in vivo *34*: 869-875 (2020)
doi:10.21873/invivo.11851

CO_2 Laser De-epithelization Technique for Subepithelial Connective Tissue Graft: A Study of 21 Recessions

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Abstract. *Background/Aim: To report cases in which we achieved sufficient width of the keratinized gingiva using a coronally advanced flap in combination with a subepithelial connective tissue graft (SCTG) obtained by the 'CO_2 laser de-epithelization technique' (CODE). Patients and Methods: Eleven patients with 21 Miller Class I, II, and III gingival recessions had surgery. To prepare SCTG, free gingival grafts were harvested and de-epithelialized extra-orally. De-epithelialization was conducted by irradiation of CO_2 laser. Postoperative examinations were performed at 12 months. Results: At 12 months, statistically highly significant root coverage was achieved in all recessions. Complete root coverage was obtained in 7 of the 21 recessions. The treatment yielded mean root coverage of 41.0%, and was associated with a mean gain of keratinized gingiva of 2.9±0.3 mm. Conclusion: The use of CODE allows harvesting grafts of excellent quality and quantity and increases the keratinization of the overlying mucosal epithelium.*

Gingival recession is a highly frequent global disorder and a long-recognized condition that can be defined as apical displacement of the gingival margin from the cement enamel junction (CEJ) that exposes the root surface to the oral environment (1). The primary concerns regarding the presence of gingival recession include esthetic/cosmetic demands, root sensitivity, and root caries. Therefore, surgical treatments of gingival recession are extremely valuable for patients to reduce these complications and to restore good oral hygiene. To treat gingival recession, clinicians need to apply suitable surgical techniques including subepithelial connective tissue grafts (SCTG), coronally advanced flaps (CAF), free gingival grafts (FGG), *etc*. The best option for the care of gingival recession has remained controversial over the years; recent network meta-analysis revealed that the CAF+SCTG combination ranked highest in the effectiveness of reducing recession and providing clinical improvement among treatments of Miller Class I and II gingival recessions (2). The authors concluded that this form of therapy could be considered the gold standard in root coverage procedures.

Root coverage and esthetic evaluation should be the primary outcomes of surgical interventions for gingival recession. In addition, a sufficient increase of keratinized gingiva (KG) could also be a matter of concern for both clinicians and patients. The presence of an adequate amount of KG has been considered a keystone for the maintenance of periodontal health. KG plays an important role in protecting the periodontium from mechanical trauma induced by toothbrushing and in facilitating plaque control, therefore contributing to the stabilization of the gingival margin at the level of the CEJ. In an observational study, Lang and Loe have reported that most surfaces with 2.0 mm or more of KG were clinically healthy (3).

FGG is known to be the most effective surgical technique to obtain enough quantity of augmented gingiva at sites with a minimal amount of KG. A retrospective observational study has reported that KG increased 4.2 mm one year after conducting FGG (4). However, scar tissue formation and differences in color and texture of the grafted site compared to its surrounding tissue limit the use of FGG for root

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Key Words: Gum disease, gingival recession, subepithelial connective tissue graft, CO_2 laser, de-epithelization.

Figure 1. *Steps of FGG de-epithelialization. (A). Laser vasopolarization by CO_2 laser (Opelaser Pro, Yoshida Dental Mfg., Tokyo, Japan). (B) Remnants of epithelium were removed by a 15 blade. (C) SCTG was prepared to transplant.*

coverage. Unlike FGG, SCTG should be fully or partially covered with a pedicle flap. In most clinical situations, the pedicle flaps covering SCTG are composed mostly or entirely of non-keratinized mucosa. Concerns have been raised with respect to the nature of the epithelium surface of the grafted area after healing. Several studies have reported a KG-increasing effect of SCTG in combination with CAF. A randomized controlled trial (RCT) in 2004 compared the effect of a combination of CAF+SCTG to CAF alone and reported additional KG increases of 0.77 mm one year of observation (5). In two other RCTs, an additional KG increase of 0.41 mm in six months (6) and 2.2 mm in one year (7) has been reported. Although these measurements are significant in the comparison of the two groups, they are noticeably less so than the change of FGG.

Cordioli *et al*. have compared the mucogingival changes following treatment with SCTG with and without coronal positioning of the flap in a retrospective study (8). They showed that the KG increase was significantly greater in sites treated with the envelope technique. Although the root coverage outcomes in this observational study showed comparable results for the two techniques, there are still some limitations for the usage of the envelope technique. Since grafted connective tissues covered with broader pedicle flap have an increased chance of survival together with better blood supply, exposure of the grafted tissue could be a risk factor for low predictability of the envelope technique.

