B-ENT, 2014, **10**, 27-34

Platelet-Rich Fibrin (PRF): an autologous packing material for middle ear microsurgery

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Key-words. Chronic otitis media, cholesteatoma, otosclerosis, tympanoplasty

Abstract. *Platelet-Rich Fibrin (PRF): an autologous packing material for middle ear microsurgery. Objectives*: To assess the use of PRF prepared using an optimised protocol in middle ear surgery as a substitute for conventional packing products of animal origin such as collagen derived from porcine skin.
Methodology: A retrospective study of 108 patients in whom optimised PRF was used exclusively to pack the external auditory canal or middle ear. The effectiveness or harmlessness of the PRF was evaluated by assessing a range of parameters. A morphological comparison was also made of PRF produced using the Choukroun procedure and our procedure.
Results: The success rate of the repair of the tympanic membrane one year after the surgery was 45/48 patients. In 5 of 63 patients in whom a retro-auricular approach and wall-up technique were used, granuloma was observed along the incision in the ear canal. Granuloma was not seen in any of the 23 patients undergoing a procedure with an endaural approach.
Conclusion: The use of a material prepared from patients themselves and not of animal origin has numerous advantages in terms of biocompatibility and safety, without any adverse effect on the success rate for general middle ear procedures. The protocol is simple and does not prolong the time spent by the patient in the operating theatre. The Choukroun technique. should be modified to prevent excessive failure rates in PRF processing.

Introduction

Middle ear microsurgery procedures involve many steps that usually require some kind of packing material. Supportive materials tend to be used for the following applications: stabilisation of an ossicular prosthesis in the middle ear cleft, support of the medial surface of a tympanic graft, fixation of the electrode of some kinds of auditory implant and filling of a cavity in the mastoid region. Usually, different types of gelatine prepared from porcine skin such as Gelfoam ([1]) or Spongostan ([2]) are used as sterile sponges in the operative field. As well as having haemostatic properties, they are resorbable[1] and do not usually seem to have adverse effects when placed in the middle ear or the external auditory canal. The use of blood products in surgery started some 40 years ago with the use of fibrin glues.[2] However, the use of autologous fibrin glues has remained largely inaccessible due to the complex protocol and the cost of preparation.[3]

Platelet-rich fibrin (PRF) is an autologous blood product which was developed in France. The specific centrifugation method for PRF preparation was established by Choukroun in 2001. The technique involves the collection of blood from the patient in plastic tubes without any anticoagulant. In the absence of anticoagulant, the coagulation cascade is triggered immediately, leading to fibrin polymerisation and clot formation. The blood is centrifuged and three layers are formed: a base layer consisting of red blood cells, a middle layer of PRF and an acellular plasma layer at the top (Figure 2).[4] PRF forms a strong fibrin matrix with a complex 3D structure.

(1) Pfizer™.
(2) Johnson Johnson™.

The effect of L-PRF was studied *in vitro* and a promising effect was found on the proliferation and differentiation of osteoblast cells[5,6] through the delivery of Platelet-Derived Growth Factor and transforming growth factor-β.[7] Recent studies have also shown that L-PRF stimulates osteoblast adhesion and the upregulation of collagen protein production, which could have a positive effect on the cicatrisation of the cavity when an open technique is selected for cholesteatoma.[8] The fibrin architecture of L-PRF is also thought be an excellent scaffold for cell migration and angiogenesis, as well as a reservoir for growth factors and cytokines, providing slow release over 7 days.[7,9] Finally, the high numbers of leucocytes in the thin layer near the red blood cells could play a role in the regulation of inflammation and prevention of infection. These properties could explain the use of PRF in oral and maxillofacial surgery. Indeed, PRF has been found to improve soft tissue healing[10] and bone graft protection and remodelling.[11-13] It is also used as the sole conductive material during sinus-lift procedures[14] and directly to fill cavities in plastic surgery.[15] PRF mixed with adipose tissue has also been used successfully in facial aesthetic lipostructure procedures.[16]

