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A novel technique for guided bone regeneration using platelet-rich plasma and osteogenic progenitor cells: Literature-based rationale and case report

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Achieving predictable guided bone regeneration in critical size defects for future endosseous dental implant therapy poses a great challenge to clinicians. A novel technique utilizing autogenous osteogenic progenitor cells, calcium sulfate activated platelet-rich plasma in addition to particulate allograft was successfully used to augment a severely deficient maxillary anterior edentulous ridge. After 6 months of healing, satis-

factory radiographic and clinical bone gain was noted with significant increase in alveolar ridge width. Endosseous implants were placed and restored successfully. The techniques with underlying clinical and biologic rationales are presented and discussed in this report. (*Quintessence Int* 2016;47: 233–240; doi: 10.3290/j.qi.a34975)

Key words: bone augmentation, dental implant, edentulism, platelet-rich plasma, ridge augmentation

Adequate quantity and quality of alveolar bone prior to implant placement is crucial for the successful use and positive long-term outcome of osseointegrated implants in partially and completely edentulous ridges.^{1,2} Atrophic ridges may provide insufficient volume or an unfavorable inter-arch relationship, which does not allow for the correct and prosthodontically driven positioning of dental implants.³ Various techniques to augment and restore these deficient ridges

have been reported.^{1,4} Autogenous graft has been considered as the gold standard in guided bone regeneration (GBR).^{1,5} However, despite their favorable results, autogenous grafts may pose disadvantages including, but not limited to potential donor site morbidity, extra surgical time associated with graft harvesting, potential graft resorption, and limited volume and dimension of available graft.^{1,5} Due to these disadvantages of autogenous grafts, commercially available biologic modifiers, such as bone morphogenetic protein-2 (BMP-2) and recombinant human platelet-derived growth factor (rh-PDGF) have been suggested and utilized in combination with particulate allograft, xenograft, or alloplastic graft for GBR of deficient ridges.^{6–8} However, although these biologic modifiers may help augment hard tissue therapeutically, the demand is growing for cell-based therapies and novel advanced material-based devices to deliver cells and healing factors.⁹ The need for additional sources of cells is particularly

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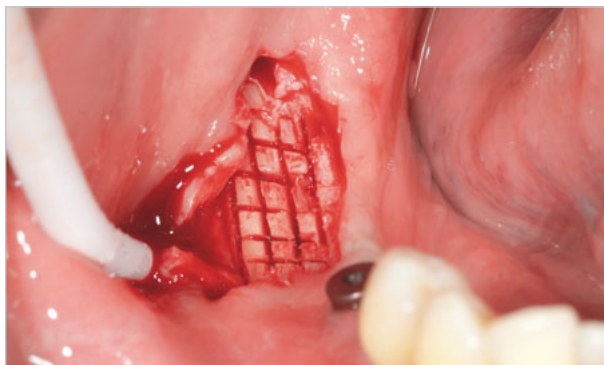
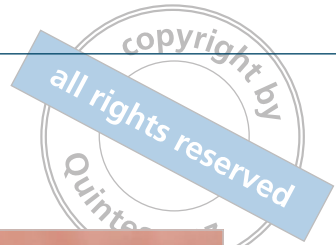


Fig 1a A trans-cortical bony defect was performed using a piezoelectric cutting tip on a mandibular right ramus.



Fig 1c The donor site after flap closure. No membrane was utilized to cover the created defect.

