

## **Comparative Histologic and Radiographic Evaluation of Alveolar Ridge Preservation in Esthetic Zone Using Concentrated Growth Factors Associated with Denaturated Albumin (Alb-CGF) and Albumin Coated Bone Allograft**

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### **Abstract**

Post-extraction alveolar ridge resorption is an inevitable process are present a challenge in upper anterior esthetic zone. Anterior maxilla present higher degrees of resorption than posterior maxilla do. Socket grafting techniques have been widely used by dentists throughout the world and a great amount of studies have been conducted to examine the effectiveness of several biomaterials. Searching for available, cheap and effective biomaterials to be used in ridge preservation and regenerative surgeries represent a great field for investigation and research, especially in blood extracts and platelets concentrates.

**Keywords:** *Extraction; Socket Preservation; Albumin Coated Growth Factors Concentrate; Albumin Coated Allograft; Sticky Bone*

### **Introduction**

After tooth extraction, structural and compositional changes of the covering soft tissues, as well as alveolar bone loss, can be expected. This various changes in the alveolar bone may lead to difficulties at the time of implant placement when a prosthetically driven implant position is required.

In order to understand the changes following tooth extraction in the esthetic zone, it is important to be familiar with the anatomic and histologic characteristics of tissues surrounding the tooth indicated for extraction. Histologically, the inner part of the socket wall contains lamellar bone, the so-called bundle bone. The thickness of this bundle bone is reported to be 0.2 - 0.4 mm. Similarly, to the root cementum and to the periodontal ligament, its existence is strictly tooth-dependent [1,2].

A recent clinical study reported that, the thickness of the labial bone plate in the maxillary anterior area was measured using cone beam computed tomography at three different positions relative to the labial bone crest [3]. It was found that the labial bone plate, in most locations in the anterior maxilla, is less than 1 mm in thickness. Additionally, about 50% of the sites investigated had a bone plate, which was (at maximum) 0.5 mm thick. This is turn, indicates that the bundle bone and the labial bone plate usually have a similar thickness in the anterior maxillary region. Therefore, we can expect that, after tooth extraction in the esthetic area, the labial bone plate will be resorbed predominantly in the more crestal region [4].

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Following extraction of the tooth, the alveolar ridge undergoes resorption in both vertical and horizontal directions [5-7]. Based on the evidence of a systematic review, the vertical dimensional reduction on the labial side amounted to 11 - 22% ( $-1.24 \pm 0.11$  mm) after 6 months, whereas the horizontal dimensional reduction on the labial side was greater, amounting to 29 - 63% ( $-3.79 \pm 0.23$  mm) after 6 - 7 months [8].

Studies demonstrated rapid alteration within the first 3 - 6 months after tooth extraction, followed by gradual reduction in dimension thereafter. Subsequently, 0.5 - 1% loss of the bone contour, per year, can be expected [9]. In summary, following single-tooth extraction, up to 50% of the ridge width will be resorbed and bone loss will predominantly occur at the labial aspect [1].

Meanwhile, the facial soft tissue thickens spontaneously in sites where progressive bone resorption of the former socket walls occurs [10]. This thickening upon thin bone wall phenotypes after 8 week healing period provides many advantages during implant surgery. First, the spontaneous soft tissue coverage after healing provides an increased amount of keratinized mucosa, which facilitates primary flap closure and favors bone regeneration [11-13].

Second, the spontaneously thickened soft tissue volume may reduce the need for additional soft tissue grafting, limiting morbidity and treatment costs. However, these spontaneously thickened tissues may mask the true extent of an underlying bone defect during the clinical examination and may subsequently mislead clinicians in the selection of the appropriate treatment protocol [14].

Regarding to these data, in the esthetic zone, the clinician is confronted with a challenging situation regarding the decision-making process required to provide an optimal treatment solution. Hence, in recent years, the healing process of the extraction socket and the related changes of respective hard and soft tissues following tooth removal has become a well-investigated research field [2].

Ideally, the therapeutic plan starts before tooth extraction and offers three therapeutic options: spontaneous healing of the extraction socket; immediate implant placement; and techniques for preserving the alveolar ridge at the site of tooth removal [2].

Alveolar ridge preservation techniques have been widely used in the past and are continuously evaluated. These techniques are performed to counteract changes in soft tissue and hard tissue that follow tooth extraction. More recent research has focused on a variety of materials and techniques and has different aims depending on the need for preservation of soft tissue and/or hard tissue, as well as on the optimization of the ridge profile [4].

