A Pilot Study to Evaluate a Tissue-Engineered Bilayered Cell Therapy as an Alternative to Tissue From the Palate

Michael K. McGuire,* E. Todd Scheyer,* Martha E. Nunn,† and Philip T. Lavin‡

Background: This study evaluated the safety and effectiveness of a tissue-engineered skin product composed of viable neonatal keratinocytes and fibroblasts and compared it to a free gingival graft (FGG) in a procedure to enhance keratinized tissue (KT) and wound healing around teeth that do not require root coverage.

Methods: Twenty-five subjects were enrolled who had at least two non-adjacent teeth in contralateral quadrants exhibiting an insufficient zone of attached gingiva requiring soft tissue grafting where root coverage was not desired. One tooth was randomized to receive an FGG, and the other was randomized to receive bilayered cell therapy (BCT). The amount of KT was measured at baseline and 3 and 6 months, and the texture and color of the grafted tissue were compared to the surrounding tissue at months 1, 3, and 6. A questionnaire was used to determine subject preference at 6 months. Biopsies and persistence studies were performed on a subset of the subjects.

Results: The FGG generated statistically significantly ($P<0.001$) more KT than the test device (BCT) (4.5 ± 0.80 mm versus 2.4 ± 1.02 mm); no significant difference in recession or clinical attachment level was detected between treatment groups ($P = 0.212$ and $P = 0.448$, respectively); and no significant differences were detected at any time point for bleeding on probing (BOP), resistance to muscle pull, or inflammation. The BCT group had significantly better color and texture match with surrounding tissue ($P<0.001$), and subject preference was significantly greater for the BCT group ($P = 0.041$). No device-related adverse events or safety issues occurred during the course of the study.

Conclusions: The tissue-engineered graft BCT was safe and capable of generating *de novo* KT without the morbidity and potential clinical difficulties associated with donor-site surgery. The amount of KT generated with FGG was greater than generated with BCT; however, 24 of 25 test sites demonstrated an increase in KT at 6 months, with more than three-quarters of the sites yielding ≥2 mm bands of KT. *J Periodontol 2008;79:1847-1856.*

KEY WORDS

Fibroblasts; keratinocytes; tissue engineering.

* Private practice, Houston, TX.
† Goldman School of Dental Medicine, Boston University, Boston, MA.
‡ Boston Biostatistics Research Foundation, Framingham, MA.

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Most teeth in a healthy environment present with a band of keratinized tissue (KT), which varies in width depending on the location in the mouth.[1] In 1972, Lang and Löe[2] proposed that ≥2 mm KT around each tooth is necessary to maintain periodontal health, and later reports[3-5] concurred that ≥2 mm KT is sufficient for the maintenance of periodontal health. Although the exact amount of KT necessary for health has not been established definitively, most clinicians, especially in the United States, agree that at least some attached gingiva (AG) is necessary to maintain periodontal health.[6-8] Consequently, clinicians have developed a variety of surgical techniques to increase the zone of KT, but it is the free gingival graft (FGG) developed a half century ago that remains the gold standard, primarily because of its high level of success.[9-12] Limitations of this technique include the need for a remote surgical site for the harvesting of donor tissue, the limited amount of this tissue available for grafting, and color and texture differences with adjacent tissues. Because the autogenous tissue retains its native phenotype, true regeneration with

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morphologically and phenotypically correct tissue does not occur.[13]

Over the last decade, attempts have been made to stimulate wound healing through the addition of bioactive molecules carried to the defect on inert scaffolds.[14,15] In theory, these bioactive molecules are intended to stimulate the native cells to differentiate, migrate, and participate in the regenerative process, provided that the bioactive molecules are delivered to the appropriate site, at the appropriate concentration, and for the appropriate length of time.[14,16,17] Although the test device§ in this study looks much like skin or a gingival graft microscopically, bilayered cell therapy (BCT) should not be considered a tissue-replacement graft, but a cell-delivery therapy that encourages healing with the subject's own tissues. Using enzyme-linked immunosorbent assays, many cytokines have been shown to be produced by the living cells contained in BCT matrix. These cytokines include growth factors known to be associated with periodontal wound healing, including platelet-derived growth factor, bone morphogenetic protein-7, and vascular endothelial growth factor (VEGF).[18] The bilayered construction of the tissue-engineered matrix tested in this study seems to have a synergistic effect on cytokine production; when present together, keratinocytes and fibroblasts each produce more factors than when present separately. Because these growth factors are delivered by living cells, the factors are released in response to local feedback mechanisms in the wound.[19-22] Used clinically since 1998 for the treatment of chronic dermal wounds, such as venous leg and diabetic foot ulcers, BCT does not contain Langerhans' cells, melanocytes, macrophages, lymphocytes, white blood cells, blood vessels, hair follicles, or sweat glands, any of which could elicit an immune reaction. Donor screening, sterility testing, and United States Food and Drug Administration (FDA) compliance ensure safety; in >200,000 subject applications, no known adverse effects have been reported.[23-27]