Of particular interest is the development of surgical techniques achieving sufficient reduction of recession, esthetic satisfaction, and increase of KG. Here, we report cases in which we achieved sufficient width of KG by "CO_2 laser de-epithelization technique" (CODE) in combination with the CAF technique. We discuss the advantage of CODE with histological evidence.

Patients and Methods

Case selection. All investigations and treatments were established clinical practices and were carried out according to accepted clinical practice and in compliance with medical principals of the Declaration of Helsinki. The Institutional Review Board of Nihon University School of Dentistry (Tokyo, Japan) approved the procedures, and all patients provided written informed consent for the treatment and the publication of the findings. A total of 21 Miller Class I, II, and III gingival recessions in 11 patients were treated by one periodontist (H.Y.) at a private clinic. All patients were non-smoker adults in good health with a full-mouth plaque score <15%, and bleeding on probing score <20% (9). Before surgery, customized oral hygiene instructions were prescribed to all patients.

Surgical procedure. Patients received systemic antibiotics (Cefditoren Pivoxil, Meiact; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) 1 hour before surgery. After local anaesthesia, root coverage procedure was performed by the combination surgery of CAF and SCTG. The technique of CAF was chosen from the options of modified CAF technique (without vertical incision) (10) and modified tunneling technique (11), considering the number of teeth and classes of gingival recession. In all the techniques, the full or partial thickness flap was extended beyond the muco-gingival junction, so that the flap could be moved coronally without any tension. Before grafting procedures, the exposed root surface was planned with Gracey curettes (Hu-Friedy, Chicago, IL, USA). For the preparation of SCTG, FGG was harvested from donor sites and de-epithelialized extra-orally. Hard palate mucosa and maxillary tuberosity were candidates as a donor site of FGG, and the choice was made depending on donor site condition and the size of graft needed. Extra-oral de-epithelialization of harvested FGG was conducted by irradiation with CO_2 laser (Opelaser Pro, Yoshida Dental Mfg., Tokyo, Japan) (Figure 1A). The CO_2 laser system has a wave-length of 10.6 μm, and was set at a power of 1.0 W and continuous mode. After laser vaporization of entire surface, remnants of epithelium were removed with a 15 blade (Figure1B and C). Grafts were positioned and adapted to the prepared vascular

Table I. *Descriptive analysis of study subjects at baseline.*

Parameter	
Age (years; mean±sd)	45.2±12.6
Gender (n)	4 males (58.3%)
	7 females (41.7%)
Type of tooth (n, %)	7 incisors (33.3%)
	7 canines (33.3%)
	4 premolars (19.0%)
	3 molars (14.3%)
Miller class (n, %)	3 Class I (58.3%)
	2 Class II (41.7%)
	16 Class III (41.7%)

Table II. *Distributions of donor sites and root cover techniques.*

Sites of harvesting graft	Palate	20 (95.2%)
	Maxillary tuberosity	1 (4.8%)
Techniques used for recipient site	Modified tunneling technique	6 (28.6%)
	Coronally advance	15 (71.4%)

Table III. *Changes in clinical indices between baseline and 1-year follow-up.*

Parameter	Baseline	1-year	Change	*p*-Value
KT (mm; mean±sd)	2.2±1.5	5.1±1.8	2.9±0.3	<0.001
RD (mm; mean±sd)	3.2±1.5	1.35±1.3	1.85±0.2	<0.001

KG: Width of keratinized tissue; RD: recession depth.

Table IV. *Mean root coverage value and frequency of complete root coverage at 1-year follow-up examination.*

Parameter	1-year
MRC	41.0%
CRC	7 (36.3%)

MRC: Mean root coverage; CRC: complete root coverage.

bed then stabilized with sutures on the mesial and distal aspects. Subsequently, the CTG was covered by the CAF and secured with a sling suture that engaged the periosteum apical to the graft, which was used to stabilize the donor. The graft and flap were sutured using 5-0 nylon sutures (Kono Seisakusho Co. Ltd., Chiba, Japan).

Post-surgically, all patients were given antibiotics (Cefditoren Pivoxil, Meiact; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) for 3 days, and two tablets of 25 mg diclofenac (Voltaren®, Novartis Pharma KK, Tokyo, Japan) to be taken when they had pain. Patients were informed not to brush their teeth in the operated areas until suture removal at 14 days post-surgery. During the 14- to 28-day post-surgery period, they brushed their teeth with a soft brush. All patients were recalled at day 1, 3, 7, 14, and 28 post-surgery. At each visit, a clinical check-up was performed and patients received professional prophylaxis procedure, including supragingival debridement and tooth polishing, as well as reinforcement of self-performed oral hygiene procedures.

