and liquid diet and avoiding hot food for the following hours. Each patient underwent a follow-up after one and three days from surgery. The sutures were removed after 10 days.

After 60 days, before implant placement, a full-thickness mucoperiosteal flap was removed by incision on the alveolar crest and a biopsy was performed with a sterile surgical blade beaver (n°64, Swann-Morton SM64) at the centre of the ridge. Then, the implant sites were prepared u ing a trephine drill and Alpha Bio SPI implants (Alpha Bio Tec.) were placed in the sockets. Finally, the implant cavity walls were laid with CGF membranes all around and mucoperiosteal flaps were sutured with 4/0 PGA suture (Omnia spa).

Histomorphometric analysis

Biopsies were fixed in 10% neutral buffered formalin for 24 hours at 4°C. After fixation, the sample was repeatedly washed in phosphate buffer (pH 7.0), decalcified in Osteodec (Bio-Optica, Milan, Italy), and embedded in paraffin according to the standard procedures. Serial sections (7 µm) were cut longitudinally by a microtome and were stained with Masson-Goldner Trichrome (Merck KGaA, Darmstadt, Germany). All sections were evaluated under an optical microscope (Olympus, Milan, Italy) by two investigators unaware of the treatment group. Percentage of vital bone (VB%) and non-Mineralized Tissue (n-MT%) was calculated within arbitrary area in five sections for each sample. Digitally

Table I. *Spilt-mouth design. Three alveola for each patient were randomly treated as CTR, CGF-WP or CGF-BC.*

Patients	Alveolar site CTR	Alveolar site CGF-WP	Alveolar site CGF-BC
Patient 1	22	24	23
Patient 2	22	23	21
Patient 3	21	24	22
Patient 4	18	17	15

Three alveola for each patient were randomly treated as CTR, CGF-WP or CGF-BC.

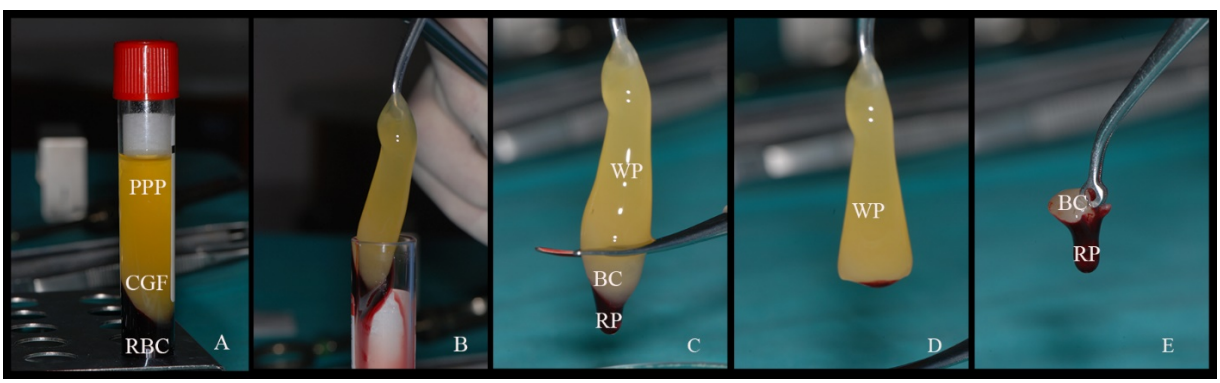

Fig. 1. *Blood sample after CGF protocol centrifugation. **A**): Three layers are obtained: upper layer, PPP-platelet poor plasma; middle layer, CGF-concentrated growth factors; lower layer, RBC-red blood cells; **B**): CGF was removed from the tube using sterile tweezers; **C**): CGF consisted of three parts: the upper White Part (**WP**), the lower Red Part (**RP**) and the middle Buffy Coat (**BC**); **D-E**): Separation of CGF into two parts using sterile scissor: **WP** and **BC**.*

fixed images were randomly analyzed using an image analyzer (Image Pro Premier 9.1; Immagini e Computer, Milan, Italy).

Quantitative values of histomorphometric analysis were reported as mean ± standard error (SE).

RESULTS

Patients and Split-mouth design

In total 4 patients, 1 male and 3 females, fulfilled the inclusion criteria and were included in this study. Patient age ranged between 64 and 66 years. The randomized split-mouth design reporting the alveolar sites and the different treatments was schematized in the Table I.

Clinical and Histomorphometric analysis

From a clinical point of view, in all patients no complications related to the grafting protocol were observed and reported over the study period. The sutures were removed after 10 days and optimal wound healing was observed without any complications. All the alveolar sites showed a complete reepithelization. After 60 days, before biopsy and implant placement, a re-epithelized mucoperiosteal layer was observed.

