Use of PRGF to accelerate bone and soft tissue regeneration in postextraction sites.

An article by:
Eduardo Anitua, M.D., D.D.S., Ph.D. Vitoria, Spain
Gorka Orive, M.D., D.D.S. Vitoria, Spain
Isabel Andía, Ph.D. Vitoria, Spain
Use of PRGF to accelerate bone and soft tissue regeneration in postextraction sites.

Evaluation of regenerated bone density.

An article by: Eduardo Anitua, M.D., D.D.S., Ph.D. Vitoria, Spain
Gorka Orive, M.D., D.D.S. Vitoria, Spain
Isabel Andia, Ph.D. Vitoria, Spain

In postextraction protocols for delayed short, medium and long-term implant placement, it is essential to develop treatments that accelerate regeneration of the soft tissue as well as alveolar bone with the purpose of reducing the patient's waiting time and improving the quality of tissue regenerated. Filling postextraction sites with PRGF and then sealing with autologous fibrin allows obtaining a sufficient quantity (densities greater than 500 Hounsfield units) and quality (type II and III) of alveolar bone both inside and, more interestingly, surrounding the walls of the alveolus which insures initial stability of implants in a time frame of 8-12 weeks compared to 12 months described in the standard Brånemark protocol. The treatment of postextraction sites with PRGF and autologous fibrin is a simple, economical and predictable biotechnological procedure for the obtention of alveolar bone and keratinized tissue, with significant reduction in waiting time for the patient without any adverse effects.

Introduction

In the last decade, the field of dentistry has evolved drastically thanks to a more detailed understanding of periodontal diseases and the development of new techniques and biotechnological protocols. One need only look to the past and recognize how protocols which at one time were considered axiomatic-like treatment with implants designed by Brånemark-now have been widely accepted. In the original standard protocol, a healing period of 12 months was recommended after a single extraction and before implant placement; during that period, 3 to 6 months passed before the second (uncovering) surgery.

Today, we utilize other criteria to determine the time between extraction (exodontia) and implantation.

According to this classification, there are implants placed immediately at the time of extraction and deferred at short, mid and long-term at 6-8 weeks, 3-4 months or 9 months, respectively.
There is no doubt that the immediate therapy offers multiple functional and esthetic advantages for the patient in addition to shortening treatment time considerably. In some cases, however, a complete regeneration of the extraction socket as well as the keratinized tissue is necessary to insure the esthetic and functional success of the implant. Certain situations such as large periapical lesions, 3-wall defects, large crater defects around the teeth, or gingival recession can become contraindications for immediate implant placement. In these cases, one must assume a longer healing time and regeneration of the alveolus.

On the other hand, a longer waiting time increases the risk of reabsorption of the alveolar process and loss of bone height and width of the alveolar crest which, in some cases, can render placement of dental implants more difficult or impossible. It is precisely in this type of situation in which it is necessary to develop protocols which accelerate the regeneration process of soft tissue and alveolar bone to obtain the quantity and quality of keratinized tissue and bone ideal for implantation, and reducing the waiting time for the patient.

A biotechnological alternative to accelerate alveolar bone regeneration is filling the postextraction alveolus with a preparation rich in growth factors (PRGF)\(^\text{7}\). PRGF consists of a small volume of platelet-rich plasma obtained and prepared rapidly and in a simple manner from a patient’s own blood; its activation yields a 3-dimensional biocompatible fibrin matrix from which a pool of proteins and growth factors (GF: Growth Factors) are released progressively and which contribute to accelerating healing as well as osseous regeneration\(^\text{8}\). Likewise, by utilizing the plasma fraction least concentrated in platelets it is possible to obtain an autologous fibrin of elastic consistency which will serve as an ideal biomaterial membrane for sealing the postextraction alveolus.

In this study, we describe the technique for filling postextraction sites with PRGF and then covering it with an autologous fibrin membrane in 11 patients with an average age over 50 years. This group of patients was selected with the purpose of observing the effects of PRGF and of the autologous fibrin. The objective of this study is to investigate if the osteoinductive effect of the Growth Factors along with the osteoconductive effect of the fibrin matrix reduces the waiting period required for adequate healing and bone regeneration.

Similarly, the capacity of the fibrin as an autologous, biocompatible biomaterial for optimal alveolar coverage will be evaluated. To that end, the bone density and quality of alveolar bone were determined between weeks 8 and 13 postextraction with CT imaging, and later it was evaluated with the BTI Scan\(^\text{8}\), an excellent diagnostic tool.\(^\text{13}\)

**Preparation**

A total of 11 patients (4 males and 7 females) between 45 and 71 years (mean age 53.8 years) were appropriately informed and constituted the trial group.