Pre- and post-operative measurement. The following clinical measurements were carried out before the surgery and at the 1-year follow-up session: width of keratinized gingiva (KG) at the treated site, measured from the most apical extension of the gingival margin to the mucogingival line; and recession depth (RD), measured from the CEJ to the most apical extension of the gingival margin. Mean root coverage (MRC) and complete root coverage (CRC) were also measured at this follow-up visit. The entire analysis was carried out by the same examiner at base line and 1 year follow up session.

Results

Demographic characteristics of the subject population at baseline are presented in Table I. In total, 11 patients (7 women and 4 men, mean age 45.2±12.6 years) were consecutively treated with CAF+SCTG. Of these, 7 teeth were incisors, 7 were canines, 4 were premolars, and 3 were molars. Distributions of donor sites and root cover techniques are shown in Table II. More than half of recessions were classified as Miller Class III. In all eleven patients, healing was uneventful and no postoperative complications such as bleeding, pus or abscess formation, flap dehiscence, or loss of the graft occurred. At 12 months, RD was statistically significantly reduced from baseline (Table III). Complete root coverage was obtained in 7 of the 21 recessions (Table IV). The treatment yielded an MRC of 41.0% and was associated with a mean gain of KT of 2.9±0.3 mm ($p<0.05$). Details of two representative cases are shown in Figures 2 and 3.

Discussion

Many techniques for harvesting connective tissues have been proposed and applied so far. Among them, the trapdoor and single-incision techniques are currently widely utilized. In these conventional techniques, the length and thickness of connective tissue is determined by the morphology of the patient's palatal anatomical structures. In many clinical situations, adequate connective tissues cannot be obtained, and there is some risk of samples containing adipose and glandular tissues, which impair root coverage.

Connective tissue obtained by de-epithelialization of the FGG is also utilized nowadays. In this technique, a 15 blade is used for de-epithelialization after harvesting FGG from the patient's palate. An RCT in 2010 compared root coverage outcomes of CTG obtained by the trapdoor approach with CTG obtained by de-epithelization of FGG (12). There were

