Use of autologous plasma rich in growth factors in arthroscopic surgery

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The objective of using autologous plasma rich in growth factors (PRGF) is to improve the surgical course by reinforcing and potentiating the physiological repair process, and also to allow a quicker regeneration and a better quality of the injured connective tissues.
The application method of PRGF in arthroscopic surgery of anterior cruciate ligament plasties is here reported. The clinical courses of 50 plasties performed without and 50 with PRGF were compared.
When PRGF is used in association with surgery, postoperative complications and inflammatory signs are reduced, wound healing and plasty integration are accelerated. The use of PRGF involves no risk or complication for the patient, and the benefits are remarkable.

Key words: knee arthroscopy, ACL reconstruction, growth factors.

Aplicación de plasma autólogo rico en factores de crecimiento en cirugía artroscópica. La utilización de plasma autólogo rico en factores de crecimiento (PRGF) tiene como objetivo mejorar la evolución quirúrgica, reforzando y potenciando el proceso de reparación fisiológica, además de permitir una regeneración más rápida y de calidad en los tejidos conjuntivos dañados. Se describe el método de aplicación de PRGF en la cirugía artroscópica de las plastias del ligamento cruzado anterior. Se compara la evolución clínica en 50 plastias realizadas sin PRGF y 50 plastias aplicando PRGF. Cuando se utiliza PRGF, asociado a la cirugía, las complicaciones postoperatorias y los signos inflamatorios son menores, se acelera la cicatrización de las heridas y la integración de la plastia. La utilización de PRGF no implica ningún riesgo ni complicación para el paciente, y los beneficios de su aplicación son considerables.

Palabras clave: Artroscopia de rodilla, reconstrucción del LCA, factores de crecimiento.

Currently, advances in the different medical fields derive from the effort and participation of certain scientific fields: bioengineering, informatics, chemistry, biology, and medicine. From this interdisciplinary collaboration, dramatic progress has resulted in the last few years.

One of the current challenges in modern medicine is to obtain practical clinical applications derived from this biological and molecular research[1].

The top priorities of these disciplines include the elucidation of the molecular mechanisms controlling cell signaling that lead to regeneration of connective tissue: cartilage, bone, and soft connective tissues.

This knowledge provides a new insight into the physiological processes involved in the repair and regeneration of these tissues and allows the development of new therapeutic approaches.

Proteins are directly responsible for the state and fate of cells in the different pathological or trauma conditions. In this biological context growth factors play a central role in controlling the evolution of regenerative and repair processes of different tissues. Growth factors regulate relevant cell functions such as cell proliferation, migration, and differentiation, in addition to extra-cellular matrix synthesis, all of the processes essential in repair and regeneration[2,3].

Recent *in vivo* and *in vitro* studies have shown significant increases in parameters indicative of cell activity, as well as improvement in the regeneration process by using different growth factors[4-6].

Also derived from these investigations, it is now known that the expression of growth factors and their receptors is modulated after an injury, and cells and tissues under regeneration are susceptible to interaction with growth factors[7,8].

Despite experimental evidence in animal models and cell cultures, so far there has not been clinical evidence of the therapeutic potential of growth factors in the field of traumatology[9,10].

Our investigation focused on the utilization of autologous plasma rich in growth factors (PRGF), obtained from the blood of the patient by a simple procedure. Our hypothesis is based on the assumption that the presence of PRGF at the lesion site accelerates the regeneration process in local tissues by a mechanism that reproduces the initial steps of tissue repair[11].

Initially, this process implies the formation of a thrombus to assure hemostasis, and trapped in this thrombus are platelets. Until recently, the recognized physiological function of platelets was the prevention of bleeding. However, it has recently been established that platelets are carriers of different proteins[12]. Once activated, platelets release various proteins that contain platelet derived growth factor (PDGF), transforming growth factor-β_1 (TGF-β_1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), insulin-like growth factor type-I (IGF-I), and epidermal growth factor (EGF)[13,14]. This combination of substances acts upon local cells triggering specific responses.

The first clinical applications of autologous PRGF were in oral surgery[15]. The clinical benefit obtained in this field is due to the ability of proteins to accelerate soft tissue repair, as well as bone regeneration in preparing future areas for dental implants[16]. These clinical results in oral implantology, together with the previously-cited experimental work on cell cultures and animal models have formed the foundations for using PRGF in traumatology.

The objective of this work was to report new strategies in PRGF use in arthroscopic surgery. We chose ACL plasties, as we have considerable experience (more than 75 patients with ACL plasties in whom PRGF was used) and more than one and a half years of follow-up (we started this procedure at the end of the year 2001).