Previous studies[28-34] examined several active and passive biomaterials as substitutes for autogenous gingival grafts. In particular, the FGG procedure has proved to be a critical model for evaluating the tissue response and clinical effectiveness of these biomaterials. In the FGG model, biomaterials are compared directly to autogenous, palatal graft controls and are subjected to the critical test of an open-wound environment. The primary endpoint of most of these studies was gain in KT or AG. However, other investigators[35,36] suggested that periodontal health should not be judged by measures of AG and KT alone and that additional measures of inflammation, color and texture match, clinical attachment, and recession should be considered as well. The purpose of this randomized, controlled, with-subject paired design study was to evaluate the safety and effectiveness of a tissue-engineered skin product (BCT). This BCT device was compared to palatal tissue for the purpose of enhancing KT and wound healing around teeth that did not require root coverage.

MATERIALS AND METHODS

Study Population

Twenty-five subjects, aged 18 to 70 years, were selected from subjects seeking treatment in the authors' private practice from September to November 2005 to participate in the study that was conducted under an approved FDA Investigational Device Exemption. Subjects had to be willing and able to follow study procedures and had to present with at least two non-adjacent teeth in contralateral quadrants exhibiting an insufficient zone of AG requiring soft tissue grafting. Root coverage was not desired or indicated. Treatment sites needed to exhibit an insufficient zone (≤1 mm) of AG associated with a history of increasing recession or inflammation in the mucosa in the presence of good home care. Molars and mobile teeth were excluded. In the event of adjacent teeth requiring grafting, only one tooth on each side acted as test or control tooth, but all affected adjacent teeth received the same treatment. If the female subject was of childbearing age, a documented negative pregnancy test was required. Subjects with systemic conditions (i.e., diabetes mellitus, cancer, human immunodeficiency virus, or bone metabolic diseases) who were taking corticosteroids, immunosuppressants, radiation treatments, and/or chemotherapeutics that could compromise wound healing were excluded. No acute infectious lesions could be present in the area of study. Smokers and subjects with known hypersensitivity to bovine collagen were excluded, as were subjects with previous soft tissue grafting at the sites of interest. Subjects had to read, understand, and sign an institutional review board–approved informed consent form.

Demographics and Baseline Evaluation

Two-thirds of the subjects were women, and the average age was 50.6 years (range: 31.1 to 69.7 years). Eighty-eight percent of the subjects were non-Hispanic white, 4% were Hispanic, 4% were Asian, and 4% were Middle Eastern, and 44% were former smokers (no current smokers were enrolled in the study). Paired *t* tests were conducted to test for baseline differences, and no significant differences were detected.

Test Material

BCT is a living product, constructed of type I bovine collagen (extracted from bovine tendons and subsequently

§ Organogenesis, Canton, MA.

purified) and viable allogenic human fibroblasts and keratinocytes isolated from human foreskin. Large cell banks are created from the donor tissue, with each extensively tested for safety and approved by the FDA. The dermal layer is formed in vitro by the combination of fibroblasts with collagen, serum, and tissue culture media in a special mold that limits lateral contraction. The collagen assembles into a gel in which human fibroblasts are interspersed, and these fibroblasts contract the network of collagen fibers. A suspension of keratinocytes was added to the surface of the collagen fibroblast layer and, after several days of growth, it was submersed in tissue culture media. At this time, the surface of the BCT was exposed to the air to promote epidermal differentiation. After 7 to 10 days of incubation under those conditions, a matured, cornified epidermis developed at the air–liquid interface. BCT is morphologically, biochemically, and metabolically similar to human skin. However, the dermo–epidermal junction is flatter in BCT than in normal human skin, but the cell proliferation rate is similar to that of human skin. Mitotic activity occurs in the basal keratinocytes of the epidermis and in the fibroblasts within the matrix. The device is supplied as a circular disk ~7.5 cm in diameter and 0.075-cm thick on a clear plastic tray of gelled support medium (agarose) stored at room temperature.

Study Design and Clinical Assessment

The primary efficacy variable was the change in the width of KT at 6 months compared between treatments, and matched contralateral sites were used to compare BCT (test) to FGG (control). AG and KT were measured at baseline and followed over 6 months to determine the absolute change. Secondary efficacy variables were further assessed by measurements of recession, clinical attachment level (CAL), bleeding on probing (BOP), inflammation, and resistance to muscle pull throughout the study. At test and control sites, tissue color and texture were compared to surrounding tissues. Subject preference and subject measures of discomfort were also used to compare the two procedures. Any adverse events and local or systemic reactions were recorded.

