Peri-Implant Soft Tissue Management through Use of Cultured Gingival Graft: A Case Report

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Abstract- Tissue-engineered gingival graft was used for regenerating facial gingiva around an implant at lower left first premolar area with insufficient attached gingiva. Engineered gingival graft was produced by mixing 250 ml bovine skin collagen with 250 ml nutritional medium containing human gingival fibroblasts (2×10^5). 3 months post-surgery, there were gains in the attached gingiva compared to pre-surgery. The histological examination revealed a keratinized tissue on the treated site. Based on the result of this investigation, this graft was safe and capable of generating keratinized gingiva.

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Introduction

It is important for patients to have keratinized tissue surrounding dental implants to successfully manage home hygiene care (1). Many reports in the literature have suggested that attached gingiva prevents peri-implant gingival hyperplasia, inflammation, pocket formation and pain associated with mechanical trauma (2). Mobile crestal mucosal tissue is subject to injury-induced pathosis by distortion from the functional pull of the lip and floor of the mouth and by physiologically or iatrogenically exposed implant threads (3,4).

Soft tissue augmentation procedures have been routinely performed using the patient's masticatory mucosa (palate) as donor material (5). However, for mucosal grafts there are the disadvantages of limitation in the size of a donor site, postoperative pain, increased morbidity, compromised aesthetic outcome, formation of exostosis and increased procedure time if a surgical complication arises such as rupturing a palatal blood vessel (6,7).

Acellular dermal matrix allograft (AlloDerm^{TM}) has been used in periodontal, plastic and reconstructive surgery since 1994. AlloDerm^{TM} may be used for soft tissue augmentation procedures around dental implants without using the patient's own palate to procedure the donor tissue (8).

Recently, there has been a substantial and growing public and scientific awareness of a relatively new field of applied biological research called tissue engineering. This field builds on the interface between materials science and biocompatibility, and integrates cells, natural or synthetic scaffolds, and specific signals to create new tissues (9). Based on this knowledge, tissue engineering technology may also be applied in implant dentistry.

A few researches have discussed the clinical use of cultured mucosal grafting for intraoral mucosal defects (10-15). Cultured epithelium fabricated with living mucosal cells has proved to be a good grafting material for any kind of mucosal defect such as peri-implant soft tissue deficiency (15). Cultivated fibroblasts onto scaffold also have been used successfully for gingival augmentation (10-12). An obvious advantage of using tissue-engineered cell sheets is that very small tissue sample from a donor site can serve as source material to cover a large recipient site (10-15).

In this case report, a patient who needed peri-implant soft tissue augmentation was treated by means of a tissue engineering technique.

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Case Report

A 40-year-old man who was referred to Shaheed Beheshti University Dental School, Iran, with a negative medical history and an absence of periodontal diseases agreed to participate in the study. This patient was informed of the purpose of the study and was required to give informed consent. The study design and consent form were approved by the ethical committee of Shaheed Beheshti University School of Medical and Dental Sciences. This patient had an implant at the area of the mandibular left first premolar with insufficient attached gingiva after uncovery stage (Figure 1). With performing Tension Test (16), gingival margin was mobile because of high attachment of buccal frenum, so we decided to augment attached gingiva. The width of keratinized gingiva (the distance between gingival margin and mucogingival line) was measured to the nearest millimeter with a Williams periodontal probe. The mucogingival line was detected with Roll Test and for verification Schiller's solution was used (17). Investigator determined the amount of attached gingiva by computing the distance from the free gingival margin to the mucogingival junction and then subtracting the probing depth.

Biopsy

On the first visit, a biopsy of attached gingiva was performed in the maxillary left area. After local anesthesia, a small portion of about 3×2×1 mm of attached gingiva (epithelial+connective tissue) was withdrawn by means of a surgical blade. Then, the sample was put in a nutritional medium (RPMI1640, Gibco, England) containing antibiotics (penicillin 100 IU/ml and streptomycin 100 µg/ml, Sigma, USA) and transferred to the laboratory. The patient was instructed to use chlorhexidine digluconate 0.2% mouthwash for several days.