MATERIALS AND METHODS

To evaluate the advantages of using PRGF, a retrospective study was undertaken, and data was analyzed from the clinical records of 100 patients undergoing ACL plasty at the Unidad de Cirugía Artroscópica, Clínica USP La Esperanza, Vitoria-Gasteiz.

Patients were divided into two groups:

Group A: fifty patients undergoing plasty without PRGF. The last 50 patients.

Group B: fifty patients undergoing plasties with PRGF. The first 50 patients.

Description of Groups Group A

Group A:

Mean age: 26.7 ± 8.4 years.

- 41 tetrafasciculated pes anserinus plasties, transcondylar transversal fixation.
- 9 allografts of frozen patellar tendon.

Additional maneuvers on meniscal and/or chondral lesions:

58% (29 knees):

- Meniscectomies: 13 internal, 4 external, 1 double.
- 8 meniscal sutures.
- 3 debridements on osteochondral lesions.

Group B:

Mean age: 29.6 ± 9.5 years.

- 39 tetrafasciculated pes anserinus plasties, transcondylar transversal fixation.
- 11 allografts of frozen patellar tendon.

Additional maneuvers on meniscal and/or chondral lesions:

76% (38 knees):

- Meniscectomies: 10 internal, 10 external, 4 double
- 9 meniscal sutures
- 7 debridements on osteochondral lesions.

SURGICAL PROCEDURE

Preparation of PRGF

A venipuncture is performed and 40 ml of blood are drawn a few minutes before surgery and before the administration of anesthesia.

Blood is collected in tubes containing 3.8% sodium citrate as anticoagulant.

Plasma is separated by centrifuging at 450 g (1800 rpm) for eight minutes (PRGF System III, BTI, Vitoria-Gasteiz) **(Figure 1A)**.

The plasma volume per 4.5 ml tube obtained after centrifugation is 2 ml approximately, when the hematocrit value is within the normal clinical range.

As a result of this centrifugation, a platelet concentration gradient is obtained; the peak amount of platelets is in the 0.5 ml plasma fraction immediately above the red-cell series **(Figure 1B)**.

Using this procedure, two plasma derivatives are obtained with different contents in growth factors. The fraction immediately above the red cell series fraction is extremely rich in platelets and growth factors. In addition to containing more platelets, the platelets contained in this fraction are more dense and have a higher content of growth factors. To activate platelets and coagulate fibrinogen, one 50 µl amount of calcium chloride is added per each plasma ml.

Depending on the specific use, this preparation will be used directly in its fluid form, so that coagulation occurs at the lesion site. Alternatively, allow it to clot in the tube or in the appropriate container and then apply directly **(Figure 1C)**.

Use of PRGF in arthroscopic surgery

The use of PRGF hardly interferes with the usual maneuvers in pes anserinus plasty procedures or HTH. Thus, once obtained, tendons (autologous or allografts) are placed in an sterile container with "activated" PRGF in order to become soaked in fibrin and growth factors **(Figures 2A and 2B)**. Fibrin serves as initial adherent, and also enhances cell migration and support of cells involved in the repair process, accelerating and enhancing the integration of the plasty.

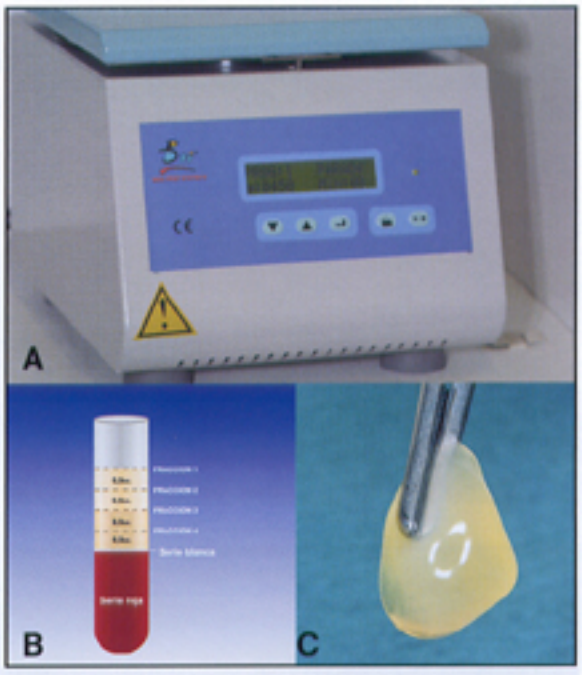

Figure 1. A) Equipment for obtaining plasma rich in growth factors (PRGF). B) Separation of the different plasma fractions in a density gradient. C) Freshly formed clot, rich in growth factors.