During subject screening, a medical history, complete dental history, and periodontal evaluation were performed. Contralateral (test and control) sites were selected to be relatively matched in terms of overall condition, i.e., recession, bleeding, width of KT, probing depth (PD), and attachment level. Preoperative documentation included the identification of the cemento-enamel junction (CEJ), the mucogingival junction (MGJ), the free gingival margin (FGM), and PD, which were measured using a probe.‖ KT was measured as the distance, to the nearest half-millimeter with a University of North Carolina 15 periodontal probe, from the FGM to the MGJ. At the outset, the MGJ was identified with and without Schiller's iodine. The amount of AG was determined by computing the distance from the FGM to the MGJ and then subtracting PD. Dental radiographs and photographs were made of the study teeth at the initial and postoperative time points.

To ensure no bias of test- and control-site designations, a predetermined computer generated randomization scheme was assigned to all treatment sites to determine which side of the mouth to treat with BCT or FGG. Training and calibration was conducted prior to the start of the study to ensure intraexaminer reproducibility with respect to outcome variables. At the time of surgery, the operator recorded the alveolar bone level and the immediate post-surgical position of the gingival margin of the test and control graft. All postoperative evaluations were performed by an independent examiner masked to the surgical procedure.

After baseline screening and surgery, subjects were evaluated at 1 week, and 1, 3, and 6 months postoperatively. At each of these visits, the position of the FGM, as it related to the CEJ, was charted. The position of the MGJ and plaque score were documented at baseline and 3 and 6 months. PD was charted at baseline and 6 months. Changes in medications, level of oral hygiene, and any adverse events were recorded. Oral hygiene instructions were reinforced as needed. Plaque scores of test and control teeth were recorded as the presence or absence of plaque at the gingival margin, and the overall plaque index was evaluated using the modified O'Leary plaque index.[37] Clinical photographs of test and control sites were taken at baseline and all postoperative time intervals. Inflammation at each site was scored, and the texture and color of the grafted tissue was compared to surrounding tissues. Resistance to muscle pull (based on whether the FGM of the tissue facial to the site moved when the adjacent cheek was retracted) was evaluated, and a questionnaire was used to determine subject preference and subject measures of discomfort at surgical and graft donor sites.

Sample-Size Determination

Prior to the initiation of this study, power calculations at the 5% significance level indicated that 20 evaluable subjects were needed to detect a difference in change from baseline to 6 months between treatments of 1.0 mm in KT with >95% power for a two-sided hypothesis test. The calculations were based on an assumed within-subject variation (standard deviation, estimated from previous studies[28-34] with similar inclusion/exclusion criteria) of 1.2 mm. The sample size was calculated based on paired analysis.

‖ Florida Probe, Gainesville, FL.

Statistical Analysis

Summary statistics were computed for baseline clinical variables by treatment group. Comparisons of side-specific baseline variables between treatment groups were made using paired *t* tests. Measures of AG, KT, recession, CAL, and PD over time were compiled for each subject. To test for differences in these variables over time between BCT and FGG treatments, repeated-measures analysis of covariance (ANCOVA) was conducted with adjustment for the initial amount of AG as a covariate. These repeated-measures ANCOVA models also took into account the paired nature of the study design where sites and sequence of treatment were randomized. Other outcomes of interest that were evaluated included tissue color, tissue texture, subject measures of discomfort, and subject preference. Inflammation scores and resistance to oral muscle pull were also evaluated. Wilcoxon signed-rank tests were used to compare these scores at each time point postoperatively. A marginal homogeneity test was used to assess subject preference. The periodontal health composite measure was assessed relative to independent success outcomes using a two-sided *t* test.

Histologic Evaluation

Seven subjects consented to have 3-mm biopsies taken for histologic evaluation at baseline and 6 months from their test and control sites. The biopsies were processed by fixation in 10% formalin for standard hematoxylin and eosin staining. The biopsy was submitted for histologic evaluation for comparison between the tissue generated through the test and control grafts. The examiner was masked to the treatments rendered.

Persistence Study

At baseline and 6 months, DNA samples were taken from two subjects biopsied to evaluate the persistence of the viable BCT cells in the subject's tissue. The samples were submitted, amplified, and typed by short tandem repeat analysis to determine DNA persistence after 6 months of healing. Polymerase chain reaction analysis of DNA collected at baseline and tissue collected at 6 months displayed identical profiles for each subject at both time points, indicating no evidence of residual DNA representative of BCT after 6 months of healing. The lack of persistence supports the concept that BCT functions by stimulating the *de novo* regeneration of the subject's tissue, not functioning as a graft.