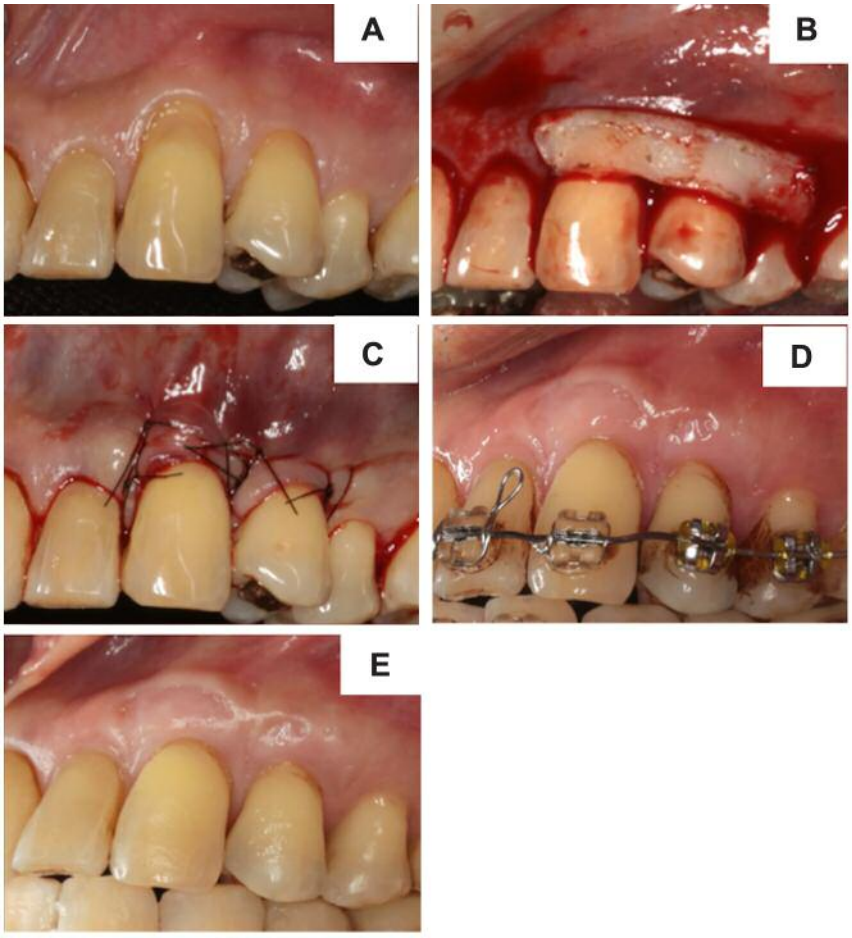

Figure 2. *A case of 69-year old male patient. (A) Miller classIrecession affecting tooth 23 and 24. (B) Modified tunneling technique was conducted prior to orthodontic treatment. Full thickness flap was extended beyond the muco-gingival junction. FGG was harvested from hard palate mucosa, and de-epithelialized with CO_2 laser. Obtained SCTG was positioned into the tunnel. (C) SCTG was sutured to the recipient site and the flap. Furthermore, sling sutures were performed with anchor teeth 33 and 34. (D) One-year post-op: Width of KG was increased. (E) Two-year post-op: Increased KG was retained.*

no significant differences in root coverage or KG increase between these two types of CTG harvesting techniques. The authors of this RCT claimed that the de-epithelialization technique enables clinicians to harvest CTG of adequate quality and quantity quickly even in patients who have thin palatal fibro-mucosa. Bertl *et al.* have examined the composition of fibrous connective and fatty/glandular tissue in CTG using harvesting techniques in fresh human cadavers (13). They concluded de-epithelialization harvested CTG contains much higher amounts of connective tissue and much lower amounts of fatty/glandular tissue than CTG harvested with split-flap preparation. Lin *et al.* have introduced a de-epithelialization technique using an Er,Cr:YSGG laser in their case series report (14). The authors insisted that it is easier to remove epithelium completely from FGG by using an Er,Cr:YSGG laser than by using a 15 blade. Although the complete removal of epithelialization is clear in their histological evidence, their technique invades not only the epithelium but also the lamina propria underlying the epithelium.

In the present study, we used CODE as a grafting procedure. We also conducted histological assessments of CODE. Specimens were collected from the remnants of connective tissue harvested for clinical usage. CTGs were irradiated with a CO_2 laser at three different intensities: 2.0 W, 1.0 W and 0.5 W. Specimens were stained with hematoxylin and eosin, and examined under a microscope (Figures 4-6). The entire epithelium and the outer surface of the lamina propria were removed in the specimen treated with the 2.0 W intensity (Figure 4), and more than half of the epithelium was left in the specimen treated with the 0.5 W setting (Figure 6). In the specimen treated with 1.0 W

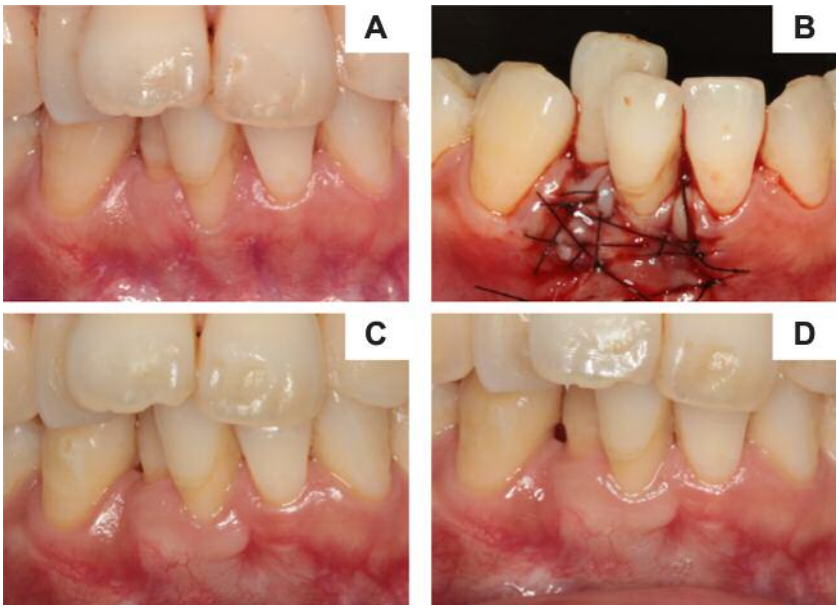

Figure 3. *A case of 37-year old female patient. (A) Miller class III recession affecting tooth 41. (B) Modified CAF technique was conducted prior to orthodontic treatment. Partial thickness flap was extended beyond the muco-gingival junction. FGG was harvested from the retromolar area of mandible, and de-epithelialized with CO_2 laser. Obtained SCTG was positioned covering exposed root surface. SCTG was sutured horizontally to the recipient site together with the flap. Several sutures were added on the mesial and distal side of the graft. Furthermore, sling sutures were performed with anchor teeth 42. (C) One-year post-op: Width of KG was increased. (D) Two-year post-op: Increased KG was retained.*