In our procedure with the "pes anserinus," the bone plug obtained from the tibial tunnel is also soaked **(Figure 2C)** and then repositioned in the same tunnel to increase the fixation of the plasty. Finally, when the intraarticular plasty is introduced **(Figures 3A and 3B)**, the entry of irrigation saline is stopped to avoid dragging PRGF and the articular fluid is aspirated to avoid dilution.

Once the plasty is placed, the activated PRGF (but still in fluid state) is infiltrated with a needle **(Figure 3C)** within the plasty, tunnels and condylar plasty.

At the donor sites (insertion in medial tibial metaphysis for "pes anserinus" or patella, TTA and patellar tendon for HTH), the activated PRGF in the coagulated form is placed to enhance hemostasis and zone repair.

RESULTS

The data obtained from the clinical records corresponding to the last 50 plasties performed before using PRGF and the first 50 plasties in which PRGF was used are as follows. *[Please refer to* **Table 1**, *which has been translated at the end of this document-Translator's note]*.

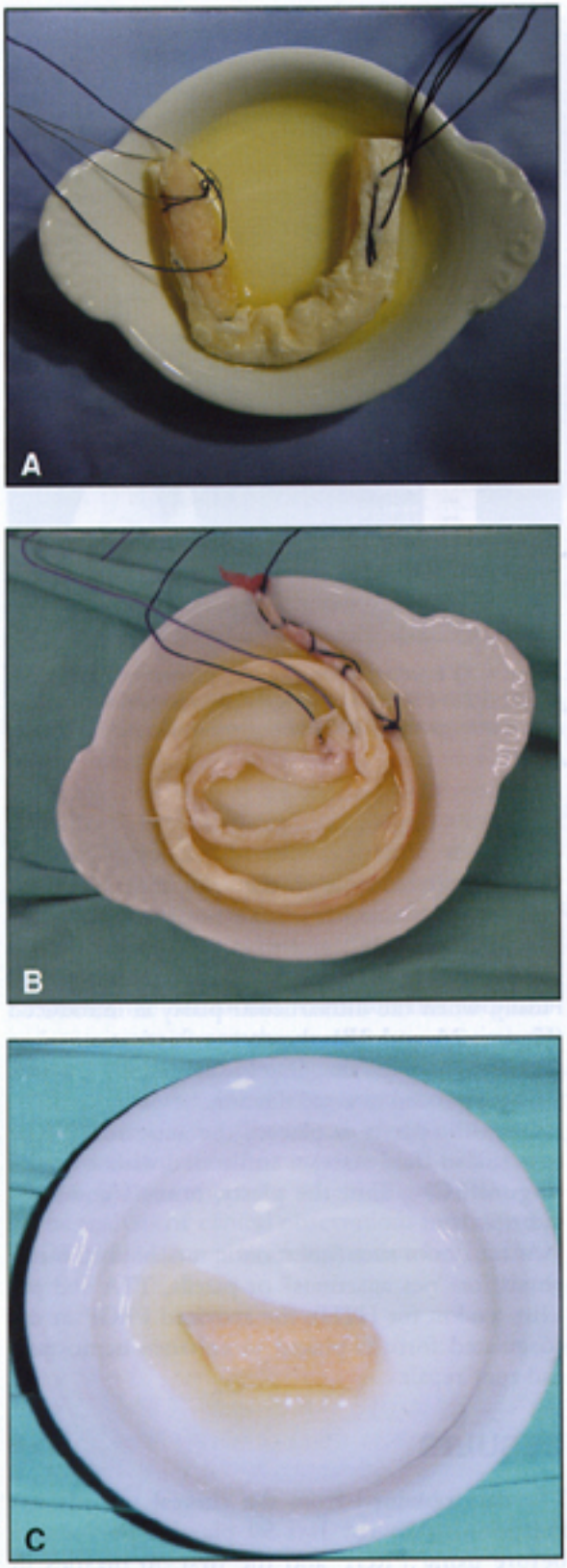

Figure 2. Washed PRGF grafts in sterile containers. A) Allograft of patellar tendon. B) Semi-tendinous and internal rectus tendons. C) Tibial bone plug.