Surgical Procedure

Upon entry into the study, each subject was assigned an identification number based on order of enrollment into the study. A predetermined randomization scheme was contained in a sealed envelope and labeled with the subject identification number. Immediately prior to surgery, the envelope was opened, and the two study sites were assigned the test or control treatment. The left side was always operated first. Following local anesthesia, a partial-thickness dissection was used to remove the mucosa and any remaining KT from the facial aspect of the test and control sites. Coronal intrasulcular incisions were made at the height of the existing mucosa extending to the mid-papillary region on the mesial and distal aspects of the study teeth. Vertical incisions were made at the mesial and distal aspects of the sites, extending apically ~7 mm from the coronal incisions. The mesial and distal incisions were connected apically, and the tissue was discarded unless the subject had volunteered for tissue biopsy. In that case, the tissue was sent to the laboratory for baseline histologic evaluation. Any muscle fibers were removed with scissors to create a clean periosteal bed. At the apical aspect of the bed, a full-thickness incision was made separating the periosteum to ensure that there was no muscle tension on the bed.

Test-Site Coverage

Immediately following the preparation of the graft bed, BCT was prepared. Under sterile conditions, the material was fenestrated with a scalpel to stimulate tissue activity and growth factor production, and the tissue was carefully separated from the polyvinyl backing and folded on itself to form a Z fold. The width of the graft was held constant at 5 mm. The length of the graft was determined, and the appropriate size of the tissue was separated from the rest of the tissue in the bioreactor with scissors and immediately delivered to the graft bed. The dermal side of the Z fold was placed in direct contact with the graft bed and sutured in place with 5/0-gut suture into the papillary region on the mesial and distal aspects of the grafted tooth. Intimate adaptation between the graft and the bed was ensured. The lip or cheek adjacent to the graft was placed under tension to make certain that the graft was free of movement during muscle traction, and a surgical dressing was placed.¶

Control-Site Coverage

A measurement of the length corresponding to the mesial–distal dimension of the graft bed was made and carried to the premolar/molar region of the palate on the same side of the mouth as the control site. A partial-thickness (~1- to 2-mm deep) incision was used to harvest a graft to the appropriate length, and the width was held constant at 5 mm. The palatal donor tissue was secured on the recipient site and covered with surgical dressing in an identical fashion to that used for the test sites.

¶ Coe-Pak, GC America, Alsip, IL.

Post-Surgical Care

Subjects were given postoperative instructions and pain medications. They were instructed not to brush teeth in the treated areas but to use chlorhexidine (0.12%) mouthrinse for 1 minute twice daily for the first 4 weeks. The test and control sites were covered with the surgical dressing, and subjects were advised to allow the dressing to fall off on its own. Subjects were instructed to avoid excessive muscle tractioning or trauma to the treated area for the first 4 weeks. At 14 days, the subjects were educated in a brushing technique that would create minimal apically directed trauma to the soft tissue of the treated tooth. At week 4, the subjects were instructed to resume gentle toothbrushing, interproximal cleaning, and chewing in the treated areas. At follow-up visits, any adverse events were recorded, changes in concomitant medications were noted, recession measurements were made, clinical photographs were obtained, and oral hygiene instructions were reviewed. PD was recorded at 6 months.

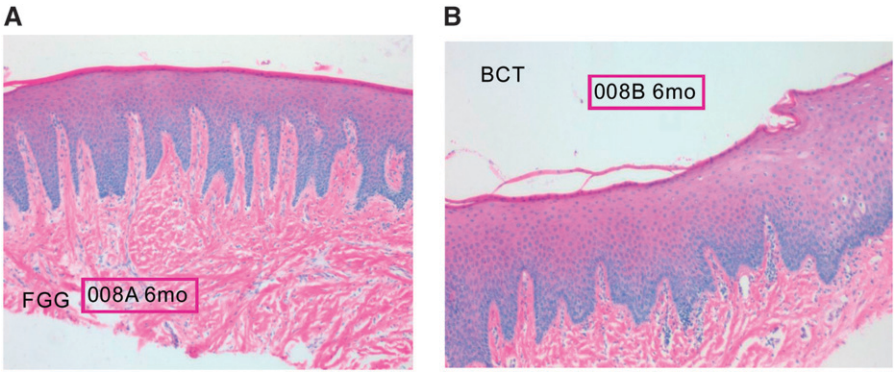

Figure 1.
*Histology of control **(A)** and test **(B)** site at 6 months.*

RESULTS

Histologic Evaluation

The biopsies sampled at baseline were representative of normal gingival epithelium with a mild degree of chronic inflammatory infiltrate indicative of periodontal inflammation within normal limits for such gingival biopsies. The 6-month biopsies (test and control) demonstrated a variable degree of chronic inflammatory infiltrate characterized by mild, perivascular inflammation beneath the epithelium. Generally, all sites demonstrated a normal epithelial architecture that showed an orthokeratinized stratified squamous epithelium typical of AG. Adjacent and contiguous with this tissue type, a parakeratinized epithelium of normal thickness was seen that demonstrated features that were characteristic of the alveolar mucosa. A transition between the two tissue types was suggestive of a regenerative response resulting in the restoration of these tissues. Test and control sites demonstrated tissues characteristic of these alveolar and gingival mucosal phenotypes and connective tissue with normal architecture. Histologic evaluation was conducted in a masked fashion (Fig. 1).