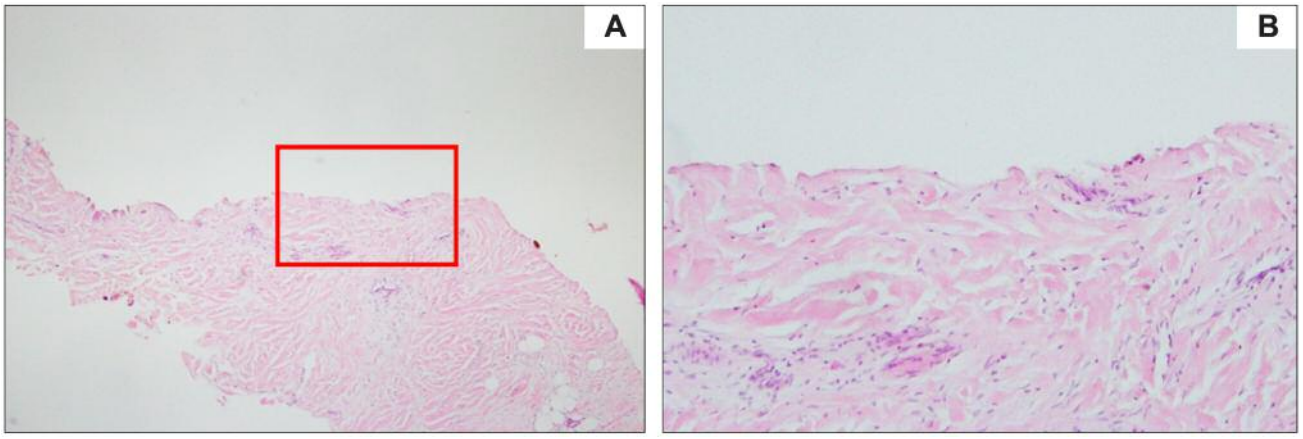

Figure 4. *A human palatal tissue specimen treated with 2.0 W CO_2 laser radiation. (A) The entire epithelium and the outer surface of the lamina propria were removed in the specimen, (B) Higher magnification.*

intensity, only the epithelium was removed completely, keeping the morphology of the rete ridge unchanged, and keeping the papillary layer of the lamina propria uninvaded (Figure 5). From this histological evidence, we decided to use CO_2 laser irradiation with 1.0 W intensity for CODE.

In the cases presented in this report, increases of KG were achieved by using the CODE technique with 1.0 W CO_2 laser irradiation. Since the mechanisms regulating keratinization of the gingiva still remain unclear, it is difficult to biologically explain our clinical results. It is well known that the basal lamina located between the epithelium and lamina propria is a critical regulator of keratinization in the field of dermatology (15, 16). Recently, the keratinized mucosa specific components were identified in the basal lamina of oral mucosal tissue (17). Our histology showed that the outer surfaces of the lamina