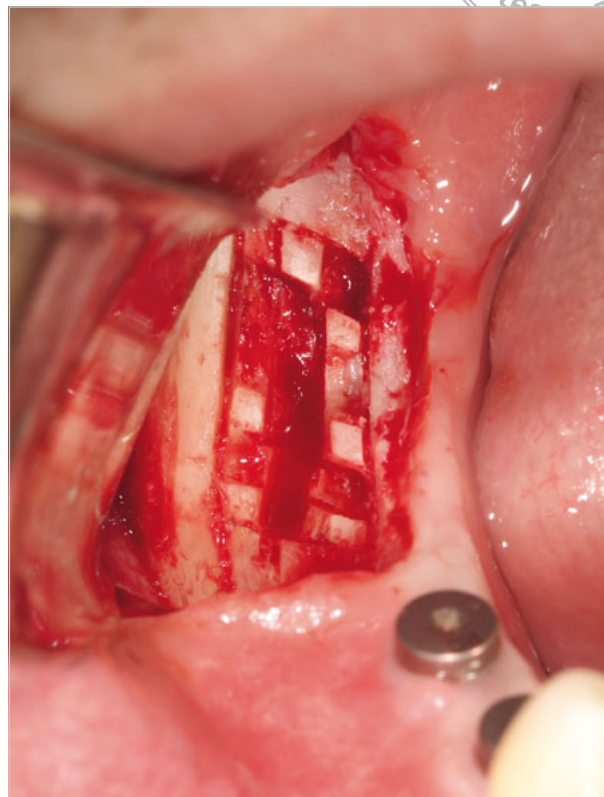


Fig 1b Using a straight chisel, the cortical layer of the alveolar bone was removed. Complete removal was not performed to prevent potential collapse of soft tissue over the defect.

important in case of severe trauma and reconstructive surgery.⁹ In this report, we suggest a novel technique, using calcium sulfate-activated platelet-rich plasma (CS-PRP), osteogenic progenitor cells, and particulate allogeneic bone grafts. Here we describe the successful use of this technique to reconstruct a severely atrophic maxillary anterior ridge. The underlying clinical and scientific rationales are also thoroughly discussed.

CASE REPORT

A 37-year-old Asian man presented to the graduate periodontology clinic at the Harvard School of Dental Medicine. The patient was diagnosed with generalized severe chronic periodontitis. The maxillary right central and lateral incisors were previously extracted by his general dentist due to severe alveolar bone loss. A clinical and radiographic examination revealed a severely

atrophic alveolar ridge with horizontal and vertical ridge resorption in this area. After successful treatment of his severe chronic periodontitis based on the principles of cause-related therapy,^{10,11} GBR was planned and executed prior to dental implant therapy. To obtain osteogenic progenitor cells, 4 weeks prior to the planned GBR a bony defect was surgically created in the right mandibular ascending ramus. This was performed after obtaining and reviewing the cone beam computed tomography (CBCT) of the relevant anatomical landmarks in the surgical area (ie, inferior alveolar nerve canal, thickness of buccal cortical plates). By using a piezoelectric device (Piezosurgery), a 20 mm × 10 mm rectangular-shaped outline was created with multiple internal horizontal and vertical connecting lines (Fig 1a). A piezoelectric tip (OT71, Piezosurgery) was further precisely inserted beyond the cortical layer into the marrow space. A straight chisel (CP7/10, Hu-Friedy)



Fig 2a The donor site was reentered after 4 weeks of healing, and the immature osteogenic tissue was collected.

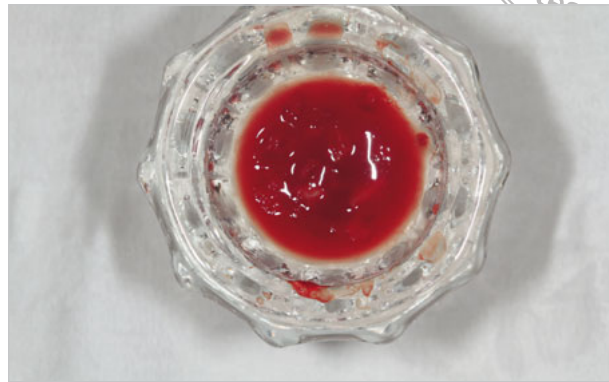


Fig 2b Immature osteogenic tissue harvested from the donor site.

was used to remove the cortical layers and create a bony defect (Fig 1b). Complete removal of the cortical bone was not performed to prevent potential soft tissue collapse into the created defect (Figs 1b and 1c). The healing was uneventful without any patient morbidity including temporary and permanent neurological damage, excessive bleeding, excessive pain, mandibular fracture, and postoperative infection. After 4 week of healing, at the time of the GBR surgery, the ramus defect was surgically reentered and the tissue, potentially containing osteogenic progenitor cells, was harvested from the defect (Fig 2). Platelet-rich plasma (PRP) was prepared collecting 20 mL of whole blood from the patient's left arm by means of venipuncture, following a methodology previously described.¹² Then, the maxillary anterior edentulous ridge was exposed. A minimal width of the alveolar ridge of 3 mm at the most crestal aspect was noted. A large bony concavity was also noted on the buccal aspect (Figs 3a and 3b). PRP was activated by mixing it with calcium sulfate (CS; ACE Surgical) using the PRP/CS ratio detailed by Intini et al.¹² The CS-PRP was then mixed with the collected osteogenic progenitor cells and 1 g of freeze-dried bone allograft (FDBA; RegenerOss, Biomet 3i; Fig 3c). Following intramarrow penetrations using a high-speed size 2 carbide round bur, the resulting composite graft was placed on to the edentulous area (Fig 3d). A titanium-reinforced dense polytetrafluoroethylene membrane (dPTFE; Ti-250, Osteogenics) was placed over the graft