PRGF was prepared from a small volume of blood (20 cc) drawn from the peripheral vein using sodium citrate as an anticoagulant (PRGF System\(^\text{8}\) citrated tubes) (Figs. 1-4). After a short, 8-minute centrifugation at 460g in equipment specially designed for the technique (PRGF System\(^\text{8}\)), the uppermost plasma fraction which retracts over 30-minutes at 37°C (98.6°F) will produce the autologous fibrin to be used to seal the postextraction site.

To produce PRGF, we will obtain by carefully pipetting a 0.5 mL fraction of plasma immediately above the red blood, always avoiding the white cells (buffy coat) which are found above the red blood (50 μL). The activation of PRGF is accomplished with calcium
chloride (PRGF activator) (Fig. 5) 5 minutes prior to using it.

Filling the extraction site with PRGF is done in the same surgery as the extraction. To do so, the recently coagulated PRGF is introduced into the alveolus and the sealing is done with the retracted fibrin clot which possesses some excellent elastic and homeostatic properties. (Figs. 6-8).

To conduct an accurate follow-up of the bone regeneration, the patients agreed to a CT between weeks 8 and 13 post-extraction. Thanks to the BTI Scan® imaging and analysis program, it was possible to determine the bone density and relate it to the bone quality classification of Lekholm and Zarb.

Accordingly, the bone densities were determined at three different points inside the regenerated alveolus and at a number of variable points, each taken at 0.5 mm inside (1 mm inside) as well as outside (1 mm outside) the future implant site. In this way, it will be possible to not only measure the osseous regeneration inside the alveolus but, more importantly, the density and quality of bone of the alveolus wall in which the implant will be placed and which will be essential to insure its primary stability.
Results

Once the PRGF is activated, a wide range of growth factors which play a fundamental role in revascularization and bone regeneration are locally secreted, inducing a mitogenic and proliferative effect on the endothelial cells and on the osteoprogenitor cells. In order to characterize the content of PRGF growth factors (GFs), we measured the principal GFs released from the platelets, including PDGF, TGF-β, IGF-I, EGF, VEGF and HGF. (Figs. 9 and 10) Specifically, the first 3 factors are the most abundant in PRGF and their effects on healing and bone regeneration have been amply described in the literature.

For example, PDGF is known to increase the proliferation of osteoblasts in vitro, while TGF-β in a determined dose boosts the synthesis of collagen-derived proteins such as type I and IV collagen in addition to augmenting the mineralization of the matrix and favoring implant anchorage. Finally, IGF-I stimulates bone forma-

Fig. 5: PRGF activator is a formulation of calcium chloride which permits the coagulation of the different plasma fractions.

Fig. 6: PRGF clot forms in between 6 and 7 minutes after activation. Its protein and growth factor content make it an ideal biomaterial to stimulate tissue regeneration.

Fig. 7: The fibrin membrane is obtained 30 minutes after activating Fraction 1.

Fig. 8: In this image, observe the extraordinary characteristics displayed by the fibrin obtained with this technique: it is the ideal biomaterial for filling, sealing and covering extraction sites.
Figs. 9 and 10: Concentrations of the principal growth factors (GFs) contained in PRGF. It is interesting to point out the relevant concentrations of IGF-1, TGF-β and PDGF.

Exerted by the secreted VEGF will be essential to provide oxygen and nutrients to the tissues being regenerated.

After the PRGF was prepared, the surgical extraction was performed and the site filled with PRGF and sealed with autologous fibrin, as illustrated and described in figs. 11 - 17. One aspect to point out is that the amount of PRGF to use will vary depending on the size of the alveolus so that the existing defect is entirely covered. Between 2 and 3 months after filling and sealing the alveolus, the patients underwent a CT; the data were analyzed using the BTI Scan® software program.

As mentioned previously, this program is an excellent diagnostic tool to assess the quality and quantity of existing bone, which will help to insure the predictability of the implant.

In fact, these two variables seem to decisively affect the success or failure of the implants, regardless of where they are placed, the percentage of failures being higher when the quantity of bone is insufficient or its quality is low, which will directly influence the primary stability.
**Fig. 12:** Filling the alveolus with a PRGF clot and an autologous fibrin membrane obtained using the same technique.

