Peri-implant keratinized gingiva augmentation using xenogeneic collagen matrix and platelet-rich fibrin: A case report

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BACKGROUND
Keratinized gingival insufficiency is a disease attributed to long-term tooth loss, can severely jeopardises the long-term health of implants. A simple and effective augmentation surgery method should be urgently developed.

CASE SUMMARY
A healthy female patient, 45-year-old, requested implant restoration of the her left mandibular first molar and second molar. Before considering a stage II, as suggested from the probing depth measurements, the widths of the mesial, medial, and distal buccal keratinized gingiva of second molar (tooth #37) were measured and found to be 0.5 mm, 0.5 mm, and 0 mm, respectively. This suggested that the gingiva was insufficient to resist damage from bacterial and mechanical stimulation. Accordingly, modified apically repositioned flap (ARF) surgery combined with xenogeneic collagen matrix (XCM) and platelet-rich fibrin (PRF) was employed to increase the width of gingival tissue. After 1 mo of healing, the widths of mesial, medial, and distal buccal keratinized gingiva reached 4 mm, 4 mm, and 3 mm, respectively, and the thickness of the augmented mucosa was 4.5 mm. Subsequently, through the second-stage operation, the patient obtained an ideal soft tissue shape around the implant.

CONCLUSION
For cases with keratinized gingiva widths around implants less than 2mm, the soft tissue width and thickness could be increased by modified ARF surgery combined with XCM and PRF. Moreover, this surgery significantly alleviated patients’ pain and ameliorated oral functional comfort.

Key Words: Keratinized gingiva augmentation; Xenogeneic collagen matrix; Platelet-rich fibrin; Case report
A 43-year-old female patient requested implant restoration of the left mandibular first molar and second molar (teeth #36 and #37).

**History of present illness**

The two implants of teeth #36 and #37 underwent osseointegration for three months. Before stage II surgery, the widths of the mesial, medial, and distal buccal keratinized gingiva of the second molar (tooth #37) were 0.5 mm, 0.5 mm, and 0 mm, respectively (Figure 1B and C), as revealed by clinical observation.

**History of past illness**

During the medical history review, the patient denied having systematic diseases and a history of smoking.
Figure 1 Three months after implant surgery. A: The cone-beam computer tomography showed that good osseointegration had formed between the implant and bone; B and C: Clinical examination and periodontal probe measurement showed that the width of the buccal keratinized gingiva of tooth #37 was 0.5 mm, 0.5 mm, 0 mm from mesial to distal, respectively.

Personal and family history
The patient denied having personal and family history.

Physical examination
Before stage II surgery, the widths of the mesial, medial, and distal buccal keratinized gingiva of the second molar (tooth #37) were 0.5 mm, 0.5 mm, and 0 mm, respectively (Figure 1B and C), as revealed by clinical observation.

Laboratory examinations
No abnormality found in laboratory examination

Imaging examinations
Cone beam computed tomography showed good osseointegration around the implant, which suggested that the implant placement was successful (shown in Figure 1A).

FINAL DIAGNOSIS
Buccal keratinized gingiva insufficiency of tooth #37.

TREATMENT
To avoid inflammation, we planned to perform stage II surgery after obtaining sufficient keratinized gingiva. There were two surgical plans for the patient to choose, ARF + FGG surgery, and ARF + XCM + PRF surgery. The patient was informed of the procedure and risks, and she preferred the second method as she was afraid of pain and infection, and written informed consent for surgery was obtained.

Therefore, an ARF technique correlated with XCM and PRF was performed to increase the reduced keratinized tissue width and thickness, while patient morbidity was reduced by avoiding a second site. Before the surgery, the operative risk and complications were communicated with the patient, and the informed written consent was obtained from the patient for the operation and publishing of the case report. Next, the patient rinsed with mouth 0.12% chlorhexidine for three times. After local infiltration anesthesia by using articaine, a linear incision that deviated lingually was made, as showed Figure 2A. As it was impacted by buccal muco-gingival movement, the buccal full-thickness flap was split into a semi-thick flap with a No. 15 blade (Figure 2B), and the upper flap was positioned apically with 5-0 protein absorbable sutures by a vertical mattress (Figure 2C and D). The graft procedure involved the following two steps. First, PRF with multiple growth factors was obtained by centrifugating the patient’s blood at a specific speed (Figure 3A), and it was adapted to the area (Figure 3B). This is beneficial for promoting healing and increasing the thickness of keratinized gingiva. Second, the XCM membrane (Mucograft®, Geistlich, Switzerland, 15 mm × 20 mm) was trimmed (Figure 3C) and used to cover the wound
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Figure 2 Surgery Process. A: Deviated lingual linear incision; B: The buccal full-thickness flap was split to a semi-thick flap by a No. 15 blade; C and D: The semi-thick flap was positioned apically with 5-0 protein absorbable sutures.