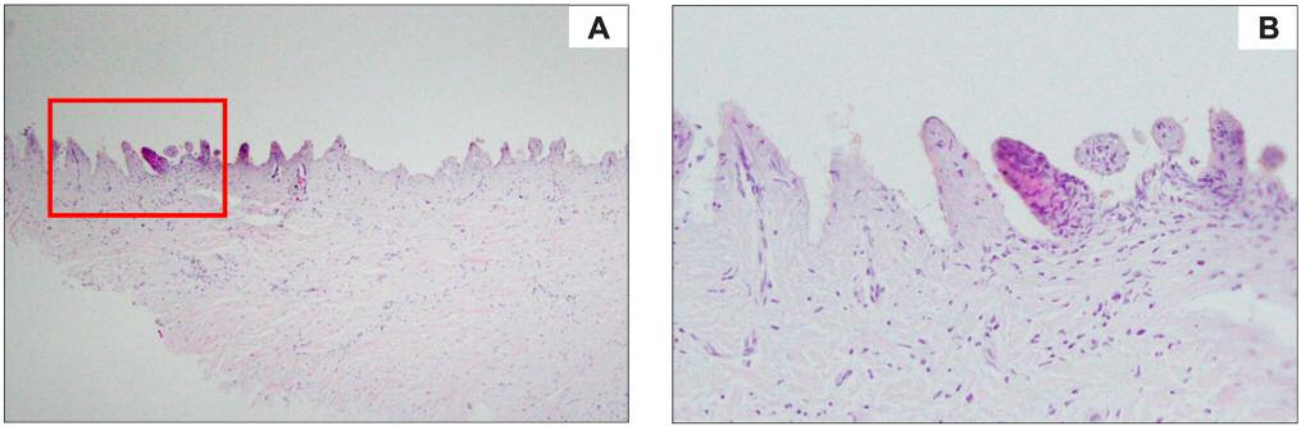

Figure 5. *A human palatal tissue specimen treated with 1.0 W CO_2 laser radiation. (A) Only the epithelium was removed completely, keeping the morphology of the rete ridge unchanged. (B) Higher magnification.*

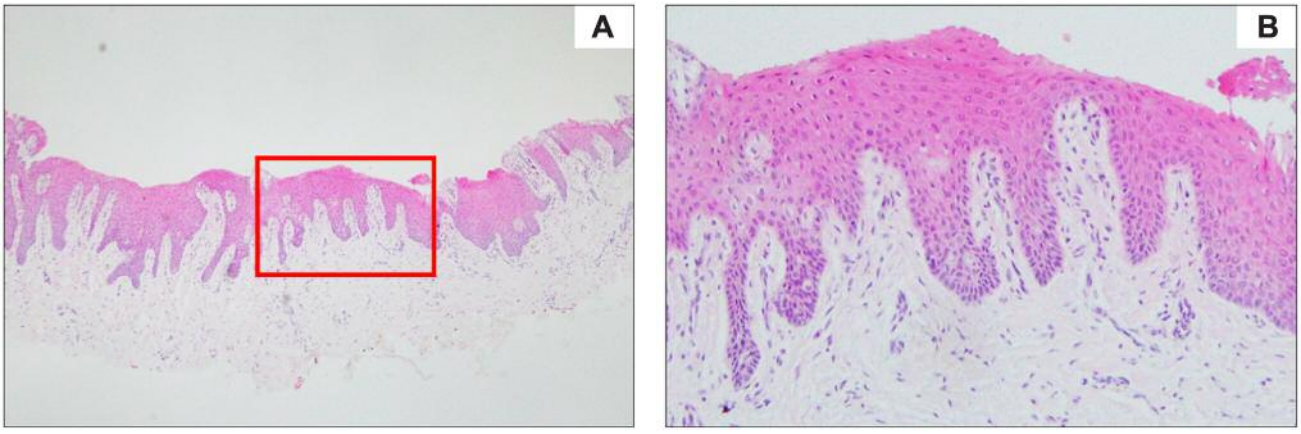

Figure 6. *A human palatal tissue specimen treated with 0.5 W CO_2 laser radiation. (A) More than half of the epithelium was left in the specimen. (B) Higher magnification.*

propria remained intact even after the de-epithelialization process, and that some retention of the basal lamina is possible. We can hypothesize that a specific irradiation setting of CO_2 laser removes only epithelial tissue outside the basal lamina while keeping both the basal lamina and lamina propria healthy.

Because this is only a presentation of a case study, further studies with more cases and with a control group for comparison are needed to confirm the outcome of CODE. In view of this, we would state in summary that *in vitro* and *in vivo* experiments are needed to elucidate the biological mechanism of our results.

Within the limitation of this case series report, the CODE technique allows for predictable harvesting of SCTG of excellent quality and quantity, and increases the keratinization of the overlying mucosal epithelium. In this respect, it meets our aim of establishing the merits of this novel technique and should provide a new more effective and safer mode of therapy for the management of gingival recession.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

All Authors made substantial contributions to the present study. HY, NS and SS contributed to conception and design. HY, DS, TK, HN contributed to acquisition of data, and analysis. HY and AH contributed to interpretation, and moreover, involved in writing and editing the manuscript. SS, NS and DS revised the manuscript before submission. All Authors read and approved the final manuscript.

Acknowledgements

The Authors thank all clinicians and researchers in Kawaguchi Institute of Periodontology and Implantology for help with development of the surgical technique. This study was financially supported by Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan (2017; A.H.).

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Received December 26, 2019
Revised January 6, 2020
Accepted January 7, 2020