Clinical Study

Interest of Mineralized Plasmatic Matrix in Secondary Autogenous Bone Graft for the Treatment of Alveolar Clefts

Florian Nadon, MD,* Benoit Chaput, MD,† Jean Périssé, MD,* Ambre de Béral,* Frédéric Lauwers, MD, PhD,* and Raphaeł Lopez, MD, PhD*

Abstract: The authors describe a new material termed mineralized plasmatic matrix (MPM), a combination of platelets, fibrin concentrate, and autogenous bone to repair alveolar cleft defects. Autogenous cancellous bone is widely used to this end because such bone affords the functionalitites (osteogenesis, osteoinduction, and osteoconduction) required for successful outcomes. To optimize these features, autologous blood products high in platelet concentrations have recently been developed. On the basis of our experience with PRP (platelet-rich plasma) and PRF (platelet-rich fibrin), we developed MPM, which contains platelets and fibrin concentrate in a liquid state; these materials can become bound to bone particles. The filling material is easy to shape and a PRF-type membrane is also generated. Ten patients with cleft lips and alveoli, with or without cleft palates (median, or uni- or bilateral) benefited from secondary bone grafts placed using our new material. We transferred autogenous bone from the iliac crest, an abundant source of cancellous bone associated with a high success rate. The 6-month outcomes of all patients were excellent in terms of both bone graft stability and closure of the oronasal fistulae. The preparation procedure is simple and the technical requirements minimal. Upon further optimization, MPM may serve as a third-generation platelet concentrate with potential applications in various fields.

Key Words: Alveolar bone graft, alveolar cleft, gingivoperioteoplasty, growth factors, platelet concentrate

Secondary alveolar bone grafting is essential in the management of cleft palate but remains a significant challenge for maxillofacial and plastic surgeons. Maxillary arch reconstruction is required to induce the eruption of permanent teeth, stabilize the maxillary dental arch, allow closure of oronasal fistulae, and permit orthodontic treatment and implant placement.1

Of the available graft materials, autogenous bone is currently preferred. Differences in gap width and height between the maxillary segments often, however, render it difficult to adequately reduce the bone defect; attainment of bone grafts of appropriate conformation remains problematic.2 Unavoidable bone resorption and the risk of fistula formation are also major issues; the rate of fistula recurrence ranges from 5% to 33% after primary palatoplasty.3,4 Many efforts to improve surgical techniques (ie, to optimize the conformation of bone grafts) have been published, and efforts have been made to promote osteoinduction and osteointegration, and reduce bone resorption in the alveolar cleft. Interest in the use of autologous blood products with high platelet concentrations has grown, since the late 1990s.

In the current study, we describe a new combination of a platelet/fibrin concentrate with autogenous bone used to repair alveolar cleft defects. The concentrate (termed mineralized plasmatic matrix [MPM]) improves the quality of the bone/fibrin mixture, creating a stable and easy to handle homogenous material. In addition, the material expresses biologically active compounds enhancing the tissue repair mechanisms of chemotaxis, cell proliferation, angiogenesis, osteogenesis, and remodeling.5

Materials and Methods

Patients

Ten pediatric patients with cleft lips (median, unilateral, or bilateral) and alveolar defects with or without a cleft palate were enrolled. All parents were thoroughly briefed on the surgical and rehabilitative procedure, and gave written informed consent. All patients benefited from primary closure of soft tissues during infancy. Alveolar bone grafting was performed at 6 to 18 years of age (average, 9.2 years). In all patients, this was the first bone graft placed in the alveolar cleft. All patients were monitored regularly for up to 6 months after surgery.