**Fig. 13:** Regeneration of the keratinized soft tissue and the post-extraction alveolus at 12 weeks.

**Fig. 14:** The extraction is performed, and the alveolus curetted and filled as we have already commented. In this case the suture stabilizes and retains the fibrin plug, added to the adhesive properties of the fibrin itself.

**Fig. 15:** The extraction 24 hours post-op.

**Fig. 16:** At 15 days, observe the epithelialization of the fibrin clot. Healing time can vary between 5 and 15 days depending on the size of the extraction site and on the patient.

**Fig. 17:** At 3 months, the epithelial regeneration is observed.
For example, type IV bone, considered as low quality bone, is characterized by a matrix of soft and low density trabecular bone in comparison to type II and III qualities of greater consistency, in which primary implant stability will be clearly superior.

The BTI Scan® allows the correlation of the bone density with the quality of bone classification proposed by Lekholm and Zarb. In this article, we propose a new classification much more precise and quantifiable wherein are established 5 classifications with their corresponding average density measurement in Hounsfield units as follow:

Bone type I: 1,000 - 1,600 Hounsfield units
Bone type II: 600 - 1,000 Hounsfield units
Bone type III: 300 - 600 Hounsfield units
Bone type IV: 100 - 300 Hounsfield units
Bone type V: < 0 - 100 Hounsfield units

During the past several years, our research group has expended considerable effort focused on an exhaustive study and description of PRGF and its possible therapeutic applications (14-6-8-10-11-13).

**Fig. 18:** Type I bone (1,000 - 1,600 Hounsfield units). It is important to evaluate the density inside as well as outside the future implant site.

**Fig. 19:** Type II bone (600 - 1,000 Hounsfield units).

**Fig. 20:** Type III bone (300 - 600 Hounsfield units).
In fact, the preliminary results obtained in experimental animals and later in humans substantiate the potential of this therapeutic product in many applications, including oral surgery\(^7\). In this current study we were able to confirm the tremendous potential for PRGF as a regenerator of alveolar bone in 50+ year-old patients, in whom osteogenic activity is reduced.

It must be pointed out that even at 2-3 months after extraction (between 8 and 13 weeks, more specifically), in sites filled and sealed with PRGF and autologous fibrin and the data processed with the BTI Scan\(^8\), the patients showed adequate bone density and quality (Figs. 23 - 25).

Specifically, an average density of 534 Hounsfield units in the center of alveolus was measured a site where 12 weeks before there had been no bone whatsoever and the density was zero.

Undoubtedly, the most remarkable thing is to observe the high density of bone obtained inside as well as outside the future implant site; it approaches

Fig. 23: In this section of the CT, we can see a control case with the measurement of the density; in addition to the surrounding area and inside the future implant site, measurements are taken at 3 points inside the alveolus of the extracted tooth. Following the extraction, the density at 3 points is 0 including negative values. The point in the center is taken between the post extraction alveolus and the other 2 points 2 mm above and below it.
levels greater than 600 Hounsfield units, which assures good primary stability. Also, the determination of the bone quality for implant placement suggests type II bone in 6 of the patients and type III bone in 5 of the patients.

These data confirm that the technique offers a great advance in immediate and delayed short and mid-term procedures since they allow a drastic reduction in the waiting time between surgeries. It must also be taken into consideration that according to the literature a postextraction site requires 12 months to heal completely, assuming that during this time the width of the alveolar crest can be reduced 50%. Therefore, as mentioned previously, it is not only a question of shortening the time between surgeries but above all one of function, esthetics and improved predictability of future treatment.

A significant observation is that none of the patients had pain, inflammation or infection throughout the extraction and filling with PRGF protocol and follow-up. In our opinion, this is due in great part to the autologous fibrin used to cover the alveoli. Some authors think that the closure of the alveolus is not a primary objective; others prefer to place a displaced flap to assure primary closure of the alveolus. However, this technique can reduce the width of the attached soft tissue around the implant, compromising the esthetic appearance of the patient.

The use of autologous fibrin, on the other hand, does not cause any side-effects and it is a safe and simple procedure for a specialist, and inexpensive and efficacious for the patient. Although the number of patients in this study is not high, we have intended to study the effect of the regeneration with PRGF and its subsequent sealing with a fibrin membrane in different teeth located in the maxilla as well as in the mandible.

The results confirm that PRGF induces excellent regenerative activity in the different alveoli studied, which attests to its therapeutic potential. The findings raised another question for us. “What happens if we study this same PRGF protocol with a new patient population over a longer time frame?”