and secured using a membrane tacking screw (Profix, Osteogenics) on the buccal and the palatal aspects of the GBR site (Fig 3e). Primary closure over the surgical site was achieved after periosteal releasing incisions, and maintained over the entire healing period of 6 months. Postsurgically, the patient was prescribed 500 mg amoxicillin three times a day for 10 days, 800 mg ibuprofen three times a day for 7 days, and chlorhexidine gluconate rinse twice a day for 14 days.

Six months following the GBR surgery, a postsurgical CBCT confirmed profound radiographic bone gain in the augmented area (Fig 4). The site was reentered, and the membrane and the buccal and palatal tacking screws were retrieved. Satisfactory gain of alveolar ridge width was observed clinically (Figs 5a and 5b). Prior to implant insertion, a core biopsy was obtained from the maxillary right lateral incisor site using a 2.5-mm diameter trephine bur. Bone level implant fixtures (3.3 × 10 mm, SLActive bone level implants, Straumann) were placed uneventfully with insertion torque of > 35 Ncm (Figs 5c to 5e). After 14 weeks of submerged healing, the implants were uncovered and restored with implant-supported single-unit restorations (Figs 5f and 5g).

Histologic analysis

The obtained biopsy specimen was fixed in 10% buffered neutral formalin immediately upon collection. Thereafter, the specimen was decalcified in Morse's solution. A paraffin block was sectioned and slides were

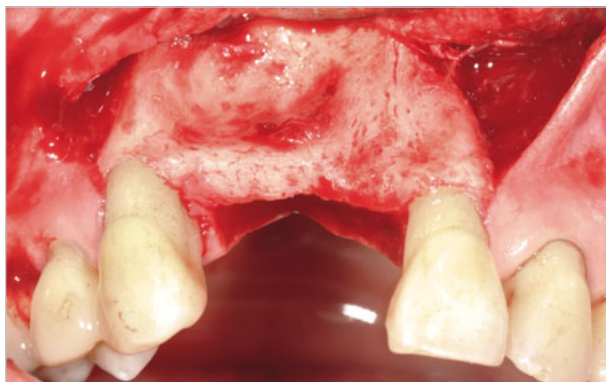


Fig 3a Buccal view of the recipient site with significant ridge resorption.



Fig 3b Occlusal view of the recipient site.



Fig 3c Composite graft consisting of calcium sulfate-activated PRP, harvested osteogenic progenitor cells, and freeze-dried bone allograft.

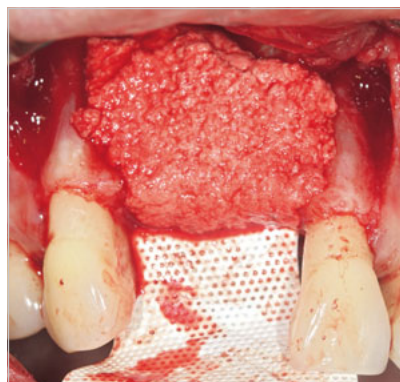


Fig 3d The composite graft was placed in the recipient site.