Postbaseline Outcomes

Table 1 lists the changes in clinical measures over time. With regard to the primary efficacy variable, change in amount of KT, at 6 months BCT produced a statistically significant increase in KT and AG over baseline, with an increase for KT of 2.40 ± 1.02 mm (95% confidence interval [CI]: 2.08 to 2.72 mm; $P<0.001$) and an increase in AG to 1.10 ± 1.01 mm (95% CI: 0.72 to 1.47 mm; $P<0.001$). FGG generated statistically significantly ($P<0.001$) more KT than the test device at 3 months (4.36 mm versus 2.54 mm) and at 6 months (4.46 mm versus 2.40 mm); both treatments achieved the desired KT goal of ≥2 mm of KT width at 6 months. Ninety-six percent of BCT sites (24/25) demonstrated an increase in KT width at 6 months, with 76% of BCT sites (19/25) yielding ≥2-mm-wide bands of KT. One hundred percent of FGG sites demonstrated an increase in KT width at 6 months and yielded ≥2-mm-wide bands of KT. When analysis was limited to sites in which multiple teeth (i.e., longer mesial–distal wound beds) were treated, 100% of BCT sites (6/6) and FGG demonstrated an increase in KT and ≥2-mm band of KT at 6 months (Figs. 2 and 3).

Repeated-measures ANCOVA with adjustment for baseline AG was conducted to test for differences between treatment groups for each clinical variable listed in Table 1. The only significant differences ($P<0.001$) noted were for AG and KT, with the control group (FGG) exhibiting greater gains for both. However, when only sites with positive AG at baseline (sites that had some AG at baseline, but removed at surgery) were evaluated (Table 2), no significant difference in AG ($P=0.184$) or KT was detected, although the difference in KT approached statistical significance ($P=0.057$). Although there was some KT and AG at baseline, all KT was removed at the surgical visit.

No significant overall difference in recession or CAL was detected between treatment groups at 6 months ($P=0.212$ and $P=0.448$, respectively).

BOP, resistance to muscle pull at baseline and month 6, and inflammation scores throughout the study were compared using the Wilcoxon signed-rank

Table 1.

Change in Clinical Variables From Baseline to 6 Months by Treatment Group

	Baseline (mean [95% CI])	3 Months (mean [95% CI])	6 Months (mean [95% CI])	*P* Value*	Change (mean [95% CI])	*P* Value†
PD (mm)						
BCT	1.41 (1.23 to 1.58)	–	1.38 (1.23 to 1.54)	0.088	0.02 (–0.18 to 0.22)	0.063
Control	1.43 (1.26 to 1.61)		1.68 (1.52 to 1.83)		–0.24 (–0.44 to –0.04)	
Recession (mm)						
BCT	2.44 (2.22 to 2.68)	2.42 (2.17 to 2.67)	2.20 (1.95 to 2.45)	0.399	0.24 (0.09 to 0.40)	0.212
Control	2.47 (2.24 to 2.71)	2.10 (1.85 to 2.35)	2.10 (1.85 to 2.35)		0.38 (0.22 to 0.53)	
CAL (mm)						
BCT	3.84 (3.51 to 4.17)	–	3.59 (3.34 to 3.83)	0.450	0.27 (0.01 to 0.52)	0.448
Control	3.92 (3.59 to 4.25)		3.77 (3.53 to 4.02)		0.13 (–0.12 to 0.39)	
AG (mm)						
BCT	0.26 (0.12 to 0.40)	–	1.10 (0.72 to 1.47)	<0.001	0.85 (0.47 to 1.22)	<0.001
Control	0.24 (0.10 to 0.38)		2.62 (2.25 to 3.00)		2.37 (2.00 to 2.75)	
KT width (mm)						
BCT	1.07 (0.89 to 1.25)	2.54 (2.30 to 2.78)	2.40 (2.08 to 2.72)	<0.001	1.33 (0.95 to 1.71)	<0.001
Control	1.17 (0.99 to 1.35)	4.36 (4.12 to 4.60)	4.46 (4.14 to 4.78)		3.29 (2.91 to 3.68)	
Plaque index						
BCT	0.18 (0.11 to 0.25)	0.32 (0.25 to 0.39)	0.24 (0.17 to 0.31)	0.425	0.06 (–0.02 to 0.14)	0.265
Control	0.26 (0.19 to 0.33)	0.30 (0.23 to 0.37)	0.26 (0.19 to 0.33)		0.00 (–0.08 to 0.08)	

– = no data.
* Based on repeated-measures ANCOVA with adjustment for baseline AG.
† Based on ANCOVA with adjustment for baseline AG.

test. No significant differences were detected at any time point for bleeding, resistance to muscle pull, or inflammation.