For several decades, different methods have been developed for promoting the regeneration of soft and hard tissue, including the use of biomaterials [15]. Interestingly, blood concentrates are promising clinically relevant products by affording an alternative source of minimally invasive autologous regeneration. Platelet aggregates can be obtained from the patients' own peripheral blood and concentrated by centrifugation, thus being able to release different growth factors (GFs) with well-documented desirable effects in tissue regeneration [16].

## **Case Description**

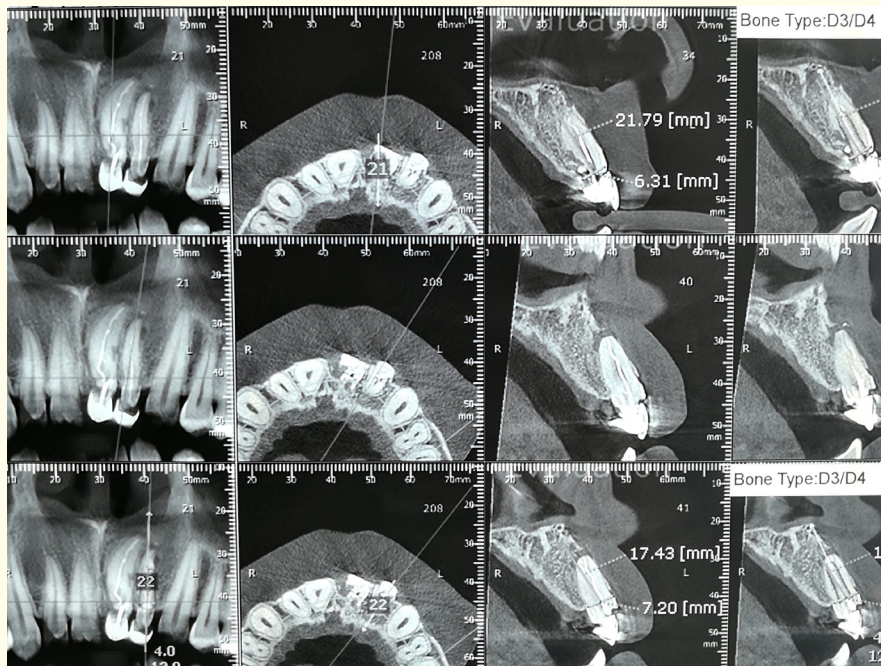
A 33 years old male patient presented with remaining roots of maxillary central and lateral incisors (left side) (Figure 1a). He asked for an implant supported fixed restoration. Upon clinical and radiological examination, we found that there was labial bone dehiscence in both sockets (type II socket) (Figure 1b).

According to the patient economic state, he asked for delaying the implant placement for a while. Upon his wish, we delayed the implant placement and for that we decided to preserve the socket to compensate for the changes that will occur upon extraction and to regenerate the labial bone dehiscence.

A decision was taken to extract the remaining roots and preserve the extraction sockets using albumin coated bone allograft manufactured by OrthoSera®) forming sticky bone for the central incisor and concentrated growth factors associated with denaturated albumin (Alb-CGF) for the lateral incisor socket. A non-traumatic extraction was conducted and a gentle debridement of the sockets was carried out using bone curette (Figure 1c).