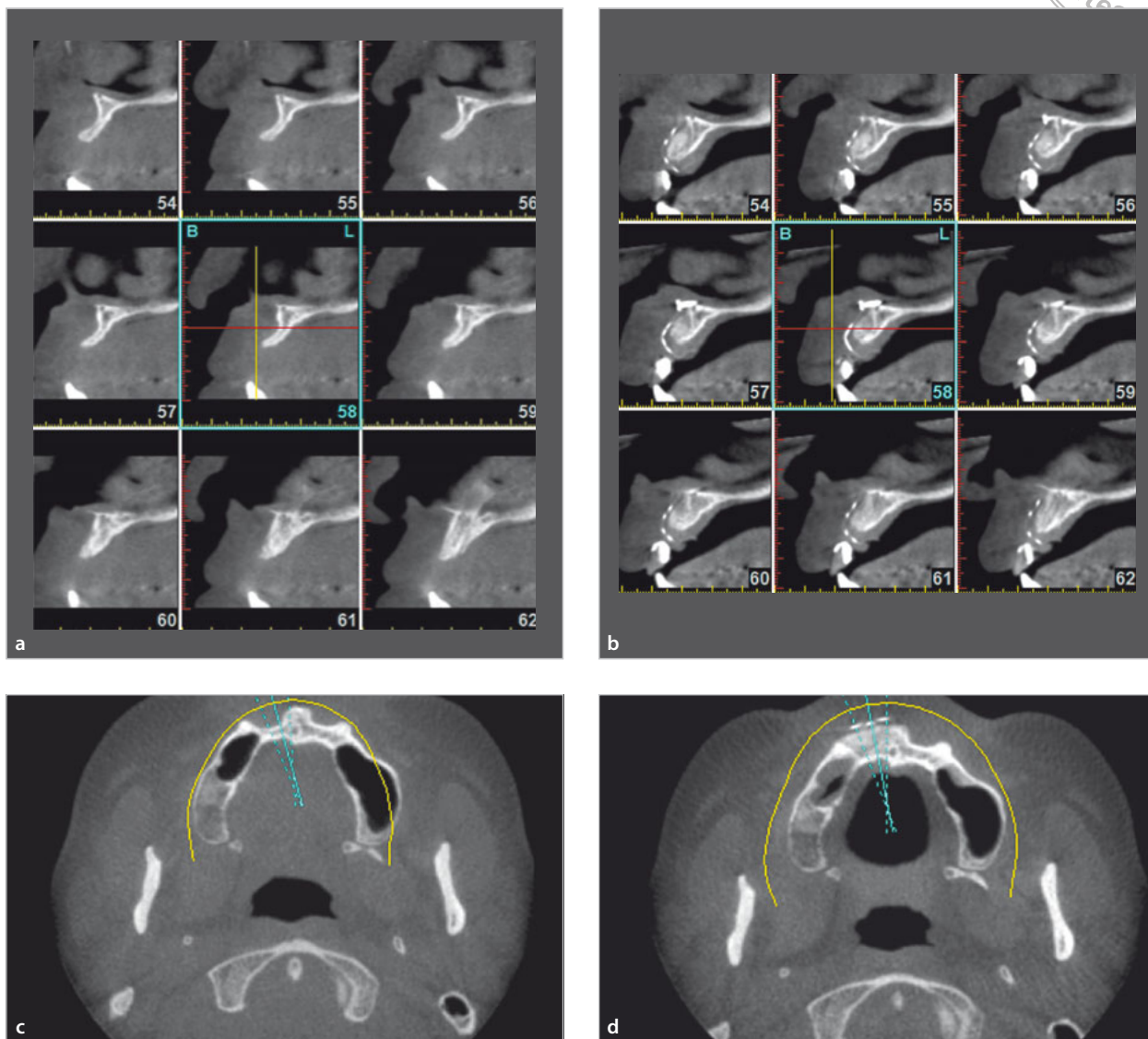
Fig 3e A titanium-reinforced dPTFE membrane was placed over the graft and secured using tacking screws.

stained with either hematoxylin-eosin (h&e) or 4',6-diamidino-2-phenylindole (DAPI) (Fig 6). Histologic examination of h&e-stained specimen showed new bone formation around the residual allograft particles. The newly formed bone tissues contained osteocytes and osteoblasts within their trabeculae. The inter-tra-

becular fibroconnective tissues contained adipocytes as well as inflammatory cells. The examination of DAPI-stained specimen revealed vital osteocytes in the newly formed bone surrounding the remnants of the allograft.