Evaluators were calibrated for the evaluation of tissue color and texture. In addition, evaluators were masked to treatment. For statistical analysis, texture and color were assigned ordinal measures, and comparisons between test and control sites were conducted using the Wilcoxon signed-rank test. Compared to the control group (FGG), the BCT group had significantly better color and texture matching with surrounding tissue at 6 months ($P<0.001$).

Subject preference was significantly greater for BCT than FGG ($P=0.041$), with 15 subjects favoring BCT, five subjects favoring FGG, and five subjects without a preference.

No unanticipated adverse events or other safety issues occurred during the course of the investigation.

DISCUSSION

Although the periodontal literature contains a few case reports of surgeons using tissue-engineering techniques to grow the subject's own cells to be used as donor tissues for FGGs,[32,38,39] to our knowledge, the current study was the first investigation of an off-the-shelf BCT in the oral environment. We chose the FGG model as a critical test with which to examine BCT's ability to create an adequate zone of KT and AG, recognizing that additional measures, such as inflammation, recession, subject preference, and proper esthetics, would be indicative of its value as an alternative to FGG. This pilot study established proof of principle that even in the open-wound environment of FGG, BCT could stimulate soft tissue regeneration similar to that achieved using FGG.

Although FGG significantly outperformed BCT in KT and AG gain, sites treated with BCT gained an average of 2.4 mm (95% CI: 2.09 to 2.71 mm) in KT width and >1 mm in AG at 6 months. This amount of KT gain was significantly more than the 1.5 mm (95% CI: 0.5 to 2.5 mm) gained at the mid-buccal site at 3 months reported by Pini Prato et al.[32,38] using autogenous fibroblasts cultured and expanded, seeded onto a benzyl ester of hyaluronic acid scaffold, and ultimately used as donor material. The amount of KT gained in this study was similar to the 2.72 mm gained at 12 months reported by McGuire and Nunn[33] using a human fibroblast–derived dermal substitute as

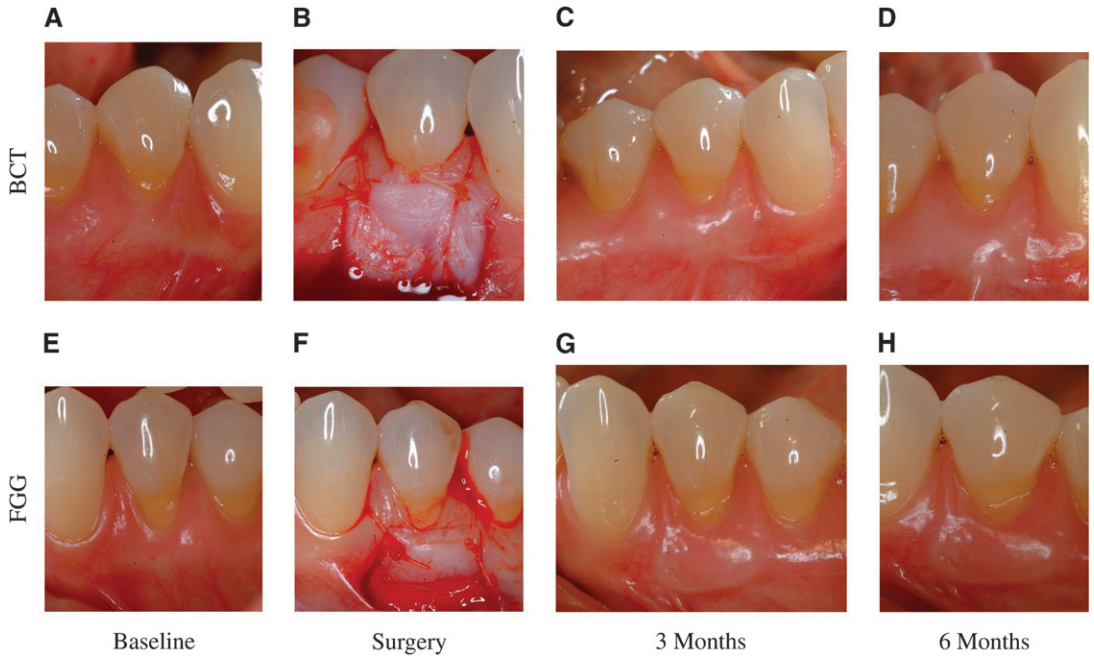

Figure 2.
Subject 1 from baseline to 6 months. ***A through D)*** *Test.* ***E through H)*** *Control.*