In 2009, Braccini *et al.*[17] reported on the use of PRF for myringoplasty using either the "Champagne plug technique" (in which a PRF cylinder is used alone to fill a small perforation without any incision of the canal) or the "hamburger" technique, which is preferred for perforations larger than one third of the tympanic surface (a temporal aponeurosis graft in underlay was optimised by the lateral application of a PRF membrane). The success rate after a minimum follow-up of 6 months was found to be 96.3% and the material was found to offer both mechanical and inflammatory protection without any undesirable tissue reaction.[17]

To date, there have been no reports on the use of PRF in ear surgeries other than myringoplasty. In middle ear surgery, it is known that products derived from porcine skin (Spongostan®, Gelfoam®) do have the properties required for microsurgery. They have been described as resorbable materials containing gelatine, with haemostatic properties for mild bleeding.[1] In terms of healing properties, gelatine-based products tend to delay the healing process by comparison with cellulose-based products.[18] Indeed, severe inflammatory changes are observed around gelatine remnants when used for the healing of the rectus abdominis muscle in mice after 3 days.[18] Although Gelfoam® and Spongostan® do not seem to have an adverse impact on otological surgeries, a substitute harvested from the patient, without an undesirable tissue reaction would be an advantage.

Given the available data about PRF, we decided to use PRF as a supportive and packing material for general middle ear surgery. A modified Choukroun's technique devised by Mardyla and Mullier was used to obtain PRF for middle ear procedures. The structure of PRF resulting from the two procedures will be described and compared in this paper. The rationale was that, with blood clots usually present after middle ear surgery, introducing clots without haemoglobin should be harmless, especially if they induce an anti-inflammatory effect. The aim of this case series was to assess the feasibility of using PRF on a regular basis as a supportive and packing material for otological procedures.

Materials and methods

The PRF was prepared in the operating theatre after the induction of general anaesthesia without any modification of the anaesthetic protocol used in our team for otological surgery. The scrub nurse and anaesthesiologists were provided with guidelines for the preparation of the PRF.

Blood was collected using an 18 gauge catheter ([3]) via venipuncture from the saphenous veins in the foot or ankle using polyethylene terephthalate tubes (Venosafe ([4])) without any anticoagulant. Mardyla and Mullier proposed systematically connecting the catheter to a one-metre plastic extension[c]. The aim of the plastic extension was to activate the coagulation cascade via the intrinsic pathway. Activation leads to a greater amount of thrombin being generated and a clot with fibrin meshing of greater density (Figure 1). Our experience showed that, without this plastic extension and strict compliance with the procedure, failures of PRF clot formation were frequent.

There should be no delay in processing the blood-filled glass tubes after they have been collected (sampling, centrifugation and PRF preparation) in order to prevent the neutralising action

([3]) CODAN Medizinische Geräte, GmbH, Lensahn, Germany.
([4]) Terumo Europe™ , Leuven, Belgium.