DISCUSSION

Evian et al¹³ conducted a time-dependent human histologic study on the healing of extraction socket. In their histologic evaluation, the greatest osteogenic potential was evident between week 4 and week 8 as demonstrated by proliferation of osteogenic cells and immature bone formation. Thus, in the presented case, as an attempt to harvest potentially osteogenic progenitor cells, the bony defect on the donor site (ie, mandibular ramus) was created 4 weeks prior to the planned GBR at the recipient site (ie, maxillary anterior edentulous



Figs 4a to 4d Comparison of pre- and postoperative CBCT. (a and b) Sagittal view: (a) preoperative; (b) postoperative. (c and d) Coronal view: (c) preoperative; (d) postoperative.

ridge). The tissue harvested from the created donor site, potentially containing osteogenic progenitor cells, was then mixed with CS-PRP as well as allograft. According to Intini et al,¹² exothermic reaction generated by the mixture of CS with PRP activates platelets contained within the PRP without the need for an agonist. The resulting activated platelets release multiple biologic factors including, but not limited to, PDGF and vascular endothelial growth factor (VEGF) in physiologically high concentrations.¹² In addition, it was found

that CS might carry and release these biologic factors slowly in a time-dependent manner.^{12,14} Therefore, CS-PRP serves as a biomaterial for GBR because it is able to sustain the nourishment of the bone defects over time via combinational delivery of calcium and platelets' multiple biologic factors.¹²

The primary effect of PDGF in bone is related to its mitogenic activity.¹⁵ PDGF achieves this by activating two structurally related cell surface receptor kinases (ie, alpha-PDGF receptor and beta-PDGF receptor), which

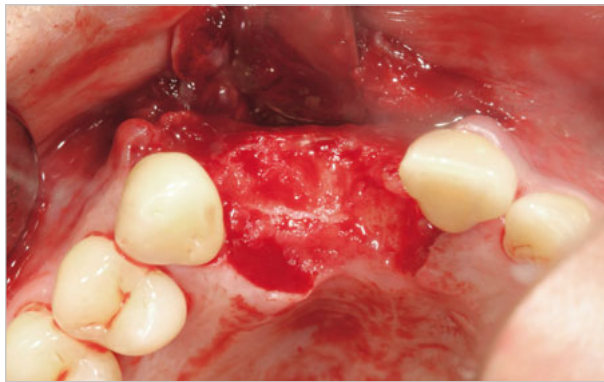


Fig 5a Occlusal view of the recipient site after 6 months of healing.

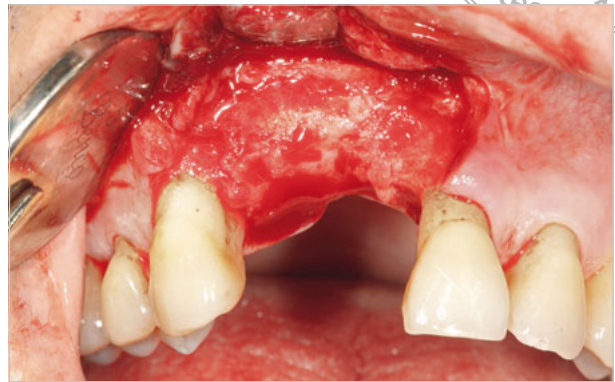


Fig 5b Buccal view of the recipient site after 6 months of healing.

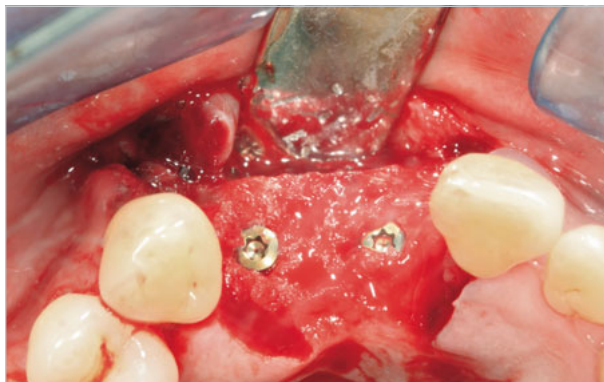


Fig 5c Occlusal view of inserted implant fixtures.