Figures 1a



Figures 1b

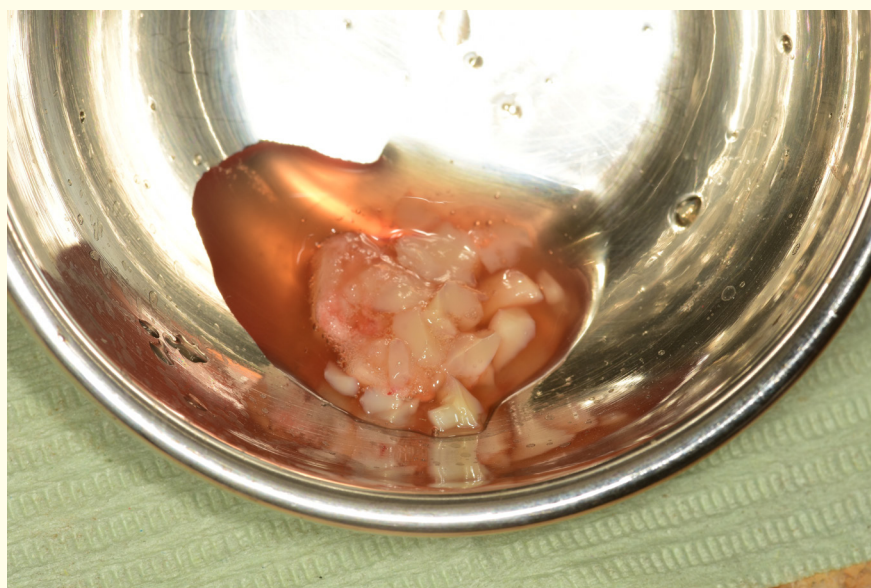


*Figures 1c*

**Production of the albumin-concentrated growth factors membranes [17] (Figure 2)**

Peripheral blood was collected from the patient, using 9 ml tubes, without adding any additives (Vacutube). For the production of the membrane, two tubes were positioned in the vertical rotor and fixed angle centrifuge, and the protocol was applied to obtain the CGF in the liquid phase (LPCGF), as described by the manufacturer Medfuge Silfradent Italy.

After processing, it was possible to observe plasma and the remaining decanted blood material containing red cells. Two milliliters of the initial portion of plasma (platelet-poor plasma [PPP]) was collected with a syringe, while the other blood portions (puffy coat, LPCGF, and red blood cells) were reserved at room temperature (20°C).



*Figures 2*

The syringes containing PPP were inserted into a device for human serum albumin denaturation activated plasma albumin gel (APAG®, Silfradent, Italy). After 10 minutes in an operating temperature of 75°C, the syringes were allowed to cool down at room temperature for another 10 min and protected from ambient light (as recommended by the manufacturer) (Figure 3).



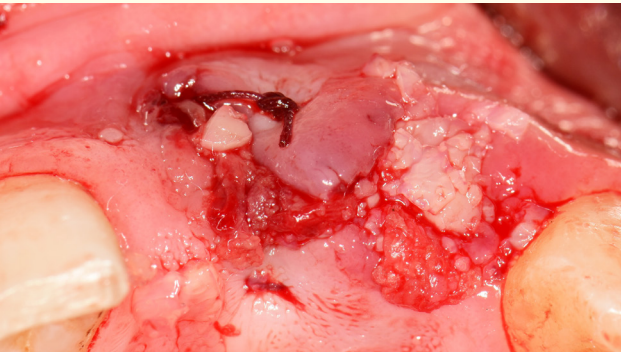
**Figures 3**

The denaturated albumin was deposited into a glass container, to obtain the desired shape. Subsequently, using a 10 ml syringe and an 18G hypodermic needle, the LPCGF and puffy coat portions containing CD34 stem cells were collected in an approximate volume of 4 ml, added to the denaturated albumin on the glass container, and then gently mixed with tweezers. After waiting for the fibrin polymerization process (approximately 5 minutes), the membrane was formed with the previously established format. Finally, we got the Alb-CGF membranes.

After preparing the LPCGF and Alb-CGF, we augmented the left central incisor extraction socket with albumin coated bone allograft and covered with LPCGF, while the left lateral incisor extraction socket with Alb-CGF only (Figure 4). A horizontal figure of 8 sutures was used to support the used biomaterial. A prophylactic oral antibiotic, Augmentin® 625 mg t.i.d. was used routinely, beginning one day prior to the procedure and continuing for five days postoperatively.

After 14 days, sutures removal was carried out. After 4 month, clinical, histopathologic and radiographic evaluations were carried out, which revealed clinically excellent soft tissue healing and fully keratinized and radiographic evidence of bone fill were recorded (Figure 5a and 5b).

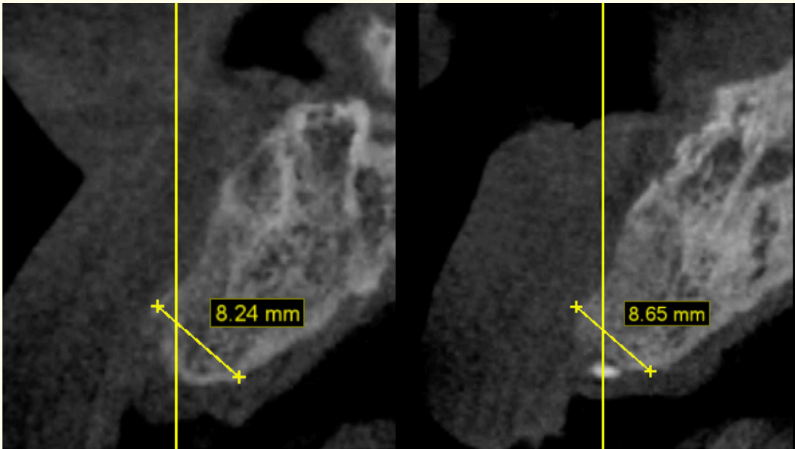
A conventional pedicle flap was elevated (Figure 6a) and two bone specimens were harvested from both sites of augmentation (Figure 6b), using trephine drill (Figure 6c) for histopathologic evaluation and then the drilling sequence for implant placement were carried out (Figure 6d and 6e).