Surgical Technique

All procedures were performed under general anesthesia with orotracheal intubation. We used only autologous anterior iliac crest bone, because its cancellous nature affords satisfactory packing. Xylocaine was initially injected into each surgical site. The buccal mucosa was incised along the cleft and around the teeth, and the mucosa covering the cleft margins detached and lifted, affording protection of the nasal bone graft. The bony margins of the cleft were well exposed; filling with cancellous bone extended from the floor of the nasal cavity to the full height of the alveolar ridge (Fig. 1). In most instances, the gingival graft was covered with a local flap, creating a physiologic environment permissive of tooth eruption. The palate graft was covered with a rotational flap formed from the palatal mucosa.
In all patients, the bone graft was mixed with the platelet/fibrin concentrate, allowing us to simultaneously create an MPM and a membrane-like PRF (Fig. 2). Blood samples were placed in 10 mL glass-uncoated plastic test tubes (2–4 per patient) without anticoagulant (BD Vacutainer®) and immediately centrifuged at 2700 rpm for 15 minutes. The upper and middle portions of each supernatant were removed using syringes and aliquoted equally. One portion was combined with the bone/platelet/fibrin concentrate mixture in 2.5 mL of physiologic saline (0.9% [w/v] NaCl) and the other was reserved to create a PRF membrane. After deflection of the lamellae of the compact bone, spongy bone chips were harvested from the iliac crest using a bone curette, added to the first mixture, and stirred immediately using a dentistry probe until fibrin strands and mineral particles began to appear. The compact mass of bone linked to fibrin was then grafted into the recipient site. Residual liquid from this mixture was added to the second portion of the blood supernatant and, within 30 seconds, a membrane-like PRF formed (Fig. 3; still image has an associated video file). The membrane was shaped to form a cover resistant to fibrin, which was placed under the mucoperiosteal flap. To minimize operator-induced bias, all surgical procedures were performed by a single operator.

### RESULTS

Clinical and radiologic follow-up examinations were scheduled regularly during 6 months. The quantity and quality of bone in the grafted sites were radiographically evaluated using cone-beam computed tomography (CBCT) (Fig. 4). All 10 patients retained their alveolar arches and all teeth adjacent to the clefts were stable. Nine oronasal fistulae were successfully closed; 1 fistula recurred in part. One patient developed minor mucosal wound dehiscence that resolved during follow-up. No other complications were observed (Table 1).

### DISCUSSION

Secondary bone grafting aims to create a periosteal tunnel that can support bone growth across the alveolar cleft. Optimally, such grafting is performed at an age of approximately 8 years. Autologous bone is the graft material of choice because it is the only material exhibiting osteogenic, osteoinductive, and osteoconductive properties. Graft failures, however, are not uncommon. To improve the biologic characteristics of the implanted material and

**FIGURE 1.** MPM preparation. A, The upper and middle portions of each tube are removed using syringes and divided into 2 equal parts. One portion (left) is mixed with bone/platelet/fibrin concentrate and the other (right) will yield a PRF membrane. B and C, The mixtures are immediately stirred using dentistry probes and fibrin strands including mineral particles gradually appear. The residual liquid in the first portion is added to the second, yielding a PRF membrane within 30 seconds. D, The PRF membrane (left) and the MPM (right). MPM, mineralized plasmatic matrix; PRF, platelet-rich fibrin.

**FIGURE 2.** The surgical procedure for secondary bone grafting in the alveolar cleft. Patients #5 (A-B) and #6 (C-D); note the gaps in the alveolar processes (A-C). MPM is transplanted into the cleft and tightly packed (B-D). MPM, mineralized plasmatic matrix.

**FIGURE 3.** Mineralized plasmatic matrix preparation. This still image has an associated video file.

**FIGURE 4.** A and B, CT scans (coronal and axial views) of patient #4 with a median alveolar cleft. C and D, Cone beam scan 6 months after surgery reveals reconstruction of the nasal floor and filling of the alveolar defect (coronal and axial views). CT, computed tomography.

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the graft success rate, autologous blood products rich in platelets and growth factors are increasingly used.

Developed in the 1990s, platelet-rich plasma (PRP) was found to promote new bone formation when mandibular continuity defects were treated, and to accelerate maturation of autologous bone grafts. Platelet-rich plasma contains high levels of platelets and several growth factors playing key roles in cell regeneration, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor, fibroblast growth factor, and insulin-like growth factor 1 (IGF1). Also, fibrin networks formed from PRP appear to reduce postoperative bone resorption. Such networks serve as osteoconductive scaffolds, providing matrices facilitating cell adhesion and osteoblast maturation. The growth factors in PRP accelerate wound healing of soft tissue. A randomized study conducted by Marukawa et al concluded that the use of PRP was more effective in preserving the width and height of the bone graft than autogenous cancellous bone alone. Platelet-rich plasma yielded higher average volume ratios of regenerated bone to alveolar cleft than controls. In a prospective longitudinal study on recurrent cleft palate fistulae, complete closure was obtained in 90.9% of patients during 6 to 24 months; the recurrence rate was less than that with other techniques. Growth factors enhance osteogenesis and remodeling of alveolar bone grafts in patients with clefts and/or palates, facilitating subsequent orthodontic therapy.