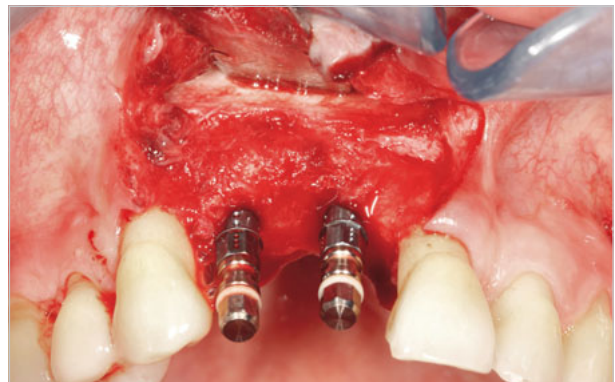
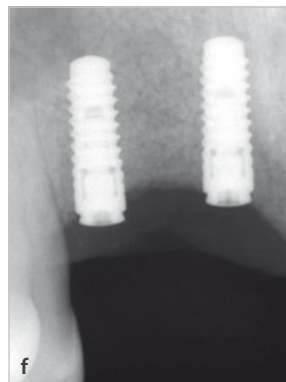
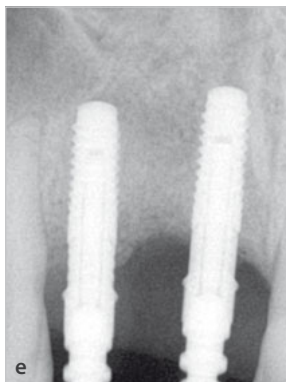


Fig 5d Buccal view of the inserted implant fixtures.



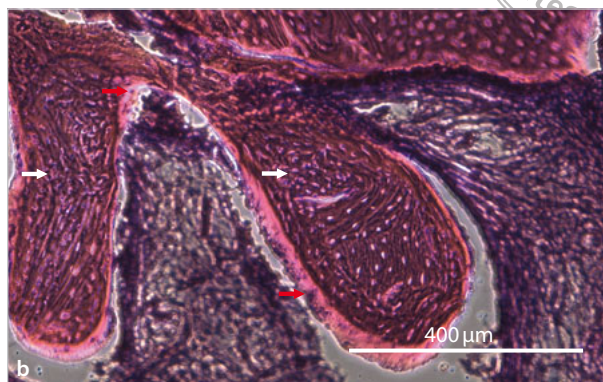
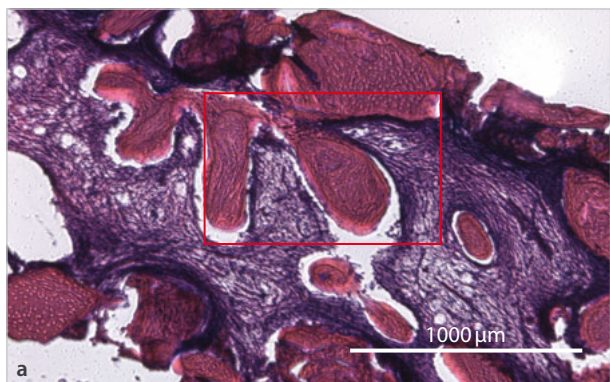
Figs 5e and 5f Periapical radiograph of the implant fixtures at their insertion (*e*) and at their 14-week follow-up (*f*).



Fig 5g The implants were restored with single-unit implant-supported crowns.

transduces mitogenic signals on their target cells.¹⁶ Target cells include connective tissue cells associated with bone healing as well as bone-derived mesenchymal stem cells.^{16,17} Therefore, the presence of platelet-released PDGF in CS-PRP may enhance the mitogenic proliferation of transplanted osteogenic

precursor cells. The presence of VEGF may also help the GBR as VEGF enhance the formation of new blood vessels (ie, neo-vascularization) at the grafted sites.¹⁵ The stimulatory effect of the activated human PRP on proliferation, migration, and differentiation of osteoblasts



Figs 6a and 6b Histologic examination of core biopsy revealed new bone formation with osteocytes (white arrow) and osteoblasts (red arrow); white bar (a), 1000 μm; white bar (b), 400 μm).