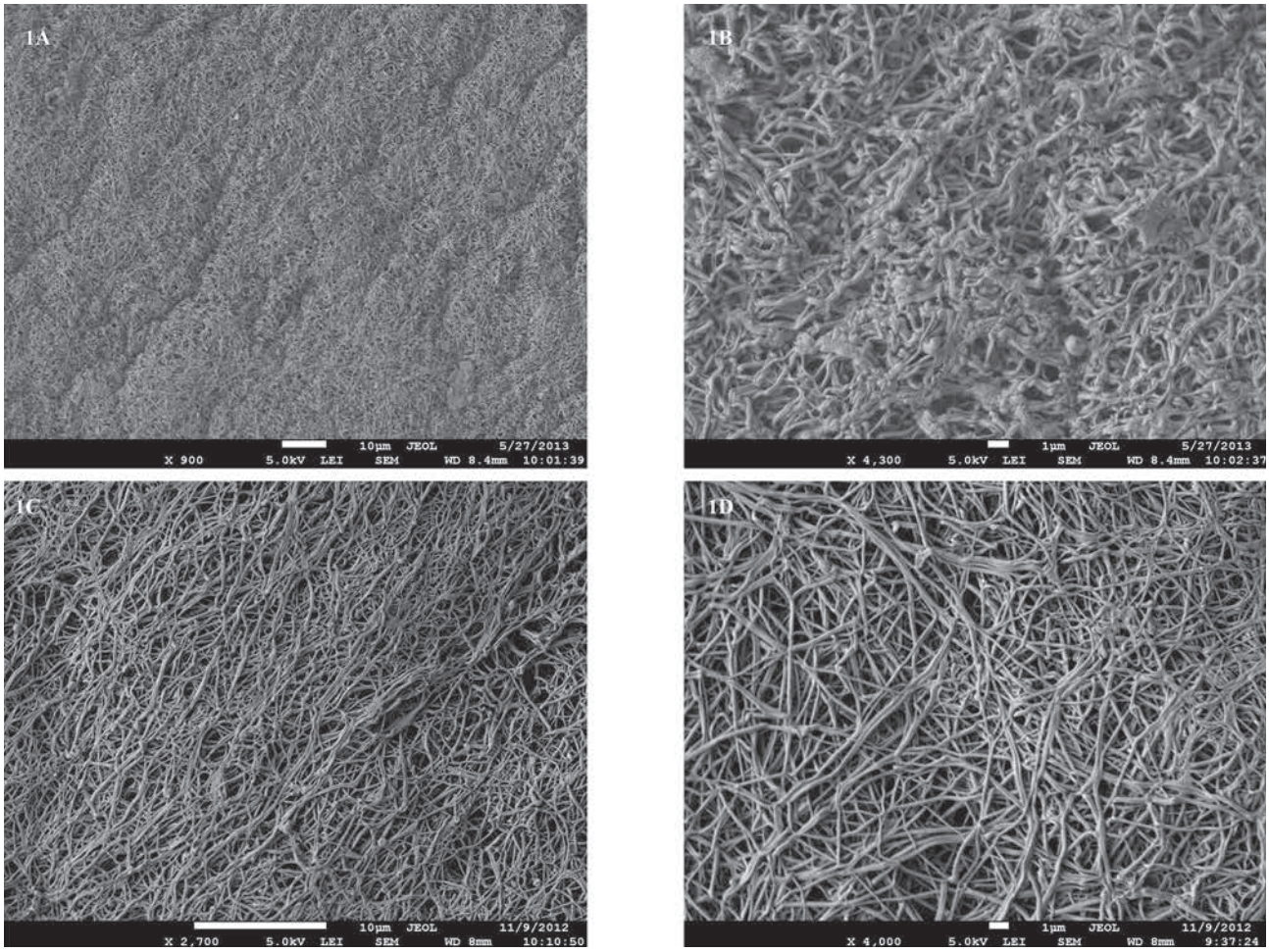

Figure 1

Scanning electron microscopy picture of platelet-rich fibrin from one healthy subject

A) PRF produced without plastic extension, scale bar 10 µm.
B) PRF produced without plastic extension, scale bar 1 µm.
C) PRF produced with plastic extension, scale bar 10 µm.
D) PRF produced with plastic extension, scale bar 1 µm.

The PRF produced with a plastic extension had more extended fibrin links, resulting in a more resistant structure.
A JSM 7500F field emission gun scanning electron microscope (FEG-SEM) (Jeol, Japan; resolution of 0.6 nm at 20 keV) was used to produced PRF pictures.

of natural anticoagulants present like antithrombin and to favour the multiple actions of thrombin in haemostasis and inflammatory processes. Blood was centrifuged for 10 minutes at 400 g ([5]), after which the tubes were emptied on the table of the scrub nurse and the PRF material was separated and kept for later use (Figure 2).

Patients who were on antiplatelet drugs or anticoagulants for reasons not related to their ear disease (coronaropathy, for example) were asked to stop medication 10 days before the surgery and, if necessary, low molecular weight heparin was used as a substitute until the day before surgery.

