Application of fibrin rich in leukocytes and platelets (L-PRF) in the reconstruction of endoscopic approaches to the skull base

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Abstract
Introduction: One of the main complications of the transsphenoidal endoscopic approach is the cerebrospinal fluid (CSF) leak, which significantly increases morbidity and consumes important economic resources. The use of biologically active hemoderivative autologous material is proposed as promoter of tissue regeneration for the rapid reconstitution of the mucosal plane to close endoscopic approaches, with the aim to reduce post-surgical CSF leak.

Materials and Methods: Leukocyte- and platelet-rich fibrin (L-PRF) membranes were produced from centrifuged autologous venous blood. They were used for the reconstruction of transsphenoidal endoscopic approaches to the sellar region in 8 patients. Incidence of CSF leak was clinically monitored during the first 30 days, and by means of fiberoptic endoscopic controls on the 2nd and 7th postoperative days.

Results: The product obtained bore the characteristics of the original descriptions of L-PRF. Standardized preparation of L-PRF membranes promoted early regeneration of the sphenoid sinus mucosa to close endoscopic approaches to the sellar region. No CSF leak or other complications within the first 30 postsurgical days were reported.

Conclusion: L-PRF membranes offer characteristics which are superior to other techniques and products, mainly due to their role as biological promoters of tissue regeneration, their low economic cost and immediate availability. However, it would be necessary to confirm these results in studies involving a larger number of patients.

Key words: L-PRF; cerebrospinal fluid leak; transsphenoidal endoscopic approach; skull base; regenerative medicine.
INTRODUCTION

The transsphenoidal approach is one of the most widely used techniques in Neurosurgery [3, 25], initially in microsurgery and then for endoscopy with the aim to treat, mainly, pituitary adenomas.

Its main advantage is the decreased morbidity associated to the transcranial approach through a more direct access. However, the narrowness of the surgical corridor prevents a hermetic closure of the dura mater, as conventionally performed in the transcranial approach. In this way, a relatively frequent complication occurs in transsphenoidal approaches, the CSF leak, whose incidence has increased due to the expanded endoscopic approaches to the skull base [21, 22].

Treatment of the leak usually requires complementary procedures, so as to stop CSF loss. Once the leak is confirmed, closure of the surgical planes is delayed, which provides access to germs mainly, but not limited to, the cutaneous mucosal flora, which may cause postoperative meningitis. Another complication due to CSF leak is CSF hypotension, whose consequences may range from orthostatic headache to subdural hematomas. CSF leak increases postoperative morbidity, intrahospital length of stay and the use of economic resources [1, 21, 22].

Scarring of the endonasal endoscopic approach implies the restitution of the planes incised, which can be identified as: rhinosinusal mucosa, bone (commonly the sellar floor, sphenoid bone or clivus), dura mater and arachnoid mater. In cases in which the arachnoid plane is incised either in a programmed or accidental manner, hermetic closure that prevents CSF leak will rarely be at the expense of the dura mater, since the edges of durotomy do not end up opposed to each other, to allow for the necessary fibroblast migration and collagen production to cover the durotomy. It is even less likely for the skull base bone tissue regeneration to provide the containment of CSF required in the immediate postoperative period, due to its slow growth pattern. For this reason, the rhinosinusal mucosa is the tissue of our interest, mainly due to its availability and proliferative potential.

The objective of this study is to report the feasibility and reliability of the implementation of innovative autologous, biologically active membranes, for the reconstruction of the sphenoidal mucosal plane in order to provide early isolation of the intracranial compartment.

Chronology of scarring and restitution of the rhinosinusal mucosa have been widely described. Endoscopically, 3 stages can be defined: 1) crust formation, within the first 10 days; 2) edema, until 30 subsequent days; and, 3) mesenchymal growth, which may take up to 3 months [18]. This sequence has its histopathologic correlation, with a particular series of cytokines and growth factors. When a traumatic injury occurs, including the surgical act, bleeding takes place first due to capillary disruption, which causes aggregation and activation of platelets, along with the activation of the coagulation cascade, which produces a fibrin net, with the subsequent formation of clot, and of hematic crusts later, due to dehydration [2, 5].