Culturing technique

On arrival at the laboratory (National Cell Bank of Iran, Pasteur Institute of Iran), the tissue was rinsed in phosphate buffered saline (PBS, Sigma, USA) and transferred into a Petri dish. The gingival tissue sample was cut into small pieces and treated with a 0.25% trypsin solution (Sigma, USA). After incubation at 37°C for 1 hour, the epithelial layer was gently peeled off from the connective tissue and rinsed with PBS. Human gingival fibroblasts were obtained by overnight digestion of gingival connective tissue with a solution of 80µl/ml of type I collagenase (Sigma, USA) at 37°C, 5% CO_2. Fibroblasts were cultured in nutritional medium (RPMI 1640) containing 10% AB human serum (Iran Blood Transfusion organization) and antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml). During this stage, the culture medium was renewed twice a week. When the culture reached 80-90% confluence for fibroblasts, the cells were detached and used to produce tissue engineered gingival graft.

Engineered gingival lamina propria was produced by mixing 250 ml bovine skin collagen (Zyderm, INAMED, USA) with 250 ml nutritional medium (RPMI 1640) containing human gingival fibroblasts (2×10^5). The mixture was poured into a well of 6-well plate. Initial polymerization of bovine skin collagen containing fibroblasts was performed at room temperature for 10 minutes in a laminar flow hood. Then, this mixture was incubated to completely polymerize at 37°C, 5% CO_2 for 30 minutes. Tissue was grown in culture medium (RPMI 1640) containing 10% AB human serum and antibiotics. On the eight day after fibroblast seeding, collagen scaffold containing the patient's cultured fibroblasts was rinsed in PBS several times to remove the AB human serum. The tissue engineered gingival graft was then transferred to the dental clinic in a sterile 6-well plate filled with nutritional serum-free medium and sealed with parafilm.

Surgical technique and follow-up

After local anesthesia, a horizontal incision was made at the mucogingival junction (submarginal) and vertical incisions were extended apically for approximately 10 mm. Then sharp dissection was done with a #15 Bard-Parker knife. Approximately 7 mm from coronal incision, a strip of periosteum in width of 2-3 mm was removed by two horizontal parallel incisions (18).

Table 1. Baseline and post-surgery clinical parameters

	Baseline (mm)	Post-surgery (mm)
Probing pocket depth	1.5	1.5
Width of keratinized gingiva	2	5
Width of attached gingiva	0.5	3.5

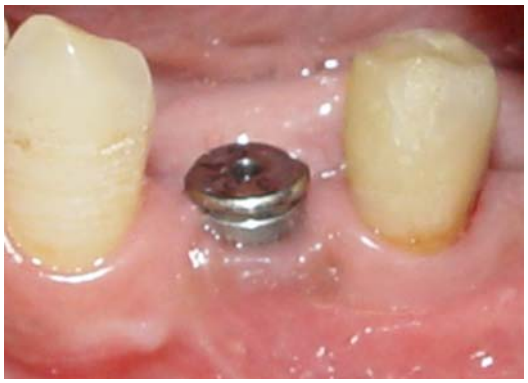

Figure 1. Insufficient attached gingiva around an implant at the area of the mandibular left first premolar

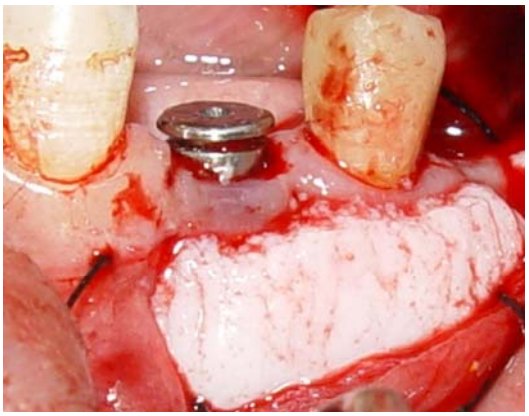

Figure 2. Tissue-engineered gingival graft is adapted to the recipient area

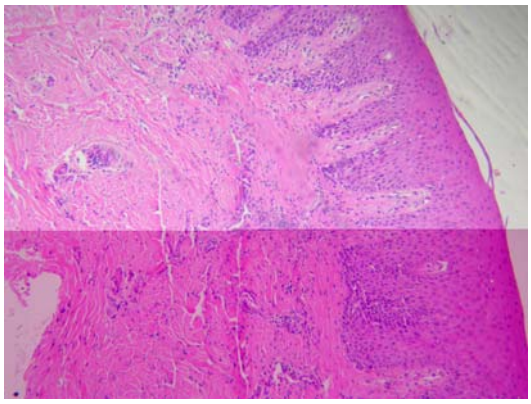

Figure 3. Histology shows a dense keratinized tissue.(original magnification × 100; hematoxylin-eosin stain)

After preparation of partial thickness recipient bed, the tissue engineered gingival graft was removed from the sterile package, shaped and adapted to the recipient site (Figure 2).