The volume of blood withdrawn was 40 ml for adults and 20 ml for children with a body weight exceeding 30 kg. For children weighing less than 30 kg, we decided not to use the PRF to prevent additional blood loss. When there was not enough material or when the consistency of the material was inadequate, Spongostan® was used and the patient was excluded.

([5]) Thermo Scientific® IEC Medilite at 3200 revolutions per minute.

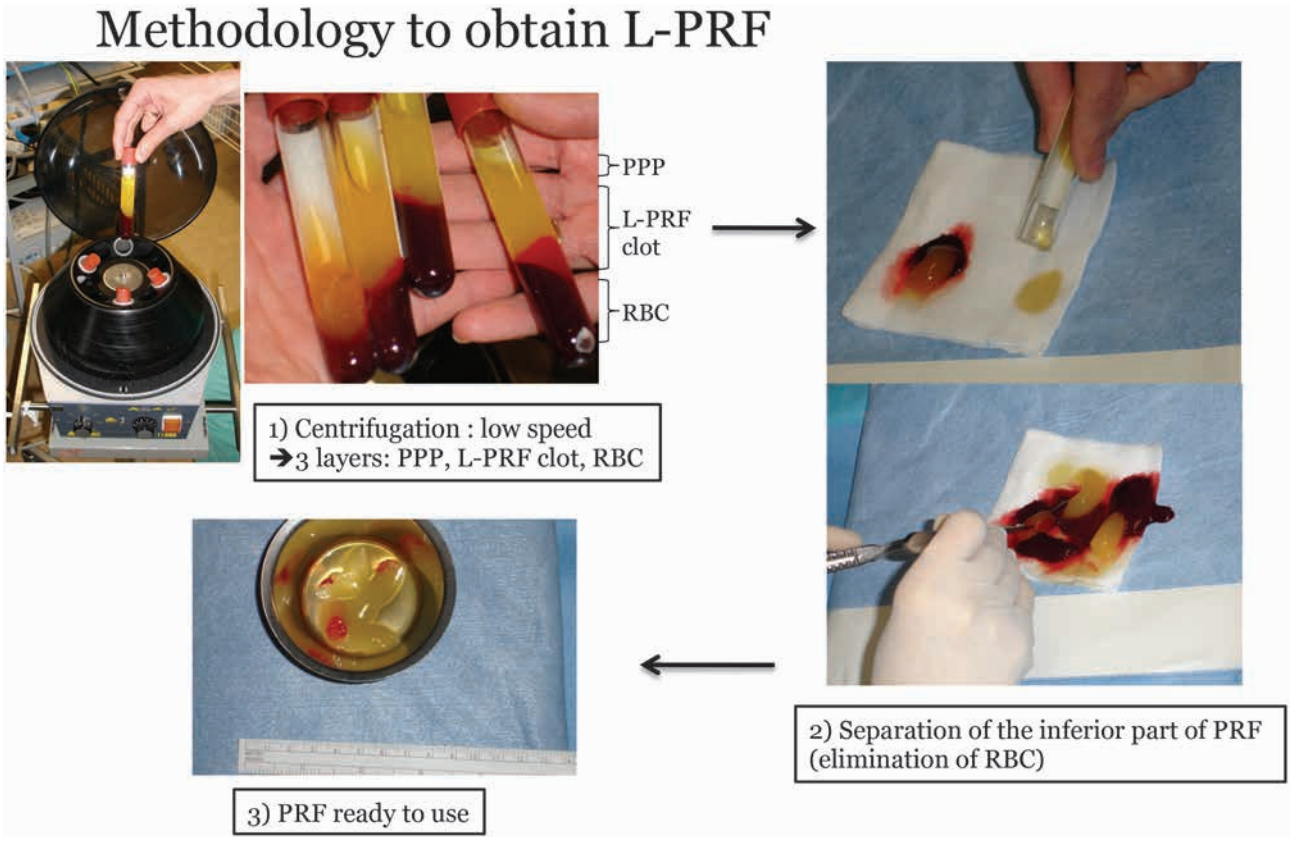

Figure 2
Procedure for obtaining L-PRF

The preparation usually took place at the beginning of the surgery and so the PRF was used about 60 to 90 minutes later.