Figure 3 Graft materials implantation. A: Platelet rich fibrin (PRF) was obtained by blood centrifugation from the patient; B: PRF was covered over the exposed wound; C: Mucograft® (Geistlich, Switzerland, 15 mm × 20 mm); D: Mucograft® was trimmed and added to cover the wound above the PRF by suture fixation.

above the PRF (Figure 3D), when it contacted with blood, thick loosened graft material can become thin and elastic, and it is good for suture fixation. No intentions and folds were made to exert external forces on the matrix in an attempt to cover the wound surface without disturbing its tridimensional structure.

OUTCOME AND FOLLOW-UP

Following the surgery, the patient was administered antibiotics (oral administration, amoxicillin 500 mg TID, metronidazole 300 mg TID) for 3 d to prevent bacterial infection. During the first 2 wk, the patient was informed not to brush the treated area, but rather to rinse the area with 0.12% chlorhexidine mouthwash twice a day.

Three days after the operation, we observed that the edge of the wound was slightly red and swollen but without infection, the surface of the wound was covered with a pseudomembrane, and the patient had no feelings of abnormality (Figure 4A). The sutures were removed after 10 days. The patient was checked at 5 d (Figure 4B), 10 d (Figure 4C), and 1 mo (Figure 5A) after the surgery.

Over time, the grafts tended to become absorbed, and the keratinized gingiva gradually grew along the Mucograft® surface until the wound closed.

After 4 wk, the wound was well-healed and the width and thickness of the keratinized gingiva reached 4 mm (Figure 5A) and 4.5 mm (Figure 5B), which was suitable for regular stage II surgery. Finally, the keratinized gingiva around the healing abutment was healthy, adequate and consistent with adjacent tissue (Figure 6A). As indicated by the periodontal probe measurement, the width of the buccal keratinized gingiva from mesial to distal reached 4 mm, 4 mm and 3 mm, respectively (Figure 6B-D). The patient was satisfied with the final esthetic outcomes.
Figure 4 Healing process. A: 3 d; B: 7 d; C: 10 d.

Figure 5 After 4 wk, stage II surgery was performed. A: The width of the buccal keratinized gingiva of tooth #37 is 4 mm; B: The thickness of mucosa is 4.5 mm.

Figure 6 The keratinized gingiva. A: The keratinized gingiva around the healing abutment was healthy, adequate and consistent with adjacent tissue; B: The mesial width of the buccal keratinized gingiva was 4 mm; C: The middle width of the buccal keratinized gingiva was 4 mm; D: The distal width of the buccal keratinized gingiva was 3 mm.

and the discomfort level was acceptable in terms of the pain, swelling, bleeding and chewing activity during the first healing period (Table 1).
DISCUSSION

There is junctional epithelium around the dental cervix and many sharp fibers between the cementum and alveolar bone, so nature teeth exhibit a stronger ability to defend against mechanical and bacterial stimulation. In contrast, dental implants are wrapped annularly by connective tissue, relying only on hemidesmosome connections [6]. Peri-implant gingiva is so easy to move, thereby causing peri-implantitis that is attributed to bacterial invasion[7]. From another perspective, the attached gingiva of healthy teeth is composed of keratinized gingiva. The epidermis layer of keratinized gingiva is stratified squamous epithelium, and the keratinized layer is full of keratinocytes. Epithelial nails were suggested to exist in the lamina propria. It is precisely because of this tissue structure that the mobility of the keratinized gingiva and nonkeratinized gingiva is different, and the former can better protect periodontal health[8,9].

