

A Randomized, Double-Blind, Placebo-Controlled Study to Determine the Safety and Efficacy of Cultured and Expanded Autologous Fibroblast Injections for the Treatment of Interdental Papillary Insufficiency Associated With the Papilla Priming Procedure

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Background: The aim of this study was to assess the efficacy and safety of using autologous fibroblast injections following a minimally invasive papilla priming procedure to augment open interproximal spaces.

Methods: Twenty-one patients with open interproximal spaces were enrolled in this study, with 20 patients retained to study completion. Two primary sites were selected and randomized to receive autologous fibroblast injections or placebo injections beginning 1 week following the papilla priming procedure; two additional injections were performed 7 to 14 days following the initial injections. Up to seven additional sites could be treated per patient, and the analyses were conducted for the primary and secondary sites. The primary efficacy parameter was the percentage change in papillary height of the primary treatment areas from baseline to the 4-month visit, as measured by a periodontal probe from the base of the contact area to the tip of the interproximal papilla. Digital image analysis and diagnostic models were used to confirm clinical measurements. A visual analog scale (VAS) was used by the examiner and subject to assess the defect change from baseline to 2, 3, and 4 months. Tissue texture also was assessed by the examiner.

Results: The primary efficacy analysis failed to show a significant treatment effect at 4 months, but the treatment areas showed a statistically significant mean percentage increase from baseline in papillary height ($P = 0.0067$; signed-rank test) at 2 months. The difference between test and placebo sites in papillary height at 2 months approached statistical significance ($P = 0.0730$), suggesting that the test treatment was superior to the placebo treatment. The examiner and subject VASs were statistically significantly different from baseline for both treatment groups, and the VAS was superior for the test sites over the placebo. Based on safety data, the test treatment was deemed safe.

Conclusions: This early-phase study using cell transplantation of autologous cultured and expanded fibroblasts following a papilla priming procedure suggests that the treatment is safe and may be efficacious for treating papillary insufficiency, especially in the early phases (2 months) of healing. The analysis of the investigator and subject VAS assessments indicates that the test treatment was superior to the placebo treatment. The finite measurement required to detect a change creates a problem that needs to be addressed in future studies. *J Periodontol 2007;78:4-17.*

KEY WORDS

Cell transplantation; fibroblasts; tissue engineering.

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Open interproximal spaces (OISs), one of the most troubling dilemmas in dentistry,^{1,2} can cause esthetic concerns, phonetic difficulties, and food impaction. The contour of the interdental tissues, as well as the color and texture of the keratinized tissues, are essential elements of anterior esthetics. OISs usually occur because of divergent roots/diastema, abnormal crown form, or hard and soft tissue loss. Fortunately, for patients and clinicians alike, most OISs occur because of the first two reasons. Treatment, although sometimes varying in complexity, is documented clearly for these two conditions. Orthodontic movement can close diastemata and align roots,³ restorations can be constructed to recreate tooth form and contact relationships,³⁻⁵ and even a combination of orthodontic and periodontal treatment can be used to close OISs in periodontally compromised patients.⁶⁻⁹ The solution for the third cause of OISs, deficient hard and soft tissues, has eluded clinicians. When proper root alignment and crown form are present and associated with an OIS, the only alternative left for the clinician is to attempt to augment the papilla itself.

Tissue loss in the interproximal regions can occur for a variety of reasons, including, but not limited to, periodontal diseases, treatment of periodontal diseases, trauma, and other iatrogenic causes. Except for limited case reports, because no one has been able to demonstrate a predictable way to regenerate or to improve deficient papillary form caused by hard or soft tissue loss, much energy is devoted to non-surgical and surgical papilla preservation. A number of papers¹⁰⁻¹⁴ have been devoted to flap designs and surgical techniques to maintain full papillary form following periodontal surgery. Other papers^{4,8,15-21} have presented surgical techniques aimed at grafting hard and soft tissue into OISs. Limited blood supply and access plague these approaches, significantly increasing the risk for failure; however, because the regeneration or improvement of deficient papillary form is such a high-value procedure, patient and clinician have been willing to attempt them. Beagle,¹⁵ in a single case report, described a surgical procedure in which a palatal pedicle graft was folded on itself and positioned into the OIS in an attempt to create a papilla. Han and Takei⁴ published a technique using a semilunar incision in the mucosa, facial to the deficient interproximal area. Intrasulcular incisions around the teeth adjacent to the OIS were connected to the semilunar incision, and the entire gingivopapillary complex was elevated coronally. A connective tissue graft was placed in the semilunar incision to support the coronally advanced tissue. The investigators claimed that the procedure could be repeated multiple times following 2 to 3 months of healing in

