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*Clinical Study*

# 15 years clinico-radiographic follow-up of regeneration using autologous fibrin combined with alloplastic bone substitute.

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**Abstract**

The study was aimed to highlight the regenerative potential of blood derivatives. A 42 years old male patient needed full-mouth rehabilitation for which block graft was taken from symphysis and ramus and were fixed with bone fixing screws. Autologous blood derivative was used to prepare adhesive bone and Cerasorb bone graft. Bone augmentation was done in layers, on top of block grafts, putty, Osteobiol® was placed followed by the Adhesive bone at the surgical site. IOPA taken after 15 years of placement of autologous cohesive mass of fibrin plus bone graft in the anterior region shows good and stable bone round implants with no bone loss in the cervical area. The follow-up of the case shows regeneration at the surgical site without any bone loss at the implant-cervical junction.

**Keywords:** Platelet Rich Fibrin, Regeneration, alloplastic, Bonegraft.

## INTRODUCTION

In today's time usage of bio-products made of blood derivative bio-products for regeneration has become a well proven treatment modality, Databases such as Pubmed have huge literature stating regenerative potential of blood derivatives. The concept of these blood derivatives is simple, to use the growth factors stored in platelets. It was in year 1954, when Kingsley first introduced platelet concentrate, created for the treatment of thrombocytopenia[1]. This concentrate later became to be known as platelet-rich plasma (PRP)[1]. It took lot of time for researchers to create a blood derivative protocol which could regenerate not only maxillofacial but in general hard and soft tissue in the body. And in the year 1988, Marx et al[2] in 1988, first proposed that PRP could be used in maxillary face reconstruction, since then for all the doctors worldwide there was no looking back and a simple evolution in the area of regeneration, henceforth resulted in many widely-used and still-applicable protocols, providing in long-lasting results.

As a rule, PRP preparation contains two spins along with an anticoagulant, to get a clot, activation is done by adding thrombin (these days taken by patient's own blood) and calcium chloride but to inject PRP a non-activated form is used. Though growth factors present in PRP aid in regeneration, the technique is not standardised hence creating logistic and technique-sensitive difference in preparation world-wide. Also, in the nascent years of its discovery the protocols were not very well established. According to Marx[3] in 2001, the author states that the first spin is the hard spin and the second spin as the soft spin, whereas, in another article by Dohan et al[4] in the year 2008, authors state that first spin is soft spin and the second spin is the hard spin. This is also the reason why so many companies have come up with different or their-own protocols and tubes for PRP preparation, giving varying degree of results and still creating confusion amongst doctors. Another disadvantage is the use of most common anticoagulant Ethylenediaminetetraacetic acid or EDTA which causes a phenomenon called as EDTA-dependent pseudothrombocytopenia (PTCP), which happens

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due to EDTA-induced aggregation of platelets resulting in a false low platelet count, causing false diagnosis and incorrect and inappropriate treatment[5]. In addition, morphology of platelets may be changed from a discoid to an irregular spherical shape[6].

Due to these technical difficulties Choukroun developed Platelet Rich Fibrin or PRF and its modifications, wherein it is just one spin without adultering the blood. Once the cycle is complete, a reddish-straw coloured liquid which gives growth factors 10 times higher that PRP for regeneration is available for injecting. Classical PRF was developed in 2001, wherein, only a straw-coloured clot could be prepared. This clot was used massively for intraoral surgeries, sheet form was used to regenerate skin and other areas wherever applicable. But, its recently developed modified forms namely advanced-PRF (A-PRF) (developed in 2014) and injectable-PRF (i-PRF) in 2016, have shown regenerative potential, specially the latter[7]. Both these forms remain in liquid state for a brief period of time, after which they clot. During their liquid state, these can be used to inject at the site of regeneration and make sticky bone. The concept of sticky bone was first developed by Sohn et al[8] in 2015 wherein a PRF gel matrix encompasses bonegraft substitute resulting in huge benefits in respect of regeneration. And that is what authors have shown in this publication, through a bone regeneration case done in year 2004 with autologous bone and Plasma, much before the advent of A-PRF, i-PRF or even sticky bone. For the sake of ease, in this article adhesive bone will be used in place of sticky bone.

## CASE REPORT

A patient (male) of age 42 years reported with poor hygiene and multiple missing and decayed teeth (**figure 1**) to the dental office. A Complete history was recorded along with the blood investigations, specially related to bleeding and clotting disorders. Patient was compliant without any medical conditions. Complete procedure and full mouth treatment wherein, root canal treatment for 16, extraction of 17, 26-27, followed by immediate implant placement and also in place of anterior missing teeth, along with GBR was explained to the patient, to which he readily agreed and after signing of the consent form, he was treated by keeping Helsinki declaration[9] into consideration. First sitting comprised a complete oral prophylasis was done and patient was kept on warm saline rinse and mouthwash twice daily for a period of 1 month prior to the surgery. On the day of the surgery for the anterior region, block graft was taken from symphysis and ramus (**figure 2**). Once block grafts were obtained they were fixed with bone fixing screws (Stryker, Germany). Autologous plasma and Cerasorb bone graft (Reismer, USA) was used to prepare Adhesive bone (**figure 3A**). Bone augmentation was done in layers, on top of block grafts, putty, OsteoBiol® (Tecnoss Dental S.R.L, Italy) was placed followed by the Adhesive bone at the surgical site (**figure 3B**). Sutures were placed routine medication of antibiotics, pain killers and mouthwash was prescribed. For the posterior immediate implants, conventional method of implant placement was followed, the extractions were uneventful and immediate implants were placed. In the jumping distance, Cerasorb bone graft (Reismer, USA) was placed and the surgical sites were sutured.