Data was obtained retrospectively during the year 2011 from 108 patients (including 20 paediatric cases: aged 14 years old or younger), with blood being collected and centrifuged for otological surgeries by the same surgeon in a tertiary referential centre. The age group ranged from 7 to 74 years old. The surgeries consisted of tympanoplasties (types I, II and III), stapedectomies, mastoidectomies, middle ear implants, repair of cerebrospinal fluid (CSF) leak and barotrauma.

Twenty patients were excluded because the volume of PRF was insufficient or because the procedure failed. The types of otological surgeries in the 88 cases in which volume was adequate and in which PRF exclusively was used were: 36 type I tympanoplasties (tympanic membrane repair only) (58% fascia temporalis – 42% tragus or concha cartilage); 11 type II tympanoplasties (eardrum + partial ossicular prosthesis to the stapes suprastructure) (55% incus transposition – 45% titanium prosthesis); 5 type III tympanoplasties (eardrum + total ossicular prosthesis to the stapes footplate) (100% titanium prosthesis); 21 stapedotomies and revision stapedotomies; 7 radical mastoidectomies using the canal wall down technique; 2 middle ear implants (vibrant sound bridge); and 6 miscellaneous procedures (CSF fistula, 2 perilymph fistulae, external auditory canal cholesteatoma or exostosis and Sup SCC dehiscence).

In all these procedures, the PRF was used to pack the external auditory canal at the end of the surgery without using Spongostan® or Gelfoam®. It should be noted that post-operative care remained unchanged: during the postoperative period, every patient was given eardrops containing ciprofloxacin three times a day and the healing process was checked under the microscope in the outpatient clinic at day 9 and day 21. Usually, the PRF packing was removed by the surgeon at day 21 in our department using the same post-operative protocol as when Spongostan® had been used in the past for external ear packing.

In addition, in canal wall up tympanoplasties, the middle ear cavity was filled with the PRF to support the medial surface of the tympanic graft and to stabilise the ossicular prosthesis.

In addition, in canal wall down tympanoplasty, PRF was mixed with autologous bone dust and fibrin glue (Tissucol®([6])) to produce an enhanced form of bone paste that was used to fill the cavity created by the radical mastoidectomy. The same type of bone paste was used to stabilise the electrode of the middle ear implant in the mastoid cavity.

In stapes surgery, a calibrated stapedotomy with a diameter of 0.6 mm was performed using the CO^2 laser. The stapes were replaced by a platinum-teflon piston (Fisch) and the gap between the shaft of the piston (0.4 mm diameter) and the platinotomy hole was filled with small pieces of PRF to prevent a perilymph fistula. Patients operated on for perilymph fistula after barotrauma were treated in the same way, with the PRF being used to fill the oval and round window niches.

Functional results were assessed by comparing the audiometry performed within 2 weeks prior to surgery and between 3 to 6 weeks after surgery in the ENT department.

The interaction between the PRF and the middle ear mucosa is difficult to evaluate in a clinical study. To establish a picture of the status of the mucosa, we used a subjective visual evaluation by the surgeon under the operating microscope, in which level 0: normal aspect of the mucosa; level I: hypervascularisation; level II: thick mucosa and level III: presence of polyps.

The effectiveness or harmlessness of the PRF was evaluated using the following parameters: the hearing results after tympanoplasties, the post-operative complications after otosclerosis, the healing of the external auditory canal, the state of the middle ear mucosa during a second-look procedure for cholesteatoma or middle ear exploration a few days post-operatively for revision surgery.