---

**Fig. 24:** Bone densities inside the alveolus obtained from the average of the 3 measurements in 3 points in the center of the alveolus.

**Fig. 25:** The measurement of the densities is done every 0.5 mm, 1 mm inside as well as 1 mm outside the hypothetical position of the future implant. This graph is between 8 and 13 weeks postextraction.
Would the density increase drastically and change the quality of bone?

We studied 8 new patients (3 male and 5 female); the BTI Scan study was conducted at 14-16 weeks postextraction, achieving very similar density values inside the alveolus (567 Hounsfield units) and somewhat lower, although not significantly different, inside and outside the simulated implant. (Figs. 26 and 27)

This indicates that we should not expect to improve the beneficial regenerative effect of PRGF after longer periods of time; on the contrary, in shorter time frames, implying improvement and benefit for the patient. Then, up to what point would a preliminary determination of the quantity and quality of bone have yielded equally positive results?

This question comes as a consequence of the excellent results obtained in some of the patients, especially in whom the CT was performed at 8 weeks postextraction. It is, therefore, worthwhile to research along this line with the aim of discovering if it is possible to achieve equally positive results in shorter time periods, which reduce the patient's waiting time.

**Conclusions**

The treatment of postextraction alveoli with the PRGF technique we discussed in this article and which was previously described by our research team (6,9) is a predictable treatment. It is probably the best biomaterial for the postextraction alveolus since it is a 100% autologous product, easy and economical to obtain (from 20 cc of blood - 4 vials).

In cases of severe loss of the vestibular plate, it can be used in conjunction with biomaterials or, preferably, with autologous bone (6,9).

In a previous 5-year multicenter clinical study in more than 2,000 patients in whom this same procedure was performed, there were no side-effects whatsoever. On the contrary: there was faster healing, less pain and less inflammation. There were no complications at all, nor were there any dry sockets. Further studies will be necessary to confirm these results. 

---

**Figs. 26 and 27**: Bone densities inside the alveolus and peri-implant at 14-16 weeks postextraction.
compare the effectiveness of this procedure with others, such as connective tissue grafts or split thickness flaps, but the regeneration of soft tissue is a more effective procedure using PRGF adjunctively, and recommended according to our criteria in all extractions whether implants be treatment planned or not.

With regard to time, we conclude on the basis of this study that in small defects, 8-10 weeks can be sufficient; and in larger defects, in 14-16 weeks a better bone quality can be guaranteed. (Figs. 28-33).

Fig. 28: Vertical fracture of the distal root of the lower first molar with probing depth of 10 mm. The mesial root has a large periradicular defect.

Fig. 29: The virtual placement of an implant in the postextraction bed at 16 weeks will give us a density measurement inside the site at 400 Hounsfield units and peri-implant of 500 Hounsfield units: type III bone.

Fig. 30: In a BTI Scan® contrast view to better evaluate the density, we can observe how the total loss of the vestibular table is regenerating. Observe that it was a substantial defect (12 mm x 16 mm).

Fig. 31: When the surgery was performed at 16 weeks following the extraction, the regenerated bone can be observed; it appears that it has regenerated the entire alveolus.
Contact information:
Dr. Eduardo Anitua Aldecoa
San Antonio 15-3º, 01005 – Vitoria, SPAIN; Email: eduardoanitua@eduardoanitua.com

Biographical Data:
- Graduated from Universidad de Salamanca in 1979
- Received M.D. in medicine and surgery
- Specialty in Stomatology – Universidad del Pais Vasco, continuing studies in many visits to the United States (Philadelphia, New York, Miami, San Francisco, Chicago) and in Europe (Italy, Germany, France and Spain).
- Delivered conferences in various Spanish universities as post-graduate assistant professor in Prosthetics and Occlusion at the Universidad de Valencia and post-graduate professor in Implantology at the Universidad de Oviedo.
- Has given more than 200 seminars and lectures on implants, prosthetics, dental esthetics and tissue regeneration in Spain and abroad (Europe, United States, Mexico, South America, and Asia).
- Director of the Continuing Education Program in Implantology and Oral Rehabilitation offered in Spain and various countries around the world.
- Visiting professor at the Universities of: Guatemala, Interocontinetal (Mexico), Javeriana (Colombia), Republic of Argentina, Uruguay, Portugal (Oporto).
- Visiting professor at the Universidad del Pais Vasco, School of Medicine.
- Scientific Director, BTI Biotechnology Institute.
- Private practice in Implantology and Oral Rehabilitation (Vitoria, Spain).

Bibliography