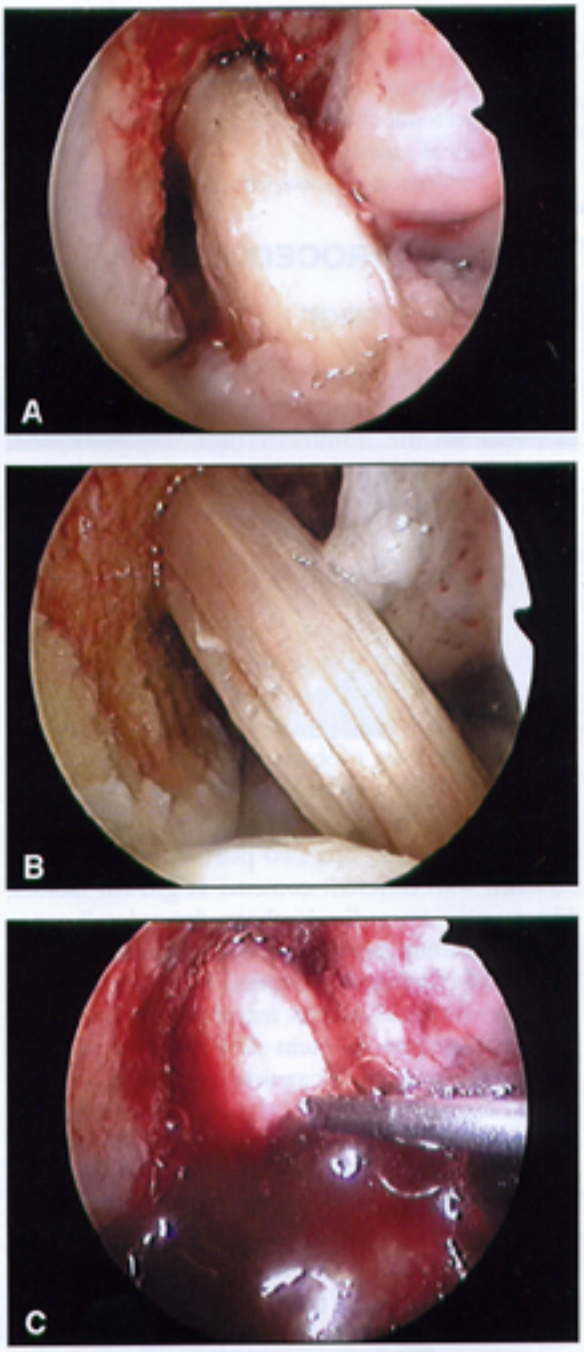

Figure 3. "Vacuum" arthroscopic images of PRGF-impregnated plasties. A) Allograft. B) Pes anserinus. C) Infiltration of plasty with PRGF.

In radiological scans, performed at month 1 after surgery, patients in group B showed tibial bone tunnels hardly visualized compared with X-ray plates of patients in group A **(Figures 4A and 4B)**.

The arthroscopic image of the plasty, in a re-intervention performed in group B (staple removal) at month 8 after surgery, shows an excellent appea-

Table I

RESULTS OBTAINED

Clinical evaluation	Group A 50 patients (without PRGF)	Group A 50 patients (with PRGF)
Presence of large postoperative hematoma with pain, pretibial edema and low-grade fever	18% (9 patients)	6% (3 patients)
Presence of minor hematomas	30% (15 patients)	16% (8 patients)
Infectious arthritis (resolved with synovectomy, arthroscopic lavage and antibiotic treatment, 6 weeks)	2% (1 patient)	2% (1 patient)
Postoperative effusions which required at least one draining arthrocentesis	26% (13 patients)	24% (12 patients)
The "Cyclop" syndrome led to one arthrolysis	6% (3 patients)	2% (1 patient)
Forced manipulations	4% (2 patients)	0
Totally stabilized knees	70% (35 patients)	82% (41 patients)
Knees with an anterior impingement in the Lachman maneuver, with negative dynamic testing (pivot-shift) or insinuation	30% (15 patients)	18% (9 patients)
Full motion	62% (31 patients)	62% (31 patients)
Flexion restriction below 120°	4% (2 patients)	4% (2 patients)
Lack of extension, not including cyclop cases: "flexo" lower than or equal to 5° and bearable	8% (4 patients)	18% (9 patients)

rance **(Figure 5A)** and an optimal integration of the plasty, which is also observed in magnetic resonance images **(Figure 5B)**.

DISCUSSION

During the last few years, a special emphasis has been placed on mechanical issues in the traumatology area. Currently, traumatologists are aware that the mechanical environment has a significant influence on the regeneration process of muscular and bone tissues, but the biological environment and cellular issues cannot be ignored. Therefore, the rationale for the treatment of trauma injures will depend upon the interaction between biological elements and the mechanical environment and their optimization[17,18].