Results

Peripheral venous blood was harvested from 108 patients and processed. Excellent PRF material was obtained in 88 (88/108) cases (81.4%), more precisely in 70% of children (14/20 cases) and 84% of adults (74/88 cases). In 11 cases (10.2%), no blood clot had formed in the tubes when centrifugation ended and, in 8 cases (7.4%), the volume of PRF obtained was too small to be useful during surgery. In one case, PRF was successfully processed but by the time it was required for use in the surgical field, it had spontaneously disintegrated. Of the 20 patients in whom PRF was not usable, only one (5%) had taken platelet-aggregation-inhibiting medication (stopped 10 days before surgery). Of the other 88 patients with good-quality PRF, 7 (8%) had taken platelet-aggregation-inhibiting medication or anticoagulants (which were stopped 10 days before surgery). Interestingly, one of these 7 patients had stopped taking his anti-platelet medication just 5 days before surgery. Moreover, in another patient with a mechanical heart valve, even though anti-platelet medication was used until 4 days before the surgery, switching to low-molecular-weight heparin until the day before surgery, PRF quality was excellent.

Evaluation of the state of the mucosa in the middle ear was impossible in the immediate postoperative period because, in the vast majority of cases, postoperative evolution was uneventful with a closed tympanic graft, and the middle ear cavity was not visible. We can report on 11 patients only, in whom a second-look procedure was performed at least 6 months later because of suspicions of recurrent or residual cholesteatoma. In 10 patients, no recurring or remnant cholesteatoma was observed. The middle ear mucosa was at level O+I in 6 patients, level II in 3 patients and level III in one patient. In one case, there was a recurrent cholesteatoma and the mucosa was at level II.

During these revision procedures, we observed an improvement of staging in 2 cases by comparison with the first surgery and a worsening in 2 cases; staging was stable in 7 cases.

One patient who underwent surgery for oto-sclerosis presented with severe sensorineural hearing loss and no vertigo on the ninth postoperative day. This patient was immediately re-operated for middle ear exploration: the PRF used to seal the oval window niche was still present, there was no granulation, and no perilymph fistula was identified. The cause of deafness remained unknown.

The success rate for repair of the tympanic membrane one year after surgery using PRF in the middle ear was 45 out of 48 patients (94%) (4 patients lost to follow-up) (tympano I + II + III).

([6]) Baxter™.

Turning to the hearing results, we did not observe any problems with over-fast resorption of the PRF in the middle ear leading to an undesired shift or bascule of the ossicular prosthesis in the middle ear cavity. Indeed, in 18 out of 24 cases (75%) (tympano II and III, VSB and others), there was a postoperative improvement in hearing of at least 10 dB at 1-2 and 4 Khz; there was no change in 5 cases. There was a deterioration by comparison with the pre-operative level in one case.

In surgery for otosclerosis, PRF seems to provide a hermetic sealing of the platinotomy since there was no vertigo in any of our patients at day 4 after surgery. When seen at the outpatient clinic nine days after surgery, 19 out of 21 patients (90%) said they had no problems in their everyday lives and there were no patients with nystagmus.

The easiest area to monitor after surgery was the healing process in the external auditory canal. Interestingly, PRF did not disappear quickly. On the contrary, it stayed in place as long as we have previously observed with Spongostan® or Gelfoam®. No postoperative external otitis was observed. Of 63 patients who underwent a retro-auricular approach and wall-up technique (tympano I-II and III, VSB and 4 others), five patients (8.9%) were seen with granuloma along the incision in the ear canal when the external ear packing was removed at day 21. The complication was easily managed with silver nitrate application under the microscope, followed by ear-drops (antibiotics + corticosteroids) at home for 10 days. None of the 23 patients who underwent an endaural procedure (otosclerosis and 2 perilymph fistulae) presented with granuloma on day 21 (0%).

Discussion

The use of a material prepared from the patient rather than material of animal origin has numerous advantages in terms of biocompatibility and safety. Moreover, patients would prefer to avoid animal/porcine-based products for personal, cultural or religious reasons. The production of PRF is inexpensive since it requires only a centrifuge, something which is already present in many operating theatres in general hospitals.