an effort to regenerate the papilla. Azzi et al.¹⁶⁻¹⁸ published three papers, each consisting of a single case report regarding a variety of surgical techniques aimed at rebuilding lost papillae. Carnio¹⁹ reported on a single case in which he intervened surgically three times at 8-week intervals using an adaptation of the semilunar technique published by Han and Takei.⁴ Carnio¹⁹ stated that the papilla remained stable for 4 years after surgery. Re et al.²⁰ presented a single case report, and Cardaropoli et al.⁸ showed 28 cases demonstrating improvement of papillary form following intrusive orthodontic movement and periodontal grafting in an intrabony defect. Nemcovsky²¹ published a report on papilla augmentation on 10 consecutive patients using an advanced papillary flap combined with a gingival graft and followed patients from 3 to 14 months. All of these papers share a common theme. Most consist of a single case report, providing no evidence of predictability, and few demonstrate long-term stability. All attempts at rebuilding the interproximal papilla have used traditional periodontal plastic surgical approaches and have been met with limited success and extensive cost to the patient, both in time and money. No controlled clinical trial has addressed the issue of restoring deficient interproximal spaces.

Tissue engineering may allow us to overcome the limitations of traditional therapy and resolve OISs in novel ways. Scientists have been able to grow periodontal ligament (PDL) cells for many years. As early as 1969, Melcher²² suggested that PDL cells have pluripotential capabilities and might have the capability of regenerating the periodontium. Other studies demonstrated that the gingival fibroblast has considerable phenotypic heterogeneity and theoretically could contribute to periodontal regeneration by providing a source of undifferentiated mesenchymal cells, which under appropriate stimulation, could differentiate toward fibroblastic, cementoblastic, and osteoblastic phenotypes.²³⁻²⁸ Under normal conditions, fibroblasts are responsible for production and maintenance of the connective tissue matrix,²⁹ and, as such, are essential for periodontal health. A recent development in the field of plastic surgery involves the injection of autologous fibroblasts expanded *in vitro* to improve soft tissue contours and cosmetic appearance of the skin.³⁰⁻³² This technique was evaluated in the oral environment in a phase I study and was found to be safe.³³ This paper reports on a randomized, double-blind, placebo-controlled study that tests the hypothesis that cell transplantation, locally delivered with injections of autologous fibroblasts, results in expansion of interdental gingival soft tissue volumes in subjects with papillary insufficiencies. Furthermore, a novel minimally invasive surgical procedure is presented, whose objective is the induction of a transient

inflammatory response in the papilla to facilitate the cell transplantation process. The project consists of an acute study completed 4 months after the first treatment and a long-term study, in which subjects were followed for 12 months after the first treatment. This paper presents the results of the acute study; a subsequent paper will report on the long-term results.

MATERIALS AND METHODS

Twenty-one subjects with interdental papillary recession defects were enrolled in the study between May 6, 2004 and August 2, 2004 at the Perio Health Clinical Research Center in Houston, Texas. One subject, after undergoing the biopsy and the papillary priming procedure (PPP), withdrew from the study before treatment because of relocation out of state. For each subject, two primary interproximal recession defects were identified and randomized: one defect to receive cell transplantation and the other to receive the placebo. The primary bilateral interproximal recession defects were always on the same arch, and the two interproximal spaces were required to be similar in size and morphology with respect to depth and width. If more than one interproximal space required treatment on one side of the arch, the interproximal space defect that most closely matched the opposite study interproximal space was chosen. If all interproximal spaces to be treated were similar, the interproximal space that was the mirror image of the study defect was chosen. As much as possible, mirror image lesions were chosen for the primary defects. Each subject could have up to seven additional interproximal recession defects that met the inclusion/exclusion criteria, which were randomized and treated. Subjects who had received a crown or pontic on one or both teeth involved in the interproximal space were excluded. Interproximal spaces next to teeth that included root grooves, furcations, tooth mobility >1, open contacts, probing depths >3 mm, and radiographic evidence of pathology also were excluded.