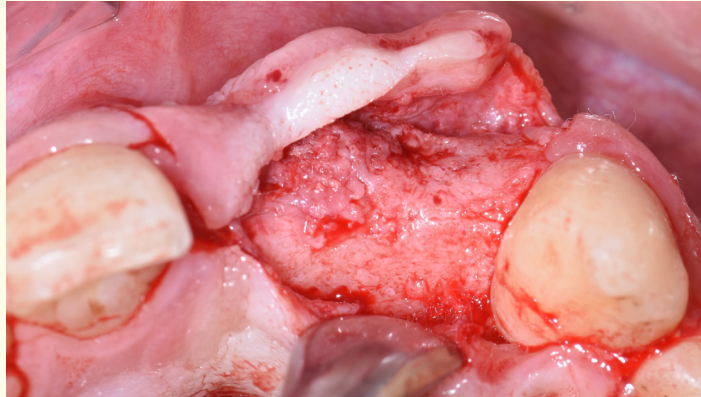
Figures 4



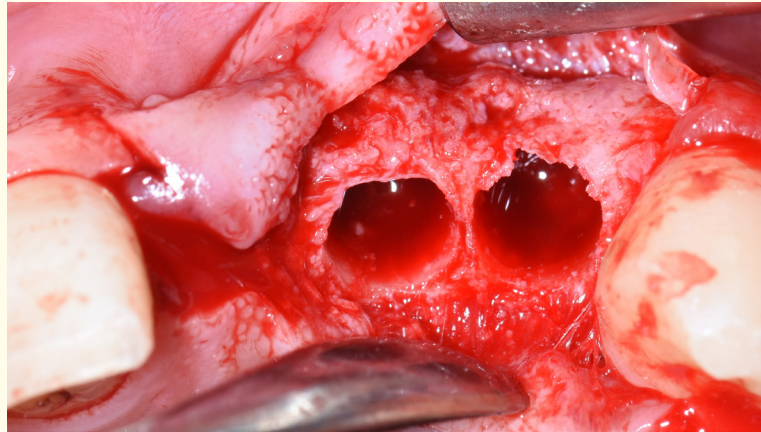
Figures 5a



Figures 5b



*Figures 6a*



*Figures 6b*



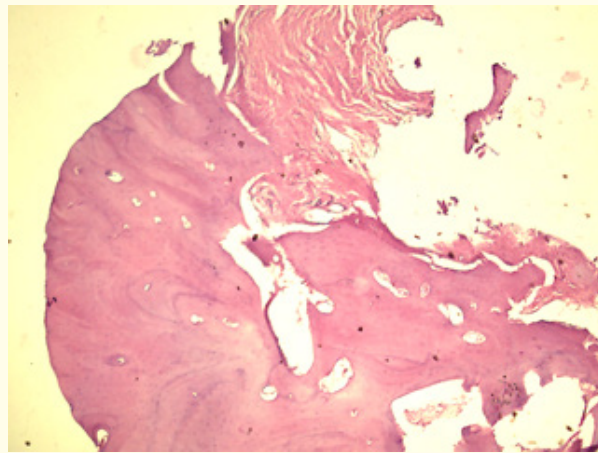
*Figures 6c*

## Results

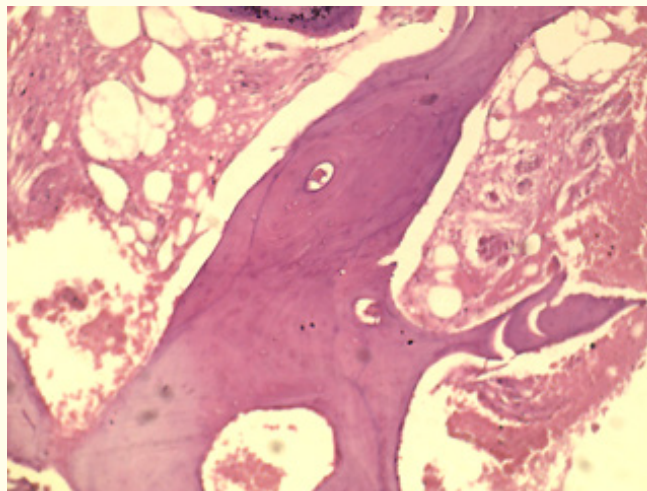
### Histological results

Section of (sticky bone) (Figure 7a) examined under light microscope revealed considerable cancellous bone formation, resulting in relatively wide bone trabeculae with numerous viable osteocytes. The bone marrow spaces revealed proper vascularity with an area of considerable fibrous tissue formation.

