Comparison of soft tissue healing around implants in beagle dogs: flap surgery versus flapless surgery

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Objective. The objective of this study was to compare soft tissue healing after implant placement in flap and flapless surgery in the dog model.
Study Design. Mandibular premolars were extracted from 10 beagle dogs. The extraction sockets were allowed to heal for 8 weeks. After healing, 3 implants on each side of the mandible were implanted using either flap or flapless techniques. One implant was installed on each side at the 0-, 4-, and 6-week time point. Eight weeks later, the peri-implant soft tissue healing was subjected to clinical and immunohistochemical analysis.
Results. It was revealed that vascular endothelial growth factor (VEGF) expression, peri-implant crevicular fluid (PICF) volume, and the aspartate aminotransferase and alkaline phosphatase activity in PICF increased significantly in the 2-week flap group compared with the 2-week flapless group. Microvascular density and VEGF expression in the 8-week flap group was statistically significantly lower than the 8-week flapless group and normal group. Buccal gingival recession was less pronounced in the flapless group than in the flap group after 4 and 8 weeks.
Conclusions. Within the limits of this study, the results demonstrate that flapless surgery contributes to better esthetic outcomes in implants compared with the flap approach. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:e21-e27)

Dental implants have become a routine and effective treatment for the replacement of lost teeth. With increasing implant survival rates, more attention has been paid to optical soft tissue esthetics and early tissue healing. Flapless surgery was first introduced by Kan et al. in 2000.[1] Becker et al.[2] showed, in a dog experimental model, that implants placed without flap reflection remained stable and exhibited clinically relevant osseointegration similar to that observed when implants were placed with flapped procedures. In recent years, a number of reports have demonstrated that flapless surgery has numerous advantages over traditional flap surgery.[3-5] Even though flapless surgery is a blind procedure, with the help of 3-dimensional imaging techniques and computer-guided implant planning, implants can be placed more accurately than in the past.[6,7]

Several reports have described the clinical outcomes of flapless implant surgery[8]; however, information regarding the soft tissue conditions after flapless implant surgery is limited. In a study by Jeong et al.,[9] the condition of the soft tissue surrounding the dental implants 1 year after flapless implant surgery was evaluated. This involved measuring the probing pocket depth, assessing the gingival index, and recording the presence of bleeding on probing. Excellent peri-implant mucosal health was observed. Mueller et al.[10] found that flapless surgery minimized postoperative inflammation and promoted reepithelialization and neovascularization of soft tissue by leukocyte count and pangenomic gene expression analysis of peri-implant mucosa 1, 2, 4, and 12 weeks after surgery in minipigs. Kim et al.[11] found that the peri-implant mucosa was more richly vascularized in a flapless group than in a flap group after a healing period of 3 months.

In pursuit of perfect esthetic finish in implants and restorations, it is important to create healthy and esthetic peri-implant soft tissue. Ideal soft tissue healing cannot be achieved without adequate blood supply and control of infection; However, there are few data on soft tissue early healing after flapless implant surgery. The purpose of this study was to perform a comparative evaluation of flap and flapless surgery in a dog model to assess early healing of the soft tissue around implants in terms of inflammatory response, microcirculation, and gingival recession.

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MATERIAL AND METHODS

Animal model and study design

Ten female beagle dogs (aged 12 months; weight 12 to 15 kg) were obtained from the Medical Laboratory Animal Center in Fuzhou General Hospital of Nanjing Military Region. The protocol was in accordance with the animal care and use policies of the Ministry of Science and Technology of the People's Republic of China and approved by the Animal care and Use Committee of Fujian Medical University.

All of the mandibular premolars of the 10 female beagle dogs were extracted. The extraction sockets were allowed to heal for 8 weeks (time 0). In total, 60 commercially pure titanium screw implants (3.3 mm in diameter, 8 mm in length, and 1.8 mm in smooth neck height; Nonferrous Metal Research Institute, Baoji, Northwest China) were placed in the 10 dogs. After healing, 3 implants were randomly assigned to each side of the mandible for either flap or flapless procedures. One implant was installed on each side at 0, 4, and 6 weeks from time 0. Eight weeks from time 0, and at various time points (8, 4, and 2 weeks, respectively) from the time of the implant, the healing of the peri-implant soft tissue was evaluated by clinical and immunohistochemical analysis. Results are discussed in terms of the number of weeks of soft tissue healing after the insertion of implants by flap or flapless surgery (8-week flap group; 8-week flapless group; 4-week flap group; 4-week flapless group; 2-week flap group; 2-week flapless group).

Surgical procedure

The operations were performed under general anesthesia: (1) flapless surgery: implantation took place after the soft tissue of the proposed implant site was punched with a 2.5-mm soft tissue punch; (2) flap surgery: implantation took place after midcrestal incision and full-thickness flap elevation. Implant insertion sites were clearly defined. The distance between the adjacent implants was more than 5 mm. Incisions were made as far as possible from the adjacent implant. Implant osteotomy and placement were performed so that the marginal level of the implant shoulder was flush with the buccal mucosa margin. All implants were inserted to obtain a torque of 35 N/cm with primary stability. Healing abutments were connected to the implant. Full-thickness flaps were secured with silk 3-0 interrupted sutures. Sutures were removed 7 days later. To prevent infection, the dogs were administered penicillin (800,000 IU, twice daily) for 3 days after the surgery and fed a soft diet. They were placed on a plaque-control regimen that included local rinsing with 2% chlorhexidine solution twice a day and tooth and implant cleaning once a day with toothbrushes and dentifrice.

Clinical monitoring and enzymatic activity analysis

Clinical status of peri-implant soft tissues were evaluated 8 weeks after first implantation by measuring gingival recession, the volume of peri-implant crevicular fluid (PICF) and the activity levels of the aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in that PICF.

PICF samples were obtained from the mesial, distal, buccal, and palatal aspects of each implant. The PICF collection sites were isolated with cotton rolls and air-dried. Any supragingival deposits present were carefully removed; #1 sterile paper strips (2×8 mm, Whatman International Ltd., Maidstone, England) were gently inserted into the sulcus until slight resistance was detected and left in situ for 60 seconds. They were then placed in Eppendorf tubes. The weight of PICF was measured by subtracting the weight of the strips and Eppendorf tubes after collection from that before collection. This value was converted into microliters on the assumption that PICF has a density value of 1. The Eppendorf tubes were immediately frozen at $-70°C$ until use. To extract the PICF from the periopaper strips, 80 μL Tris-HCl containing 0.25% bovine serum albumin (pH 8.0) was added to each tube. Tubes were shaken at room temperature for 1 hour. Then, the strips were removed and the elute was centrifuged at 3000 cycles per minute for 10 minutes. The supernatants were measured in an automated analyzer (Hitachi 717, Hitachi, Tokyo, Japan) to evaluate the levels of AST and ALP in PICF. The reported PICF volumes and cytokine levels were the average of 4 samples (mesial, distal, buccal, palatal) collected at each implant. AST and ALP levels were expressed as U/L in PICF. The dogs were then killed and their mandibles were retrieved via sharp dissection and fixed with 4% paraformaldehyde for 1 week.

Gingival recession was calculated by subtracting the height of healing abutment from measurement of the distance from the top of the healing abutment to the lowest point of buccal gingival margin with a sliding caliper.

Preparation of specimens and immunohistochemical analysis

Buccal full-thickness mucosa samples were excised and processed through a series of alcohol and xylene before paraffin embedding. The samples obtained from the first molar served as normal controls. Serial 4-μm-thick sections were used for immunohistochemical staining