All patients were required to be systemically healthy non-smokers, between the ages of 18 and 70 years, who maintained adequate plaque control (<20% O'Leary plaque index).³⁴ The patient population, ranging in age from 35 to 68 years (mean age, 51.4 years; SD: 7.8), included three (15%) men and 17 (85%) women. A written Institutional Review Board–approved consent form regarding the study was obtained from each patient. All patients agreed to participate in the study and gave their informed consent. Of the 20 patients who received treatment, 17 (85%) were of white descent, two (10%) were of African American descent, and one (5%) was of Asian/Oriental descent.

All interproximal spaces included in the study were associated with keratinized tissue. Occlusal interfer-

ences were identified and eliminated through occlusal adjustment, and hard acrylic bite guards were constructed for those patients with parafunctional habits. The study was designed so that extraneous factors, such as oral hygiene and compliance, would be controlled within each subject.

Clinical Evaluation

At baseline and all subsequent study visits, the treated sites were examined clinically, defect measurements were recorded, and photographs were taken at a standard (1:1) magnification. Radiographs and study impressions of sites were taken at baseline and 4 months. The primary efficacy parameter was the percentage change in papillary height of the primary treatment areas from baseline to the 4-month visit; this was assessed by the examiner using a periodontal probe measuring the distance from the tip of the interproximal papilla to the base of the contact area for each study lesion separately. The secondary efficacy analyses included change of the following parameters from baseline to the 4-month visit: distance from the tip of the papilla to the alveolar crest and from the base of the contact area to the alveolar crest, probing depth, interproximal width of papilla, plaque index, inflammation score, tissue texture and color, and patient and clinician perception of change in the Nordland Class Score. Assessment of subject safety included an analysis of the incidence of adverse events.

Summary of Clinical Assessments

The study was conducted by two investigators, one blinded and one unblinded. Additionally, there was an examiner who was blinded. The unblinded investigator administered all test/placebo treatments, whereas all study measurements and assessments were performed by the blinded investigator and the examiner. Training and calibration prior to the study were conducted to ensure intra- and interexaminer reproducibility with respect to measurement of outcome variables. The subjects were not informed and had no way to deduce which of the study lesions was the test or control site. Data were collected on all lesions treated. The O'Leary plaque index³⁴ was used to evaluate the entire mouth; all other assessments were performed only on the treated lesions (test and control).

The distance from the tip of the papilla to the base of the contact area was measured to the nearest millimeter by the examiner, using a University of North Carolina (UNC) 15 periodontal probe, a digital caliper on a model of the site, and analysis of digital photographs of the sites. The distance from the base of the contact area to the alveolar crest was measured to the nearest millimeter, under local anesthetic, by sounding to bone with a UNC 15-mm periodontal probe. The distance from the tip of the papilla to the alveolar crest

was calculated by subtracting the distance from the tip of the papilla to the base of the contact area from the distance from the base of the contact area to the alveolar crest. Interproximal width was measured with the same periodontal probe, from the mesial height of contour to the distal height of contour of the interproximal tissue.

The examiner, using a UNC 15 periodontal probe at six locations around each study tooth, measured probing depths. All probing depths were rounded down to the nearest millimeter, and bleeding on probing was recorded. The inflammation score was recorded according to the criteria of the modified gingival index presented by Lamster et al.³⁵ The examiner assessed tissue texture by comparing test and control sites to surrounding tissues and scoring it as more, less, or equally firm. Similarly, color of test and control sites were compared to surrounding tissue and scored as more, less, or equally red. A visual analog scale (VAS) evaluation was completed for each site by the examiner and by the subject. Evaluation ranged from 0 to 100, where 0 indicated "worst imaginable defect" and 100 indicated "no defect." Change from baseline in the Nordland Class Score³⁶ also was assessed by the examiner. Digital photographs (1:1) were taken of each site. The distance from the alveolar crest to the base of the contact was recorded 5 to 7 days prior to first treatment and at the 4-month visit. All other clinical assessments were recorded at each study visit.