Using a strict protocol for PRF processing, we succeeded in preparing a good quantity of PRF with a high rate of success (81%) thanks to the excellent collaboration of the anaesthetist and theatre nursing staff. The protocol is simple and does not prolong significantly the time spent by the patient in the operating theatre, but every step and every technical parameter has to be applied rigorously, otherwise the preparation of PRF fails. For example, the blood tubes have to be centrifuged immediately without any delay between sampling and centrifugation. The addition of the 1m plastic extension to the initial protocol as described by Choukroun *et al*.[11] is, in our experience, necessary in order to prevent excessive failure rates for PRF processing.

Interestingly, it seems possible to obtain the PRF even in patients on antiplatelet agents or preventive treatments with low-molecular-weight heparin.

Although PRF has become our first choice, it is always prudent to have Spongostan® as a back-up available in the operating theatre in case a significant amount of PRF cannot be obtained.

In a clinical study like ours, the interaction between PRF and the mucosa in the middle ear cavity is difficult to evaluate. Because PRF is an autologous product derived from the blood of the patient, and because the blood of the patient is always present in the middle ear in the immediate postoperative period, we assume that PRF is not harmful. In fact, we can affirm that there is no visible immediate postoperative reaction in the middle ear cavity. Patients did not complain of pain, vertigo or tinnitus, and there was no otorrhoea or other signs of an inflammatory reaction. In 10 patients, the same surgeon was able to view the middle ear mucosa again at least six months after the first surgical procedure using PRF. These patients underwent a second-look procedure for cholesteatoma; no recurrence or residual disease was found, and the PRF did not induce any intense scarring process in the middle ear such as the adherence of the tympanic graft to the promontory. Interestingly, when the appearance of the mucosa was normal during primary surgery (6 cases), it was also normal during the second-look procedure.

Another question is the speed of resorption of PRF in the middle ear: does it provide enough stability to support the tympanic graft and when placed around an ossicular prosthesis? Our success rate for tympanic membrane repair is 94% for tympanoplasties I – II and III. This result is in line with quoted figures in the region of 90% for underlay techniques.[19,20] There were three residual tympanic perforations among our patients. One of them successfully underwent revision surgery

with fat myringoplasty and the two others refused the proposed revision surgery and were lost to follow-up. The surgeon was the same for all the cases and the technique was, in most cases, the retro-auricular approach with cartilage or fascia temporalis placed using the underlay technique. The three failures were always with fascia and two cases were paediatric cases. The technique used has been the same for many years and we did not find more complications than previously, when we used Spongostan®.

We found an improvement patient hearing in 75% of cases involving ossiculoplasty (tympanoplasties II and III, VSB and others) (18/24). These results are comparable with those of other teams.[21,22] Furthermore, our results are similar to those we obtained previously, with the same surgical procedure, the same ossicular prostheses and the same surgeon, when Spongostan® was used on a regular basis as a packing material.

The PRF can be used to seal the oval window in otosclerosis surgery. Previously, we used Spongostan® to fill the tiny gap between the platinotomy and the piston. Currently, we prepare tiny pieces of PRF to prevent perilymph fistulae. None of our 21 patients suffered from significant vertigo in the postoperative period.

Turning to our healing rate for the skin of the external auditory canal, this skin tolerated the contact with the PRF very well, and the incisions healed very efficiently. A rate of 8.9% for granuloma is difficult to comment on given the lack of publications on this specific topic, but it would seem to be quite high. This is probably due to the relative poor quality of the skin in some external ears, for example in cases of prolonged otorrhoea, which explains the decision to adopt a retro-auricular approach. Nevertheless, this rate is similar to our complication rate with Spongostan®; and, when the pathology allowed an endaural approach, there were no cases of granuloma.

Conclusion

Platelet-Rich Fibrin (PRF) can be easily obtained from the blood of patients after harvesting at the outset of the surgical procedure using an inexpensive centrifuge and without extending the operating time. PRF can be placed in the middle ear and act as a transient supporting structure for a tympanic graft and/or an ossicular prosthesis; it can be used as a packing material in the external auditory canal to protect the ear in the postoperative period without interfering with the eardrops usually prescribed and maintaining its volume for at least three weeks. The observed effects of PRF seem comparable to those of the gelatine-based products currently used, with the advantage of preventing the use of products of animal origin.

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