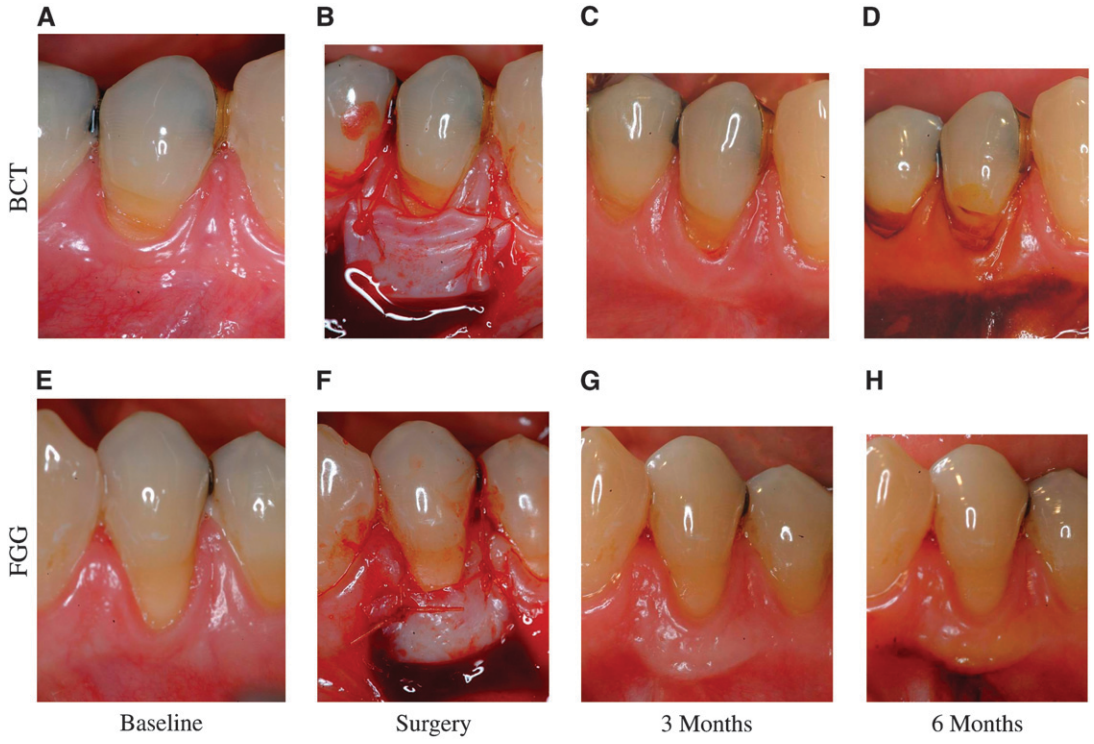

Figure 3.
Subject 2 from baseline to 6 months. ***A through D)*** *Test.* ***E through H)*** *Control. D and H display staining with Schiller's iodine solution to delineate the MGJ.*

Table 2.

Clinical Variables at 6 Months for Sites With AG at Baseline

	Baseline (mean [95% CI])	3 Months (mean [95% CI])	6 Months (mean [95% CI])	*P* Value*	Change (mean [95% CI])	*P* Value
AG (mm)						
BCT	1.00 (0.59 to 1.41)	–	2.10 (1.39 to 2.81)	0.423	1.10 (0.28 to 1.92)	0.184
Control	0.83 (0.36 to 1.30)		2.60 (1.79 to 3.41)		1.77 (0.83 to 2.71)	
KT width (mm)						
BCT	2.00 (1.59 to 2.41)	3.05 (2.23 to 3.87)	3.20 (2.11 to 4.29)	0.118	1.20 (0.38 to 2.02)	0.057
Control	1.83 (1.36 to 2.30)	4.38 (3.44 to 5.32)	4.37 (3.12 to 5.61)		2.53 (1.59 to 3.47)	

– = no data.
* Based on repeated-measures ANOVA.

donor material. Another study[39] investigated tissue-engineered donor materials, but the amount of KT gained was not reported.

In the present study, the mean amount of KT generated with FGG was greater than that generated with BCT (4.5 mm versus 2.4 mm); however, 24 of 25 sites treated with BCT demonstrated an increase in KT width at 6 months, with more than three-quarters of the sites yielding ≥2-mm-wide bands of KT. Although BCT did not generate as much KT as FGG, it created up to 4 mm of KT without the need for a donor site. The FGG created up to 5 mm of KT. In all sites where multiple teeth were treated, BCT generated a ≥2-mm band of KT. The reason for this improved outcome on longer grafts is unknown, although it could be attributed to increased vascularity of the graft because of the larger bed, resulting in less shrinkage, or access to a greater number of the host's cells. The improved outcome for multiteeth sites might also be explained by the greater dose of cells, cytokines, and matrix proteins available to the wound because more BCT was applied in these cases.