Twelve biopsies were fixed and sent to the Section of Anatomy and Physiopathology of the University of Brescia for the subsequent histomorphometric analysis. However, only 9 biopsies were adapted

Table II. *Quantitative data by histomorphometric analysis. Quantitative values were reported as mean ± standard error. (**NA** - Not Applicable).*

Patients	Alveolar site (treatment)	NB%	n-MT%
Patient 1	22 (CTR)	59.272 +/- 1.115	40.728 +/- 1.115
	24 (CGF-WP)	55.572 +/- 1.225	44.428 +/- 1.225
	23 (CGF-BC)	**65.923 +/- 1.402**	**34.077 +/- 1.402**
Patient 2	22 (CTR)	NA	NA
	23 (CGF-WP)	NA	NA
	21 (CGF-BC)	**61.663 +/- 1.328**	**38.337 +/- 1.328**
Patient 3	21 (CTR)	NA	NA
	24 (CGF-WP)	**60.446 +/- 0.892**	**39.554 +/- 0.892**
	22 (CGF-BC)	47.798 +/- 1.105	52.202 +/- 1.105
Patient 4	18 (CTR)	4.84 +/- 7.53	95.16 +/- 7.53
	17 (CGF-WP)	7.68 +/- 13.44	92.32 +/- 13.44
	15 (CGF-BC)	**26.2 +/- 22.46**	**78.3 +/- 22.46**

Quantitative values were reported as mean ± standard error. (NA - Not Applicable).

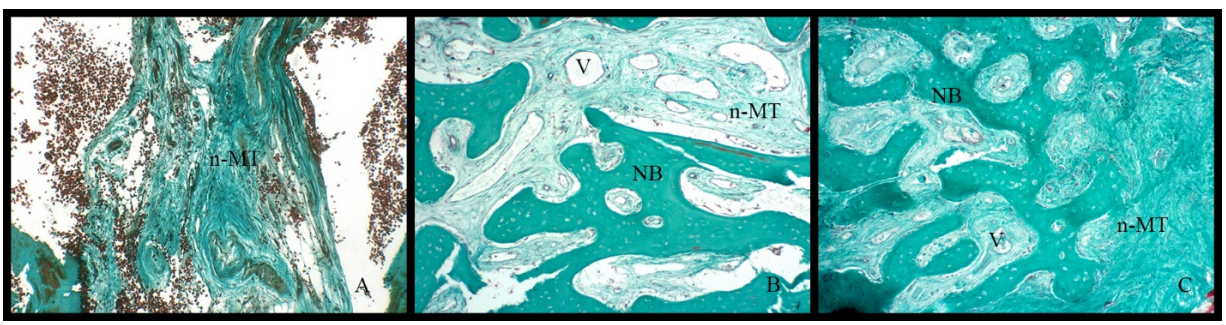

Fig. 2. *Comparison among the three different treatments of the alveolar sites: **A**): CTR; **B**): CGF-WP; **C**): CGF-BC. **NB**: New Bone; **n-MT**: non-Mineralized Tissue; **V**: Vessel. Final magnification X100.*

for histomorphometric evaluation; the other samples were shuttered in little pieces and appeared as fibrous or granulation tissue, suggesting a not fully newly formed and compact tissue.

Histomoprhometric analysis of 9 processed samples showed the presence of newly formed trabecular bone (NB%) in the graft site, together with non-mineralized tissue (n-MT%). Quantitative data are reported in Table II and representative images were reported in Fig. 2. The data showed a significant presence of NB% in 3 cases treated with CGF-BC (Patient 1, 2 and 4) respect to CTR and CGF-WP treatment, in which the values were lesser, or the analysis was not applicable. Patient 3, on the contrary, presented a higher NB% with CGF-WP treatment respect to CGF-BC; in this patient, evaluation of the percentage in the control site was not applicable.

DISCUSSION

To date, different techniques and material grafts are available for the preservation of the alveolar socket before implant surgery (1-4). Since an ideal technique does not exist, the oral surgeons have to choose the best for all specific cases and CGF can represent an ideal alternative.

CGF is the third generation of platelet concentrates and several studies reported its ability to improve bone and tissue regeneration (12, 16, 23, 24). It is composed of cross-linked fibrin network full filled with several autologous growth factors (VEGF, PDGF, IGFs, etc.) and with the presence of autologous cells such as platelets and leukocytes, including CD34 positive (CD34+) cells (11, 22). In particular, platelets and lymphocytes were found in a very thin space called "buffy coat" localized between the white upper part and the lower red part of the CGF (22).

Considering this peculiar distribution, the aim of this study was to evaluate the effect of two different parts of CGF (CGF-WP and CGF-BC treatment) in the post-extractive alveolar ridge preservation. As we reported in our results, the comparison among the three different treatments within each patient was not always possible, since some samples appeared not adapted for the quantitative histomorphometric evaluation, showing a shuttered sample made up of fibrous and granular tissue. However, the results of this study showed a major increase in the percentage of new bone formation in the alveolar sites treated with CGF-BC compared to CGF-WP. These data were in accordance with the study conducted by Borsani and collaborators in 2015 (22), in which leucocytes and platelets were principally found in the buffy coat. About the presence of CD34 positive (CD34+) cells, their presence in CGF has been previously reported (11), but to date there are not data concerning the major presence of CD34+ in the buffy coat respect to upper white part and lower red part. However, considering the higher concentration in the buffy coat, it is possible to speculate that CD34+ cell could also be present in this thin layer, promoting cells in neovascularization, angiogenesis and bone and tissue regeneration (22, 25, 26).

Limit of this study consist in few patients enrolled, so we decided to consider it only a preliminary study. Further investigation together with the description of different distribution of CD34+ cells in the CGF part have to be planned to confirm the choice of buffy coat respect to white part of CGF to promote bone and soft tissue healing and regeneration.

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