A vaseline gauze in an appropriate size was placed on the graft surface and then a foil was adapted. These components were fixed with circumferential and interdental sutures.

During the first 2 weeks, chlorhexidine digluconate 0.2% mouthwash was not prescribed to avoid damage to the fibroblasts and tooth brushing was discontinued. Sutures were removed 2 weeks following the surgery. Supragingival professional tooth cleaning along with oral hygiene instructions were performed weekly for the first 6 weeks post-surgery and then once a month for up to 6 months post-surgery.

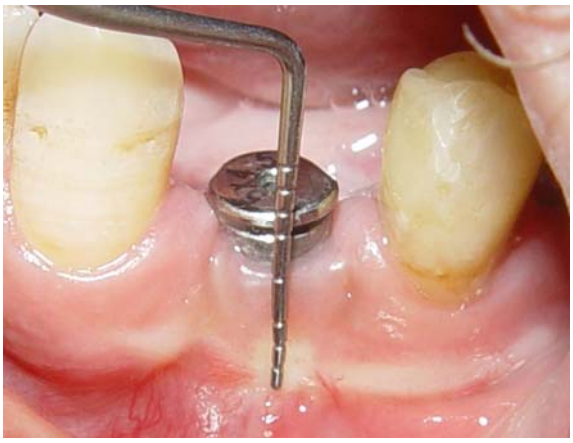

Figure 4. Three months post-surgery, tissue augmentation is obtained

Postsurgical assessment

The patient had a mild pain at first 2 days after surgery. Six weeks later, the grafted site appeared epithelialized. Three months later, a biopsy was taken from the newly formed tissue for histological examination.

The histological features demonstrated a fully keratinized tissue. The gingival epithelium was acanthotic and parakeratotic. Anastomosed rete-pegs were seen. Lamina properia was a fibrovascular tissue with mononuclear inflammatory cells infiltration (Figure 3). The clinical parameters (probing pocket depth, width of keratinized gingiva, width of attached gingiva) were controlled 3 months after surgery (Figure 4). The data of probing pocket depth (PPD), width of keratinized gingiva and width of attached gingiva at baseline, at 3 months after surgery and differences are reported in Table 1.

Discussion

Recent development of biomedical engineering as well as basic biology and medicine has enabled us to induce cell-based regeneration of body tissue to self-repair defective tissue or substitute biological functions of damaged organs. The most successful application of cell transplantation involves the development of a tissue engineered skin equivalent (19). Skin tissue is needed to treat burn victims and patients with diabetic ulcers. This need led to early research on the engineering of skin tissue, and resulted in the first FDA-approved tissue-engineered products for clinical use (20,21). In oral surgery, cultured gingival keratinocyte grafts have been applied to cover epithelial defects in preprosthetic surgery (13). These transplants are usually cultured according to the technique of Rheinwald and Green with a feeder layer of γ-irradiated 3T3 mouse fibroblasts (22). A new technique of soft tissue management for implant therapy was the use of cultured epithelial graft. This technique has a difficulty related to the mechanical weaken of cultured epithelium. Cultured epithelium possesses only the epithelial layer, and it is too thin in epithelial sheets to visit mechanical trauma during the healing period (15).

Therefore, an autologous cell hyaluronic acid graft was introduced for gingival augmentation in mucosal surgery (11). An increased amount of keratinized tissue on all treated sites after 3 months was reported. The mean average of the increased amount of keratinized tissue was 2.00 ± 0.41 mm in the middle site (12).

The safety and effectiveness of a tissue-engineered skin equivalent, a living human fibroblast-derived dermal substitute (HF-DDS), was evaluated and compared to a gingival autograft (GA) consisting of donor tissue harvested from the patient's palate in a procedure designed to increase the amount of keratinized tissue around teeth that do not require root coverage.

The GA generated more keratinized tissue and shrank less than the HF-DDS graft, but the test graft generated tissue that appeared more natural (10).

The use of cultivated gingival fibroblasts without keratinocytes is justified because it has been demonstrated the keratinization of gingival epithelium is controlled by morphogenetic stimuli of the underlying connective tissue so we used only cultivated gingival fibroblasts (23). In present study, the amount of increased gingival width was 3 mm. This clinical result was confirmed by histological evaluation performed after 3 months.

This case showed a keratinized epithelium supported by dense connective tissue. Application of this technique has several advantages: 1) a very small donor site 2) sufficient amount of keratinized tissue obtained 3) minimal discomfort for the patient.