In this regard, there are currently many lines of research that study how to improve, for instance, the integration of the prosthesis to the bone, the early regeneration of soft tissues or the accelerated formation of the fracture callus, in an attempt to obtain an optimal functional recovery as quickly as possible. In this context, many reports have recently been published regarding the study and clinical use of morphogenetic proteins, BPMs[19-21] and growth factors[22,23]. The aim of these studies was to determine the most appropriate combinations of these

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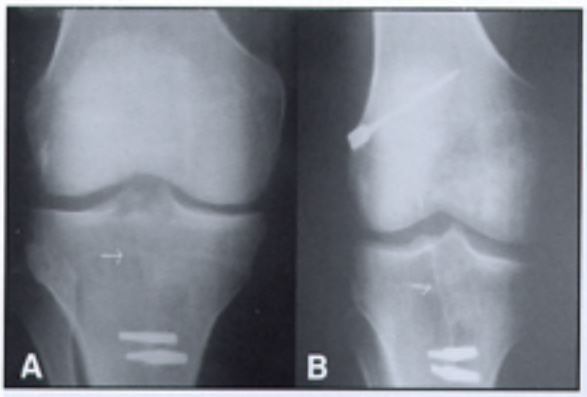

Figure 4. Postoperative radiographs one month after surgery. Note the different integration of tibial tunnels (arrows). A) With PRGF. B) Without PRGF.

proteins, the greater potency, the most efficient exposure time, the most effective therapeutic doses, as well as to define the most appropriate carriers for their release.

While works are in progress in this field, the clinical use of growth factors is an efficient alternative. With respect to intra-articular tissues, difficulties in the repair process may be due to poor vascularization after the injury. Experimental works are currently being conducted in animals in order to evaluate the role of VEGF and its receptors in the process of remodeling tendon grafts used in the reconstruction of the anterior cruciate ligament[7].

Our clinical data suggest that the use of PRGF minimizes the hematoma formation risk and inflammatory signs during the postoperative period; the recovery process is better tolerated, as pain decreases, and is also shorter. Plasty integration also appears to be accelerated, a fact that we can observe when evaluating the integration of tunnels in plain radiographs **(Figure 4A)** and resonance images **(Figure 5B)**.

The analysis of clinical observations indicates that PRGF treated group has a higher number of stable

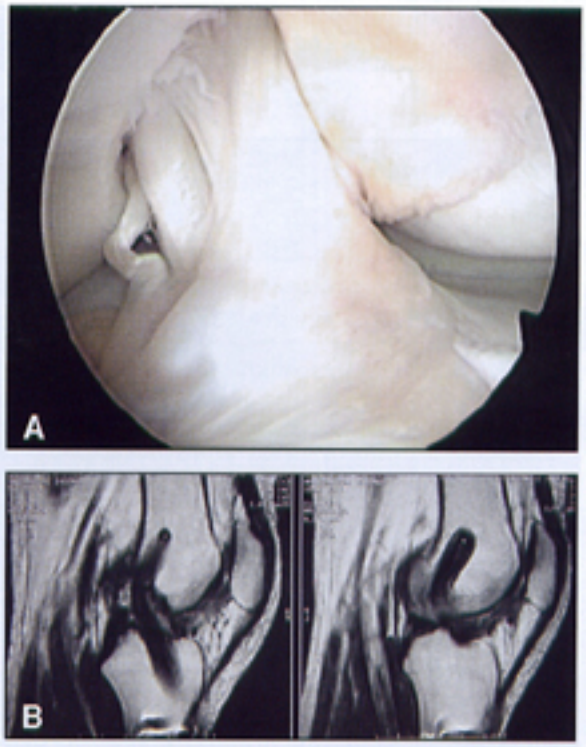

Figure 5. A) Arthroscopic appearance of tetrafasciculated pes anserinus plasty of anterior cruciate ligament treated with PRGF after eight months. B) RM appearance.

knees; examination tests (Lachman and Pivot-shift), performed six months after surgery indicate that PRGF-treated group has a higher index of stability.

The excellent results obtained by Dr. E. Anitua in the dental implantology field encouraged us to incorporate the use of PRGF in our specialty. In October 2001, we initiated the use of PRGF for different conditions and situations such as cutaneous ulcers, tendon and muscular ruptures[10], fractures, pseudo-arthrosis, chondral lesions[9] and ACL plasties, and considerable benefits for our patients were obtained.

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