**Figure 1.** Pre-op radiographic and clinical images.

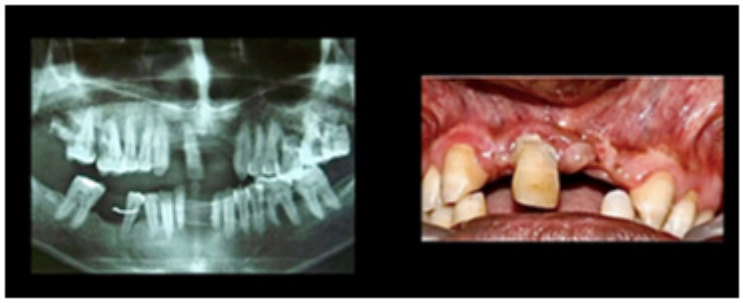


**Figure 2.** Procurement of block grafts.

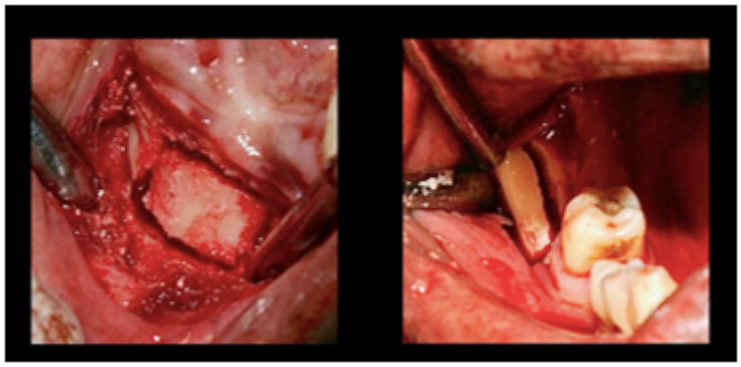


**Figure 3a.** Procurement of Adhesive bone

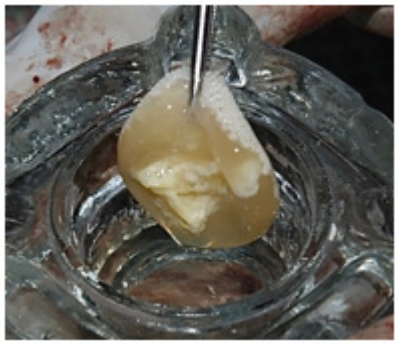
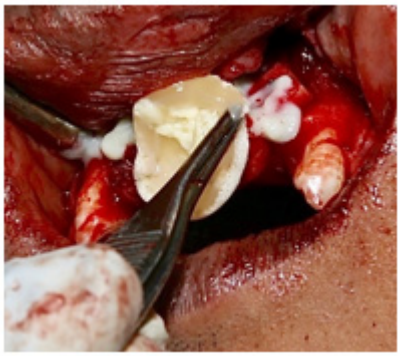


**Figure 3b.** Placement of Adhesive bone at surgical site



**Preparation of the autologous fibrin matrix**

As aforementioned, in the early years of PRP discovery the protocols to procure the same were not well established. Hence, here, PRP was prepared by collecting the blood in a plain plastic test-tube and was spinned only once at 2000 rpm for 5 mins in a Remi manual centrifugation (Remi®, india). The resultant obtained was in liquid state in 3 layers (due to higher RPM and time), with tiny bits of clots, suggesting that autologous fibrin would soon clot. The upper straw-coloured liquid was pipetted out from the test-tube and added to the bone graft substitute. Once the clot was formed, it became a cohesive mass containing bone graft as well (figure 3b) which was then placed at the surgical site.

The healing was uneventful. Patient on consecutive follow-ups did not complain or show any signs of discomfort. At 6 months CBCT shows implant placement in anterior region and first quadrant along with good bone formation (**figure 4**). Last follow-up of patient was done in 2020. The last x-ray of the anterior region shows stable implants. Good and stable bone formation can be seen around implants with no bone loss at the implant-cervical junction (**figure 5**).