Platelets produce a wide range of growth factors, mainly the platelet-derived growth factor (PDGF), and chemotactic factors which induce migration of both mesenchymal and
epithelial cells, as well as of mediators of cellular immunity. The initial process continues with neutrophil migration during the first two days, which increases the production of proinflammatory cytokines (transforming growth factor -TGF- α, TGF-β1, interleukin -IL- 1). This also causes vasodilation of the surrounding capillaries, which favors extravasation of fluid, producing mucosal edema. On days 4 and 5, platelets attract macrophages, fibroblasts and endothelial cells as well [12, 34]. Macrophages allow the transition between the inflammatory and proliferative phases, mediated by growth factors such as the tumor necrosis factor-α (TNF-α). The proliferative phase results in the generation of granulation tissue, where fibroblast cells replicate, which produce the extracellular matrix. This matrix, containing collagen mainly, reorganizes and strengthens, a process called “remodeling”, in which type III collagen is replaced by type I [13]. Angiogenesis, proteoglycan production and, finally, epithelial coating, in a concentric way at a speed of 20 μm/h [17], occur as from the 3rd day and may take from 2 to 3 weeks. Scarring may take from 3 months [16] to 2 years [27], for the mucosa to recover optimal function. As generally occurs in the transsphenoidal endoscopic approach, the removal of the basement membrane of mucosa involves a scarring process estimated in at least 6 months [29].

The importance of a rapid restitution of the sphenoid sinus mucous plane has been demonstrated with the description of the pedicle graft technique, which consists of dissecting a portion of the nasal mucosa, generally from the underlying nasal septum, preserving its vascularization by means of an arterial pedicle which, once placed in the sinus wall defect, maintains its vitality, thus increasing the chances of scarring and closing the approach [3, 15, 24, 37]. Other common strategies in the daily practice are the use of autologous abdominal free-fat or fascia lata grafts [20] as bone tissue resected during the approach. The use of synthetic material is described mainly as the application of membranes produced from different compounds, such as collagen or cellulose, or the use of dural sealants [33]. These are fibrin-based plasma derivatives, initially proposed as CSF physical barrier. However, the use of commercial products alongside the classic free or pedicle grafts has not completely solved the occurrence of CSF leaks.

Despite the variety of commercially available products, plasma derivatives (e.g. platelet-rich plasma – PRP) continued to be developed to be used in tissue regeneration therapies. In this study, we propose the specific use of leukocyte- and platelet-rich fibrin (L-PRF).

Structurally, L-PRF has a dense fibrin network which, unlike PRP, provides with a matrix where mainly leukocytes and platelets are immersed. Because L-PRF retains these cells, both its cellular and humoral mechanisms (generation of biologically active molecules) will remain constant and steady in time. Furthermore, this dense fibrin network provides the structure for immune cell migration, and for the migration of mesenchymal and epithelial cells from the periphery to the site of the defect. Its biological characteristics, which have been widely described, include:

- presence of viable autologous cells, in proportions higher than serum [8],
- production of growth factors such as TGF-β1, platelet-derived growth factor AB (PDGF-AB), and vascular endothelial growth factor (VEGF) [7, 30],
chemotaxis, attracting more immune cells and mesenchymal stem cells partly mediated by IL-1b to the inflammatory area [30],

angiogenesis, which implies endothelial cell migration from healthy tissue forming new capillaries, which supply the L-PRF graft, and vasculogenesis, i.e., the formation of capillaries inside the L-PRF graft, which connect to the local circulatory circuit. Both processes, mediated mainly by VEGF, promote vitality of tissue and graft, allowing the extravasation of the blood constituents to reinforce the scarring process [7, 30],

bactericidal action, due to cell and humoral activity mediated by both platelets and leukocytes [6]. It is worth mentioning that no cases of local or systemic infections have been reported associated to the application of L-PRF in any type of tissue, and

production of extracellular matrix proteins, such as fibronectin, vitronectin and thrombospondin-1[7].

L-PRF is produced from 10 ml of a patient's venous blood. In two steps, centrifugation and pressing, a flexible membrane of approximately 30 x 15 x 2 mm is obtained, which is waterproof and highly resistant to mechanical forces. It does not require any special kind of aggregate. The interaction of these membranes (L-PRF) with the sinus mucosa surrounding the surgical endoscopic access site will try to promote a rapid reconstitution of this anatomical plane, thus reducing the risk of CSF leak.

MATERIALS AND METHODS

Ethical considerations

This protocol was approved by the Research Protocol Ethics Committee of Hospital Italiano de Buenos Aires, nº 2849. Participation in this study was in all cases voluntary and certified by informed consent, as well as by consents regarding the specific surgical procedure and general anaesthesia, according to institutional protocols. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Population

Patients with indication of endoscopic skull base surgery, particularly in the sellar region, were included.