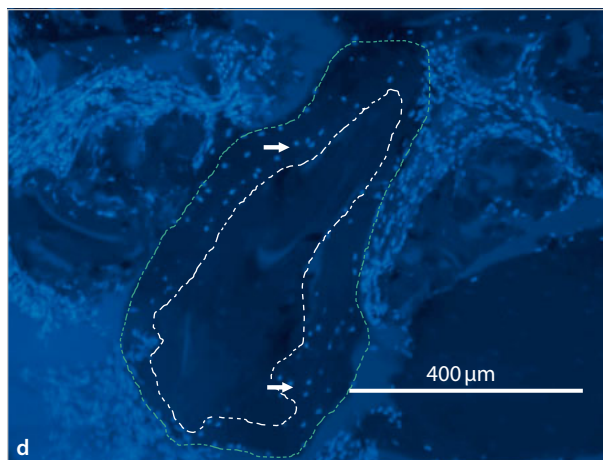
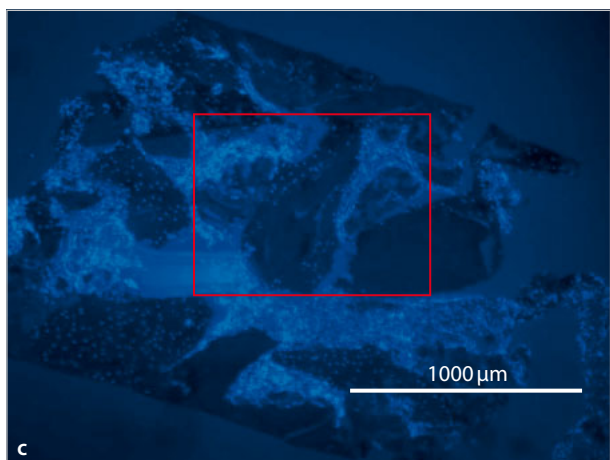
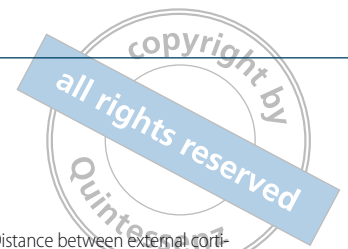


Fig 6c and 6d Histologic examination of core biopsy with DAPI stain showing the presence of vital cells (DAPI+, depicted by white arrows) surrounding an allograft particle (demarked by white dotted line). White dotted outline, acellular allograft residue; green dotted outline, newly formed cellular bone surrounding the residual graft; white bar (c), 1000 μm; white bar (d), 400 μm).

was demonstrated in the previous studies,¹⁸⁻²² which further support their use in GBR.¹⁵

It must be noted that the primary objective of using CS was to activate PRP, and serve as a device for releasing biologic factors slowly over the healing period. Furthermore, the objective of harvesting and transplanting the progenitor cells in the recipient site was to provide the additional source of cells with osteogenic potential, considering the severity of the recipient site being a critical size defect.⁹ It must be also noted that wound stability, space maintenance, and osteoconductive potential were primarily derived from FDFA and securely tacked titanium-reinforced dPTFE membrane.

Clinicians should also be aware of potential postoperative complications associated with preparing and obtaining the potentially osteogenic progenitor cells from mandibular ramus regions. These may include excessive pain, excessive swelling, postoperative bleeding, mandibular fracture, and inferior alveolar nerve damage.¹⁵ In this reported case, in order to minimize the aforementioned complications and patient morbidity, the horizontal and vertical relationship of the potential donor site to the inferior alveolar canal and other relevant anatomical structures was pre-determined using CBCT.²³



Successful insertion and restoration of endosseous implant fixtures was achieved; however, the long-term success of the inserted implants in the augmented area using this particular approach should be assessed with a longer follow-up and a larger subject size. For a comparison, according to a recent systematic review on the success of endosseous implants placed in the augmented alveolar ridge, the success rate of the implants ranged from 61.5% to 100% with at least of 6 months of loading.³ It is noteworthy that the lowest success rate of 61.5% was reported in the only randomized clinical trial study with the lowest risk of bias with a 3 year follow-up.³

Furthermore, future studies should be conducted to fully characterize the osteogenic progenitor cells obtained from the donor site, assessing their osteogenic potential.

CONCLUSION

The presented novel technique based on the combined use of CS-PRP, osteogenic progenitor cells, and particulate allogeneic bone grafts was successfully utilized to reconstruct a severely atrophic maxillary anterior ridge. Future studies should be performed to further validate this methodology.

ACKNOWLEDGMENT

The authors thank Dr Katarzyna Wilk for her assistance with histologic examination and analysis.

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