Platelet-rich plasma, however, may rapidly release all contained growth factors before cell outgrowth from surrounding tissue, which would significantly limit stimulation of bone regeneration. Platelet-rich plasma is relatively difficult to prepare; at least 40 to 50 mL of whole blood is required from each patient, collected in 4 sterile pipettes containing anticoagulants (citrate phosphate, and dextrose). Two centrifugations are required to obtain platelet-rich fractions that constitute only 10% of the volume of the initial blood sample. Finally, at the time of grafting, the suspension must be mixed with 2% (w/v) calcium chloride to trigger gelation and promote growth factor release. Sometimes, bovine thrombin is added. Many variations in PRP preparation have been described, which may be broadly divided into 2 types. These are complex techniques using hematological cell separators and simplified techniques (ready-to-use commercial kits featuring 2 centrifugation steps).

Platelet-rich fibrin (PRF) is a second-generation platelet concentrate yielding fibrin membranes enriched in platelets and growth factors, made using anticoagulant-free blood. Both PRP and PRF membranes form resorbable fibrin-like networks facilitating efficient cell migration and proliferation (and thus cicatrization) to guide tissue regeneration. Platelet-rich fibrin affords slow, sustained release of significant quantities of key growth factors for up to 28 days. Thus, PRF stimulates tissue regeneration for an adequate time during wound healing, as its natural fibrin framework protects growth factors from proteolysis. Recently, PRF has been shown to regulate HSP47 and LOX protein expression in human osteoblasts. These proteins facilitate cell attachment, proliferation, and matrix synthesis. Thus, PRF may aid in bone healing, regeneration, and repair.

Platelet-rich fibrin preparation involves collection of whole blood samples (without anticoagulant) in 10 mL glass-coated plastic tubes followed by immediate centrifugation at 3000 rpm for 10 minutes. A fibrin clot appears in the middle of the tube; the upper region contains acellular plasma and the lower region red corpuscles. The fibrin clot is easily separated from the lower region of centrifuged blood. This PRF clot is gently formed into a membrane using sterile dry compression. Platelet-rich fibrin preparation is thus very simple, requiring no anticoagulant, bovine thrombin, nor any other gelling agent. Data on the biologic properties of PRF in terms of bone regeneration, however, are sparse.

Mineralized plasmatic matrix preparation features the simplicity of the PRF protocol, but yields a liquid platelet/fibrin concentrate that can become bound to bone particles. Scanning electron microscopy (SEM) reveals that MPM creates a dense fibrin network woven around the mineral blocks. Bone grafts can be readily conformed and the surgical site fortified by the various contained products. The surgical procedure is essentially unchanged but becomes easier and safer. The cost of the additive is very low (5–9 cents per tube).

Mineralized plasmatic matrix features the use of plastic tubes without additives, deferring initiation of the intrinsic pathway of coagulation. The contribution of a mineral phase (high level calcium and thromboplastin) in the plasma fraction from the tubes induces the extrinsic pathway of coagulation. Usually, a homogeneous filling material, a fibrin membrane, and the beneficial biologic properties of PRF (listed above) become simultaneously available. In addition, the volume of product obtained is high, which is of great importance in clinical practice. Rapid blood collection and patient compliance during the operation, however, are essential. Finally, MPM is devoid of any risk of xenopathic transmission.

We hypothesize that MPM and PRF have similar properties because their production processes (1 centrifugation step, no need for additives, and use of blood collection tubes devoid of silica) are identical. Platelet-rich fibrin, however, differs from PRP, which may be explained by the fact that large amounts of plasma rich in growth factors and platelets are lost during manufacturing of PRF. The various platelet concentrates have very different biologic

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+A, with alveolar cleft; BCL, bilateral cleft lip; C, complete; MCL, median cleft lip; +P, with palate cleft; P, partial; UCL, unilateral cleft lip.
CONCLUSIONS

The use of MPM for cleft palate closure using local mucoperiosteal flaps is innovative; the preparation protocol is very simple. Mineralized plasmatic matrix is totally autologous and thus devoid of risks of infection or rejection. Mineralized plasmatic matrix is a cost-effective source of growth factors and is easy to prepare. Mineralized plasmatic matrix is effective, as judged by reference to our experience with PRP and PRF; our success rate was excellent. Furthermore, work with more patients, however, is necessary, and the biologic qualities of MPM must be better defined.

REFERENCES


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