Selection criteria

Inclusion criteria:

- Patients with indication of transnasal endoscopic surgery requiring indispensable dissection of the rhinosinusal mucosa by means of that approach.

- Patients over 18 years of age.

Exclusion criteria:

- Patients who refused either to participate or the informed consent process.
• Concomitant pathology or treatment which affected the viability of the respiratory mucosa.
• Hematologic disorder or congenital or acquired blood dyscrasia.
• Patients with nasal fibrosis, or any other condition affecting the rhinosinusal mucosa (polyposis, etc.).

Production of L-PRF membranes
Between 10 and 20 ml of blood were obtained from each patient's peripheral venous blood, after induction of anesthesia, and placed in 10 ml hermetically sealed sterile glass tubes, without prior preparation or aggregate. 10 ml of blood were required for each membrane. The amount necessary depended on the size of the defect caused by the surgical approach.

Extracts were immediately centrifuged at 2700 rpm for 12 minutes, in a low-vibration centrifuge (EBA 21, Andreas Hettich GmbH & Co. KG, Germany). Then, the L-PRF clots were removed using sterile tweezers, and were compressed with a surgical spatula, carefully but firmly for a few seconds, until they obtained a 1-2 mm thick membranous appearance.

Macroscopic and histological characterization of L-PRF membranes
Macroscopic characteristics of the membranes, such as their proportions and resistance to manipulation and instrumentation with conventional surgical material, were considered.

In addition, samples of L-PRF membranes were taken from each patient, which were later examined by means of optical microscopy with hematoxylin-eosin (H&E) staining and Masson's trichrome.

Surgical technique
The use of L-PRF membranes did not involve modifications to the transnasal endoscopic surgical approach. They were used during reconstruction stage and were placed in close contact and in apposition to the borders of the remaining sphenoid sinus mucosa in the periphery, until they covered all the defect of the epithelial lining of sinus. All surgeries were performed by two neurosurgeons with experience in the technique.

Postoperative care
All patients received standard systemic antibiotic regimen. Amoxicillin 1000mg-Clavulanic acid 500mg was given at the anaesthetic induction. It was maintained every 8 hours and discontinued after removing the nasal plug at 48 hours after the surgery. No nasal rinsing or nasal clearance manoeuvres were performed. Neurologic and endocrinological assessment was provided as usual.

Postoperative follow-up
Determination of CSF leak
Postoperative follow-up was carried out during both inpatient and outpatient periods.
Nasal packing was removed 48 hours after surgery. The presence of rhino-liquorrhea was investigated by means of daily directed questioning and physical examination during hospital stay, and on outpatient care days 7 and 30.

Fiberoscopy

Control of the scarring caused by the approach was carried out by means of endonasal fiberoscopy, on the 2nd day (48 hours) and on the 7th postoperative day.

Trophic status of membranes was assessed through signs of neovascularization and epithelialization, and their correct position was also evaluated. Furthermore, the presence of crusts was confirmed using an adapted crust formation score proposed by a previous description [32], described on Table 1. The results of these data (presence of vascularization and epithelialization, correct positioning of membrane, crust evolution) were expressed as percentages.

RESULTS

The protocol was applied to a series of 8 patients, operated on between September 5th and November 30th, 2016. Demographic and anatomopathological data are summarized on Table 2. In all cases, a transsphenoidal endoscopic approach to the sellar region was performed.

Production of L-PRF membranes

Two 30 x 15 x 2-mm membranes were obtained from each patient, from a total 20 ml of peripheral blood, enough to widely cover the sella r floor defect in all cases.

Production time was 13-14 minutes, 12 of which corresponded to centrifugation. The process was parallel to each surgery; thus, L-PRF production time did not affect duration of surgery (average: 113 minutes; minimum: 90 min; maximum: 220 min).

Macroscopic and histological characterization of L-PRF membranes

Histologically, a dense fibrin network was observed, containing platelets and leukocytes, and scarce residual erythrocytes, evidenced by H&E (Fig. 1) and Masson's trichrome (Fig. 2) staining techniques. As reported in the original description, 3 sectors were identified inside the membrane: fibrin matrix, leukocyte hood and erythrocyte residue.

Their macroscopic appearance was pearly-white, with a firm but flexible consistency. They did not suffer any damage due to manipulation or the use of conventional surgical instruments.