For the subset of sites that started with at least some AG at baseline, there was no statistical difference between test and control in the gain of KT and AG. The reason for this relative improvement in BCT results is unclear; although some test and control sites had some AG and KT at baseline, all sites began the study with no AG or KT, because it was removed at the surgical visit during the preparation of the recipient bed. The periosteum remained, and it may have played a role in determining the type of tissue established during healing.[40,41]

The test sites demonstrated significantly better color match and tissue texture than the control sites. The color and texture of tissue are important subject-based outcomes. FGG yielded a traditional, grafted appearance, whereas BCT provided a matched and cosmetically superior result, possibly because the cells of the grafted palatal tissue retained their phenotype, whereas BCT encouraged the native cells adjacent to the graft to migrate into and over the graft, yielding a graft composed of cells typical of the local anatomy.

Subject perceptions, including assessment of pain and preference for each site treated, were determined from questionnaires. When considering FGG treatment and its concomitant requirement for palatal tissue harvesting, subjects preferred BCT treatment. Overall, compared to traditional FGG therapy, BCT reduced the duration of pain and sensitivity of treatment.

Because the thickness of the graft seems to influence its revascularization and shrinkage, FGGs used as a control in this study were ~1 mm thick, similar to a number of reports[42-44] in the literature. In this study, BCT was placed onto the graft bed in three layers by creating a Z fold, not to increase the thickness of the graft, but to deliver an increased dose of cells and related cytokines to the bed. Based on medical experience with the device, we were unconcerned that layering keratinocytes and fibroblasts in this way would lead to complications; on the contrary, we believed that layering would maximize both cell types interacting synergistically in the graft bed. One of the most important factors in the success of any graft is the presence of adequate vascularity. One of the unique qualities of BCT is its ability to produce a variety of angiogenic growth factors, such as VEGF and fibroblast growth factor (basic FGF), which are important in the early phases of wound healing to increase vascularity to the site.[22] This attribute of BCT may be particularly important in the future when this material is used in other applications.

Another interesting component of the device is its inherent antimicrobial qualities. When human skin is injured, the body produces certain peptides in an acute fashion, such as β-defensin, which has antimicrobial properties. BCT produces β-defensin on an ongoing basis, potentially reducing the bacterial

bioburden near the defect and ultimately leading to more rapid and uneventful healing.[22]

The precise mechanism of action of BCT is not known. Griffiths et al.[45] hypothesized that young active fibroblasts and keratinocytes stimulate healing by producing new matrix material (fibronectin, vitronectin, and proteoglycans), cytokines, and growth factors. In vitro, BCT was shown to regulate cytokines and growth factors in response to the wound healing.[19-22] It is likely that the device influences healing by contributing cells (fibroblasts and keratinocytes) and extracellular matrix, as well as by influencing the angiogenic and inflammatory pathways through a variety of cytokines. A synergism is evident between the fibroblasts and keratinocytes, both of which produce more cytokines when they are present together than each does alone.[22]

Evidence of the living nature of metabolically active BCT is that when placed in a wound site, it upregulates cytokine expression to meet the needs of the host. More growth factors are produced at 4 days following application than when the graft is initially placed. The "living cell" nature of this bioactive matrix is demonstrated most dramatically by its ability to "heal itself"; when wounded, migration of keratinocytes is shown as early as 12 hours after wounding, and by 5 days, restoration of the epidermis is seen.[22]

Autogenous grafts are the gold standard for several fields of medicine. Autogenous (saphenous) veins provide the best patency for cardiovascular surgery, and autogenous bone is the preferred graft for orthopedic surgery. Yet xenogenic, allogenic, and synthetic substitutes are routinely used within each specialty. Because of the morbidity, scarcity, and time associated with the harvest of autogenous tissues, substitute biomaterials are viable standard-of-care alternatives.

Given BCT's performance in the critical test open-wound FGG model, the therapy seems to offer promise as a reasonable alternative to autogenous tissue from the palate. In this pilot study, even when fully exposed as an FGG, BCT stimulated up to a 4-mm band of KT. Given these results, further investigation is warranted; a multicenter, appropriately statistically powered, pivotal trial is underway to examine BCT's full potential and optimize its predictability in clinical practice.

CONCLUSIONS

The purpose of this randomized, controlled within-subject paired design, single-center study was to evaluate the safety and effectiveness of a tissue-engineered BCT device as an alternative to tissue taken from the palate to enhance oral soft tissue regeneration and wound healing. The results demonstrated that BCT is safe and capable of generating KT. The amount of KT generated with FGG was greater than that generated with BCT; however, 24 of 25 sites treated with BCT demonstrated an increase in KT width at 6 months, with more than three-quarters of the sites yielding ≥2-mm-wide bands of KT. BCT was similar to FGG in other measures of periodontal health, such as CAL, recession, inflammation, and resistance to muscle pull. BCT yielded a better result than FGG sites in color and texture match to surrounding tissue, whereas subject perception of the duration of pain and sensitivity was also reduced in the BCT sites. Subjects preferred the BCT treatment over the FGG procedure.

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Correspondence: Dr. Michael K. McGuire, 3400 S. Gessner, #102, Houston, TX 77063. E-mail: mkmperio@swbell.net.

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