With the extension of tooth missing time, keratinized gingiva tends to decrease, and the patients of this type should generally restore missing teeth along with keratinized gingiva augmentation. ARF is the earliest technique that has been applied to increase the keratinized gingiva around implants. The half-thick flap is opened through horizontal internal oblique incision and bilateral vertical incision, pushed apically and then sutured and fixed, so the exposed periosteal area can self-heal and form keratinized gingiva[10]. Basegmez et al[11] demonstrated that the application of ARF increased keratinized tissue by 1.15 mm at 1 year after the operation, although operation process was simple and time-saving, the postoperative tissue contraction was severe and the augmentation effect was unstable. To prevent tissue contraction, stability and curative effect predictability, clinicians attempted to combine ARF with free gingival graft (FGG) or connective tissue graft (CTG), and the research demonstrated that combined application could achieve more effective outcomes, although there are some serious shortcomings (e.g., limited autograft tissue, second operation area, risks of pain and infection, texture and color differences after the transplantation). Therefore, clinicians’ and patients’ choices should be limited to a certain extent. In the era of “patient-centered” medical treatment, while pursuing the results, the indicators of pain and satisfaction also need to be considered, therefore, clinicians are seeking an alternative to FGG or CTG[12]. Currently, acellular dermal matrix (ADM) and XCM are extensively accepted as soft tissue substitutes that are in the market. The ADM was originally applied to cover burn wounds and diabetic ulcer wounds, increase keratinized gingiva, deepen vestibular sulcus, cover dental root exposure, etc.[13]. However, as demonstrated from several clinical studies, some cases of recession occurred in the long term[14]. The other option is the XCM, a porcine absorbable XCM membrane, consisting of collagen type I and type III, a double-layer structure with one side as a porous layer for cell growth, early vascular discourse and tissue integration, and the other is a smooth and dense layer to facilitate cell adhesion and wound protection[15,16]. A randomized, controlled clinical trial by Cairo et al[17] showed that XCM and CTG obtained similar amounts of apical-coronal keratinized tissue after 6 mo, and XCM was correlated with shorter surgical time, lower postoperative morbidity, less anti-inflammatory tablet consumption and higher final patient satisfaction than those of CTG. At present, increasing the width of keratinized gingiva by ARF combined with XCM is still being explored. Biological graft substitutes are so expensive that autologous biological products can be employed to perform an economic treatment for patients, and PRF, the second generation platelet concentrate reported by Dohan et al[18] is one of the representatives, covering abundant autologous growth factors that facilitate cell proliferation and migration. Its three-dimensional (3D) fibrin network is close to the physiological state, which can promote neovascularization, and wound healing and accelerate tissue remodeling[19].

The principle of increasing keratinized gingiva of XCM refers to guiding the growth of keratinized tissue cells and fibroblasts from the edge to center by exploiting its unique 3D scaffold[20]. Therefore, the incision design should maximize the reservation of keratinized tissue, which contributes to keratinized tissue cell migration from the edge of the incision. In the case of this study, because of the severe atrophy of the buccal keratinized gingiva, the incision was slightly inclined to the lingual side, which
means that certain keratinized tissue was reserved on both sides of the incision. Moreover, we did not use a vertical incision, just a simple oblique incision to maintain the blood supply. Most of the blood vessels in the gingiva are parallel to the gingival margin from back to front[21]. This is a modified ARF as a reference[22]. As a result of long-term edentulous, the alveolar ridge atrophied, the vestibular sulcus became shallow, and the positions of the frenulum and muscle varied and were higher, thereby increasing the difficulty of the operation. In addition, the vertical width of XCM implantation was limited. Thus, the muscle attachment was partially relaxed during the operation, and then the semi-thick flap was fixed to the root with protein thread to stabilize the implantation area of XCM. Furthermore, three PRFs were added under the Mucograft® to promote tissue healing, increase the thickness of keratinized gingiva, and lay the foundation for the later cuff depth[23]. The final Mucograft® exhibited open healing, thereby promoting the keratinized cells on both sides of the incision to migrate to the center and proliferate. The increased keratinized gingiva was consistent with the surrounding tissues. The average width of buccal keratinized gingiva was nearly 4 mm, and the patient satisfaction also reached 8 points on average (Table 1).

CONCLUSION
A modified ARF combined with XCM and PRF, as an alternative to FGG, was adopted to increase the keratinized gingiva in the posterior area in the mandible, and the outcomes were satisfactory. The width of keratinized tissue increased from 0.5 mm to 4 mm. It was demonstrated that this method could have a certain curative effect. For some cases meeting the indications, this method could be selected for soft tissue augmentation. Moreover, subsequent exploration will be conducted with a longer tracking time and more case summaries.

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