Surgical technique

L-PRF membranes were easily placed performing the mentioned approach using standard instruments (Fig. 3). Their placement did not involve any setback for the surgeon or assisting staff. No intraoperative complications were reported. It was not necessary to modify any step in the conventional surgical technique. Except one craniopharyngioma, all operated cases were adenomas, and no intraoperative leak was reported.
**Postoperative follow-up**

**Determination of the presence of CSF leak**

Clinically, no CSF leaks (0%) were reported during the inpatient (72 hours) or outpatient period (30 days), or any other kind of neurosurgical intercurrence such as infections, bleeding, pain, fever, or other complications associated to the placement of L-PRF membranes. No deviations from the normal postoperative course were observed. Length of stay was 72 hours.

**Nasal fiberoscopy**

By means of fiberoscopic controls, restitution of 90% of the mucosal plane was confirmed 48 hours after surgery, with no evidence of CSF leak. In addition, vascularization signs inside the membrane and mucosa were observed. Finally, mucosal lining was macroscopically identified almost completely on day 7 (Fig. 4).

Degree of scarring was assessed by means of an adjusted crusting score, distributed as seen on Table 3. No variations to the score were reported after 48 hours compared to day 7 (n=5), and absence of crusts (grade 0) was reported in all cases on day 30 (n=4). No patient presented with grade-3 crusts in any of the fiberoscopic instances (0%) (n=8).

**DISCUSSION**

In this presentation we propose the use of membranes produced from a hemoderivate, L-PRF, to close endoscopic approaches to the sellar region. The interaction of these membranes with the surrounding mucosa in the surgical access site allows fast reconstitution of this anatomical plane; thus, CSF leaks are prevented. Patients' clinical evolution was monitored with serial fiberoscopy, actively looking for signs of complications during a 30-day postoperative period, which provided us with reliable data to determine the safety of this type of product in patients.

The regenerative capacity of the rhinosinusal mucosa is the concept that led to the description of the pedicle graft technique. However, this technique requires surgical dexterity, operative time, as well as the preservation of the arterial pedicle and of the mucosal lining which, because they are part of the working channel of the endoscopic approach, and they are not always available in optimal conditions at surgical closure. Furthermore, the apposition of this graft to the skull base defect requires traction and torsion on the pedicle, which may compromise its vitality and displace the graft from the site the surgeon initially placed it. Even when the pedicled graft maintains its vitality and stays in place, its regenerative properties (chemotaxis, angiogenesis, extracellular matrix production, re-epithelialization) are debatable and it can be assumed that, in their maximal expression, they are similar to the remaining mucosa, unlike L-PRF, which induces these processes. Finally, this technique involves the denudation of another region of its mucosa nasal cavity, which may take months to re-epithelialize, as was described in the “Introduction” section.

Regarding the use of the so-called dural sealants, besides the fact that they do not bring a definitive solution to the CSF leak problem, there are other disadvantages as well, such as the use of synthetic materials (Adherus®), or the combination of human heterologous sources with bovine materials (Tissucol®), without underestimating the financial cost of these commercial
presentations.

The use of hemoderivative products in neurosurgery for the closure of neurosurgical approaches has been described in few indexed articles, generally in humans [14, 19, 31], and in particular for endoscopic approaches to the skull base in humans [36]. Although their commercial name indicates that they are in fact platelet-rich fibrin (PRF), they are actually products of the platelet-rich plasma family (PRP) [11], which implies a weak consistency instead of a dense fibrin network which provides mechanical support both to contain the CSF in the intracranial compartment and to retain, in its interweaving, the cells that promote tissue regeneration at the level of the approach. Other hemoderivative products, of inexact composition, generally termed autologous growth factors (AGF), have shown dissimilar results regarding intervertebral fixations [4, 23, 26, 35]. Furthermore, the use of L-PRF in neurosurgery has only been described by one group, who wrote about their preliminary experience in closing the transsphenoidal endoscopic approach to the skull base [32], without further details regarding its implementation or histological characterization.

Generating this type of autologous products, which are biologically active, easy to produce and instrument, and with minimal economic cost, makes of this product a very interesting alternative, also as an adjuvant to other membranes and surgical grafts.

Original descriptions of the production of L-PRF have made emphasis on the use of Intra-Spin L-PRF commercial centrifuge system and of Xpression preparation box system (Intra-Lock Inc., USA), both of high economic cost, based on a series of publications [9, 10, 28] which show differences in their vibration pattern, affecting product quality. However, in the analysis of the production of biologically active substances, comparison is only made with the poorest centrifuge performance [9]. In our interpretation, these studies do not rule out that L-PRF can be obtained by means of other low-vibration devices, as we used in this study. In fact, the macroscopic and histological characteristics of the membranes produced by our group, added to their performance as regards the regenerative potential of the mucosa evidenced in-vivo, prove them to be comparable to the original descriptions. Nevertheless, further studies are warranted to determine the specific technical characteristics required to standardize production of L-PRF.