The other extraction socket (Figure 7b), where Alb-CGF was applied when examined under light microscope revealed still favorable cancellous bone formation. Thin bone trabuculae are formed around relatively large red bone marrow spaces with viable vascularity.



*Figures 7a*



*Figures 7b*

Both sections were free from any inflammatory infiltrate.

### **Radiographic results**

According to CBCT, the labio-palatal dimension of the central incisor socket was 6.3mm, and after 4 months of preservation using sticky bone and CGF, it becomes 8.03mm. For the lateral incisor socket, the labio-palatal dimension was 7.2mm and it becomes 8.2mm after 4 months of augmentation using Alb-CGF.

### **Discussion**

When it comes to the esthetic zone, the clinical concept in today's dentistry has clearly changed in a way that the treatment plan and the decision-making process should take place before a tooth is extracted. This allows the patient to benefit from the multiple treatment options that are available at the time of tooth extraction.

Alveolar socket preservation techniques have been used in the past and are continuously evaluated. These techniques are performed to counteract changes in soft tissue and hard tissue that follow tooth extraction. Recent research has focused on a variety of materials and techniques and has different aims depending on the need for preservation of soft tissue and/or hard tissue, as well as on the optimization of the ridge profile [18-21].

The main goals include: the compensation, or at least a limitation, of post extraction socket alterations; the promotion of healing of soft and hard tissue within the former extraction socket; and facilitating the placement of dental implants in a prosthetically ideal position without the need for further augmentative procedures [19,20]. From a clinical point of view, the decision to perform a certain alveolar ridge preservation technique depends mainly on: (i) the time-point chosen and the ability to place a dental implant; (ii) the quality and quantity of soft tissue in the region of the extraction socket; (iii) the remaining height of the labial bone plate; and (iv) the expected implant survival and success rates [4].

From a patient's point of view, dental implants should be placed immediately. Practically, this technique is associated with a number of limitations and may not be suitable for all cases. This is mostly because of existing deficiencies in terms of bone and soft tissues.

The use of serum albumin in the tissue engineering field is widely investigated and documented, which is a protein abundant in the human body and easy to isolate, from blood plasma precipitation with high purity and homogeneity. Furthermore, it provides a compatible structure for cell proliferation, biomaterials enriched with albumin showed dimensional stability over time, suggesting less degradation *in vitro* [22]. Adding to that, a study has showed that the association with albumin can modulate the fibrin network ultrastructure and permeability, inducing fibers with increased thickness and a coarse nodular appearance [23]. According to these data, the association with denaturated serum albumin could represent a possible modification in the PRF-based framework, which is a totally autologous, biocompatible and possibly more durable material with a longer duration of action.

Processing of LPCGF and denaturated albumin on a glass vessel resulted in a solid, opaque moldable membrane. A very dense surface of the Alb-CGF membrane, with very evident deposition of a layer of denaturated protein, which is clearly coating the fibrin fibers and surrounding the trapped cells and platelets, was showed upon ultrastructural evaluation. Regarding the presence and distribution of cells within the membrane, the nucleated cells are detected at similar densities both at the center of the membrane and in its left and right borders [17].

It was showed that, the membrane is able to release GFs such as PDGF, VEGF, and FGF2 in considerable concentrations within the 1st hour. Also, similar levels of noncumulative release of VEGF and FGF2 were observed after 7 days, without significant difference from 1 hour, indicating a continuous release with time for at least 1 week. PDGF presented a strong release at 1 hour and a reduction on the mean GF concentration after 7 days of incubation [17].

Upon these facts, no wonder in our study to see close results with non-significant differences between preserving the socket with either sticky bone or Alb-CGF alone, which have been investigated histologically and radiographically. Further investigations with large patient samples are recommended to prove the efficacy of using Alb-CGF, which may provide in the future a good substitute for bone graft and/or membrane.

## Conclusion

The Alb-CGF is a promising biomaterial, which was proved that it is capable of both immediate and prolonged release (after 1 week) of important GFs related to tissue regeneration such as PDGF, VEGF, and FGF. It may also represent an important step toward the development of autologous moldable and stable biomaterials for use as soft tissue barriers and potential for different applications in the oral cavity, as in periodontal regenerations.

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