The method we have presented can be recommended as a modality of peri-implant soft tissue management in those cases in which it might be expected the planned implant will later be surrounded with insufficient or no healthy attached gingiva. In conclusion, based on the result of this investigation, the tissue engineered gingival graft was safe and capable of generating keratinized tissue without the morbidity and potential clinical difficulties associated with donor site surgery.

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References

1. Horning GM, Mullen MP. Peri-implant free gingival grafts: rationale and technique. Compendium 1990;11(10):604, 606-10.
2. van Steenberghe D. Periodontal aspects of osseointegrated oral implants modum Brånemark. Dent Clin North Am 1988;32(2):355-70.
3. Block MS, Kent JN. Factors associated with soft- and hard-tissue compromise of endosseous implants. J Oral Maxillofac Surg 1990;48(11):1153-60.
4. Mitchell DL, Synnott SA, VanDercreek JA. Tissue reaction involving an intraoral skin graft and CP titanium abutments: a clinical report. Int J Oral Maxillofac Implants 1990;5(1):79-84.
5. ten Bruggenkate CM, Krekeler G, van der Kwast WA, Oosterbeek HS. Palatal mucosa grafts for oral implant devices. Oral Surg Oral Med Oral Pathol 1991;72(2):154-8.
6. Moy PK, Weinlaender M, Kenney EB. Soft-tissue modifications of surgical techniques for placement and uncovering of osseointegrated implants. Dent Clin North Am 1989;33(4):665-81.
7. Pack AR, Gaudie WM, Jennings AM. Bony exostosis as a sequela to free gingival grafting: two case reports. J Periodontol 1991;62(4):269-71.
8. Silverstein L, Callan D. An acellular dermal matrix allograft and a palatal graft for tissue augmentation. Periodontal Insights 1996;6:3-6.
9. Baum BJ, Mooney DJ. The impact of tissue engineering on dentistry. J Am Dent Assoc 2000;131(3):309-18.
10. Wilson TG Jr, McGuire MK, Nunn ME. Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblast-derived dermal substitute. II. Comparison to the subepithelial connective tissue graft: a randomized controlled feasibility study. J Periodontol 2005;76(6):881-9.
11. Okuda K, Momose M, Murata M, Saito Y, lnoie M, Shinohara C, Wolff LF, Yoshie H. Treatment of chronic desquamative gingivitis using tissue-engineered human cultured gingival epithelial sheets: a case report. Int J Periodontics Restorative Dent 2004;24(2):119-25.
12. Pini Prato GP, Rotundo R, Magnani C, Soranzo C. Tissue engineering technology for gingival augmentation procedures: a case report. Int J Periodontics Restorative Dent 2000;20(6):552-9.
13. Prato GP, Rotundo R, Magnani C, Soranzo C, Muzzi L, Cairo F. An autologous cell hyaluronic acid graft technique for gingival augmentation: a case series. J Periodontol 2003;74(2):262-7.
14. Raghoebar GM, Tomson AM, Scholma J, Blaauw EH, Witjes MJ, Vissink A. Use of cultured mucosal grafts to cover defects caused by vestibuloplasty: an in vivo study. J Oral Maxillofac Surg 1995;53(8):872-8.
15. Ueda M, Hata KI, Sumi Y, Mizuno H, Niimi A. Peri-implant soft tissue management through use of cultured mucosal epithelium. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;86(4):393-400.
16. Vincent JW, Machen JB, Levin MP. Assessment of attached gingiva using the tension test and clinical measurements. J Periodontol 1976;47(7):412-4.
17. Fassake T, Morgenroth K. Comparative stomatoscopic and histochemical studies of the marginal gingiva in man. Parodontologie 1958;12:151-60.
18. Carranza FA, Carraro JJ. Effect of removal periosteum on post-operative results of mucogingival surgery. J Periodontol 1963;34:223-6.
19. Morrison M. Advances in the skin trade. Mechanical Eng 1999;121:40-3.
20. Parenteau N. The organogenesis story. Sci Am 1999; 280: 83-84.
21. Naughton G. The advanced tissue sciences story. Sci Am 1999; 280: 84-5.

22. Rheinwald JG, Green H. Formation of a keratinizing epithelium in culture by a cloned cell line derived from a teratoma.Cell 1975; 6: 317-30.

23. Karring T, Cumming BR, Oliver RC, Löe H. The origin of granulation tissue and its impact on postoperative results of mucogingival surgery. Journal of Periodontology 1975; 46: 577-85.