It is worth highlighting that the economic cost of production and use of L-PRF membranes in this study was extremely low, because we used the centrifuge in which we usually perform hemoderivatives compatibilization. Furthermore, glass tubes are resterilizable, with an eventual replacement cost of AR$ 10 (USD 0.67), and no substances or instruments apart from those described in the “Materials and Methods” section are needed.

CONCLUSION

The literature is continuously growing, both in basic and clinical sciences, where L-PRF has proved to be widely versatile, innocuous and with highly promising results in tissue-regeneration therapy. However, it would be necessary to confirm these results in studies involving a larger number of patients.

With the proposed protocol, L-PRF membranes were satisfactorily produced to be used in the closure of transsphenoidal endoscopic approaches to the sellar region. Thus, autologous
biological material was obtained from the patient's peripheral blood, in a standardized and faithfully reproducible way, with minimal production cost and immediate availability.

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REFERENCES


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Table I. **Crusting score.** (Adapted from Soldatova et al, 2016).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Aspect of the surgical bed</th>
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<tbody>
<tr>
<td>0</td>
<td>No crusts</td>
</tr>
<tr>
<td>1</td>
<td>Minimal isolated crusts</td>
</tr>
<tr>
<td>2</td>
<td>Moderate presence of crusts (partial covering of sphenoid mucosa)</td>
</tr>
<tr>
<td>3</td>
<td>Severe crust formation (sphenoid sinus modeling)</td>
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</table>

Table II. **Demographic and anatomopathological data.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Complications</th>
<th>Length of stay (days)</th>
<th>Duration of surgery (minutes)</th>
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</thead>
<tbody>
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<td>Acromegaly</td>
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<td>120</td>
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<tr>
<td>64</td>
<td>M</td>
<td>Macroadenoma</td>
<td>no</td>
<td>3</td>
<td>200</td>
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<tr>
<td>62</td>
<td>F</td>
<td>Cushing's disease</td>
<td>no</td>
<td>3</td>
<td>90</td>
</tr>
<tr>
<td>61</td>
<td>M</td>
<td>Macroadenoma (Pituitary apoplexy)</td>
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<td>3</td>
<td>90</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>Craniopharyngioma</td>
<td>no</td>
<td>3</td>
<td>110</td>
</tr>
<tr>
<td>56</td>
<td>F</td>
<td>Macroadenoma</td>
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<td>90</td>
</tr>
<tr>
<td>48</td>
<td>M</td>
<td>Acromegaly</td>
<td>no</td>
<td>3</td>
<td>105</td>
</tr>
<tr>
<td>66</td>
<td>M</td>
<td>Macroadenoma</td>
<td>no</td>
<td>3</td>
<td>105</td>
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Table III. **Postsurgical nasal fiberoscopy scoring.**

<table>
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<tr>
<th>Crusting Score</th>
<th>48 h</th>
<th>7 days</th>
<th>30 days</th>
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<tr>
<td>2</td>
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<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
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</table>

N/A: not available
Fig. 1. **L-PRF membrane, hematoxylin and eosin.** A. The area corresponding to erythrocyte residue (red-color cells) is clearly evidenced, in close apposition to a leukocyte layer (violet-nucleus cells), from which the fibrin matrix begins to be observed (pale pink tissue) (4x); B. Integration of leukocytes to the dense fibrin and leukocyte network (4x). C. Idem B (10x).
Fig. 2. **L-PRF membrane, Masson's trichrome.** A. The dense interweaving of the polymerized fibrin network is highlighted, containing the constituent elements (40x). B. Platelet clusters are observed between layers of nucleated cells, in close interweaving with the fibrin networks (40x). C. Distribution of the components described in the membrane thickness (4x).
Fig. 3. Surgical field, transsphenoidal endoscopy. Membrane resistance and ductility are observed, as instrumented in the narrow surgical corridor, without losing consistency or tearing. For illustrative purposes: A. By means of disc clamp. B. With pituitary clamp. C. With straight edge clamp. D. With a curette.
Fig. 4. **Postoperative control, nasal fiberoscopy.** The red dotted line shows sites of partial mucosal lining; the black dotted line shows vasculogenesis foci. A. Postoperative control on day 7. B. Postoperative control 48 h after the procedure; a small superior region without mucosal lining is observed. C. Same patient as in B, postoperative control on day 7: mucosal defect is clearly smaller.