**Figure 4.** 6 months CBCT showing good bone formation.

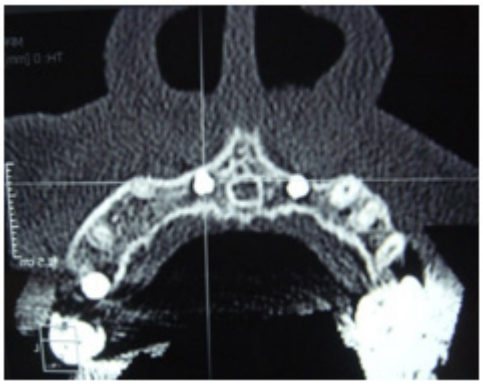
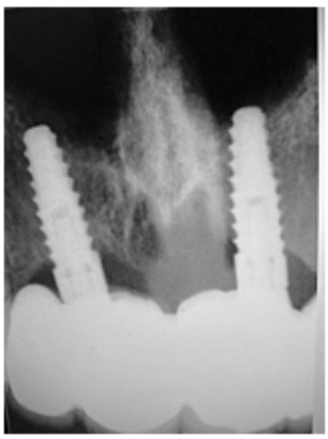


**Figure 5.**15 years follow-up with stable implant and bone levels



## DISCUSSION

When it comes to the topic of regeneration or blood derivatives, huge research is available, describing uses and properties of PRP, since it was first discovered. It is a well-established fact by now that blood derivatives aid in regeneration. And contain growth factors, such as Transforming growth factor (TGF)-beta, Insulin-like growth factor (IGF-1), Platelet-derived angiogenesis factor (PDAF), Platelet-derived growth factor (PDGF), Vascular endothelial growth factors (VEGF), Platelet-derived epidermal growth factor (PDEGF), Platelet factor 4 (PF-4) and many other which aids in regeneration and the same has been highlighted in this article. However, as the protocols of PRP were not well established at that time, what authors prepared was the PRF and sticky boneTM, if today's terminologies are to be used to describe the autologous cohesive mass prepared. However, the preparation protocols do not match as that of conventional PRF, A-PRF or i-PRF. And hence not to create any conflict-of-interest authors have used the term adhesive bone.

The advantages of using adhesive bone are many, such as, no spillage or spread of the bio-product into the surrounding area[8]. The bio-product made can be cut into desired size or rolled to be placed in the socket8. It is cost effective and excellent source of regeneration[8]. Other advantage is that it adapts well to the defect[8] and the graft remains in its place even after 15-20 days of the surgery and is not dislodged. Post-operatively, since the material is autologous, there are no chances of infection, as PRF secretes growth factors the healing is faster without any pain or swelling at the surgical site. PRF has a solid fibrin and strong final architecture[10] which lasts for a min period of 25-28 days[11]. In an article by Giannini et al[12], the authors have compared PRP, PRGF and PRF and affirmed that PRF contains highest value of platelets (thus growth factors) such as TGF, PDGF and VEGF and shares extremely representative network of fibrin, Vitronectin and Bronectin and 65% of leucocytes. Authors also stated that the osctecondictive potential of PRF is high whereas that of PRP and PRGF is poor.

With time the techniques and understanding of blood derivates has evolved, newer age PRF namely APRF and i-PRF are based on low-speed centrifugation concept or LSCC[13] where the blood is spined at very low G-force just enough to separate RBCs from the blood but retain all the other cells. The concept of retaining all the other cells is because they help in regeneration. leukocytes are involved during bone regeneration[14]. Neutrophils also aid in bone regeneration[15,16] and help in monocytes recruitment, which also aid in regeneration and vascularization of the wounded area[17]. Macrophages also help in collagen production and wound repair by releasing several immunomodulatory factors and cytokines[18] and express osteogenic molecules such as bone morphogenetic protein 2 (BMP-2)[19]. Since the introduction of these two types of PRFs, the science of hard and soft tissue regeneration and has evolved and modelled. A faster healing, no pain and no swelling is possible when PRF is used at the surgical site[20].

Coming to the bone graft used, usage of block graft was done to give width and height to the edentulous arch. Autogenous bone is the gold standard and provides oeteogenic and osteoconductive properties aiding in regeneration. Osteobiol® is a cortico-cancellous heterologous bone mix containing 80% granulated mix and 20% collagen gel, with particle size of up to 300 µm. since it is a putty, it has exceptional malleability and plasticity, creeping into small gaps and sealing micro spaces. The product also facilitates blood clotting and shows osteoconductive behaviour[21]. In the adhesive bone the graft used is an allograft, however the choice of graft does not alter the properties of adhesive bone, aforementioned advantages remain the same.

## CONCLUSION

The strength of our report lies in the fact that the case was done in 2004, wherein, autologous fibrin was used encompassing the bone graft to create a matrix rich in regenerative potential. It has to be emphasised that since the protocols to make PRP were not well established at that time, what authors prepared was indeed PRF and sticky boneTM but authors do not wish to create any conflict of interest in respect of taking credit as the 'first preparation' of this bio-product. The follow-up of the case shows regeneration at the surgical site without any bone loss at the cervical portion of the implant. Placement of the implant and the masticatory forces acting also have a role to play in bone resorption at the cervical portion, however not considering that factor, the bone surrounding the implant has shown good regeneration and stable levels over the years.

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**Conflict Of Interest**
none declared
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