Cell Transplantation Technique

Biopsy. Following local anesthetic, keratinized tissue from the maxillary tuberosity was harvested with a 3-mm punch biopsy instrument. The tissue was placed in a sterile biopsy vial, and a cyanoacrylate dressing was applied over the biopsy site. The biopsy sample was express mailed to the laboratory,[§] where fibroblasts from the biopsy were extracted and propagated using standard tissue culture techniques. Unless medically contraindicated, the patient could be given a non-steroidal anti-inflammatory agent for discomfort.

Investigational agent. The investigational agent is the autologous cell suspension generated by the laboratory following cultivation and expansion of gingival fibroblasts obtained from a biopsy taken from the oral cavity. Most cells in the study were transplanted within 12 hours of receiving them.

For each treatment, two vials were produced in the laboratory: one containing the cell suspension in an adequate carrier to preserve cell viability and the other containing the cell culture media. The two vials were labeled with the contents (test or placebo), subject number, subject name, identification of the study lesion(s) for which the vial was intended according to the randomized treatment assignment, and a vial bar-

code identification. A randomization list was generated by a centralized randomization agent prior to initiation of the study. Subject's lesion treatment assignments were assigned sequentially from this list, based upon the order in which the subjects were registered. On the day of treatment, ~15 minutes before the injections were to be administered, the unblinded investigator drew up the solution from each vial into a separate, labeled 3-ml syringe with a 30G needle. No preparation of the injectable material was required.

Placebo. The placebo used in the study was cell culture media. The culture process is proprietary, but used standard tissue culture technique similar to other described processes for growing and isolating fibroblasts. Fetal bovine serum was used during the culture of the cells.

PPP. Five to 7 days prior to the initial injection of cells or placebo, the papilla received a controlled surgical insult. This procedure was intended to induce a transient, acute inflammatory response in the papilla, temporarily increasing the volume of tissue to be treated, which would, theoretically, permit injection of a larger volume of cell suspension than previously found possible. Following the administration of local anesthetic, each test and control papilla received the PPP. An Orban knife was inserted into the base of the papilla on the facial aspect. The clinician carefully avoided penetration of the lingual aspect of tissue or extension into the mesial or distal sulcus. The blade of the Orban knife was pivoted off the alveolar crest, opening the incision line and surgically creating a small space. A 12B blade was inserted into the space, and the incision was carried toward the apex of the papilla perpendicular to the initial incision. The investigator carefully avoided cutting through the tip of the papilla or perforating to the lingual aspect. The patient was advised to use acetaminophen as needed, 1,000 mg three times a day, to manage discomfort, and a prescription for 0.12% chlorhexidine was provided with instructions to rinse with 0.5 ounce twice daily after meals. The patients were instructed not to brush or floss the study sites until after the first injection.

Cell transplantation/placebo injections. Prior to all injections, study assessments and digital photographs were taken by the blinded investigator or the blinded examiner. All bone sounding measurements were made by the blinded investigator. Local anesthetic was administered apically on the facial and lingual aspect adjacent to the study papilla. After expansion, cells were transported to the clinic where they were prepared for cell transplantation injection as described previously. Injections of a concentrated cell suspension of 20×10^6 cells/ml or placebo was administered

§ Isolagen Technologies, Exton, PA.

by the unblinded investigator. The first treatment occurred 5 to 7 days after the PPP. The second treatment was administered 7 to 14 days after the first treatment, and the third treatment was administered 7 to 14 days after the second treatment (Fig. 1). The study was designed for each subject to receive three treatments of a concentrated cell suspension of 20×10^6 cells/ml of cultured and expanded fibroblasts. The recommended dose was 1 ml total injection volume given at $100 \mu\text{l}/\text{cm}$ linear lesion or $50 \mu\text{l}/5 \text{ mm}^2$ and divided over all treatment sites. The placebo treatment consisted of cell culture media in the same volume. The two primary treatment sites were injected in an effort to deposit as much of the solution into the papilla as possible. Approximately 1.0 ml of test solution and 1.0 ml of control solution were available for injection into all test and control sites at each treatment visit. The amount of test or control liquid injected was recorded, and the primary test and control sites were always injected first to ensure that they received ideal treatment. The remaining amount was injected equally among the non-primary treatment sites. The injection technique used was as follows. The first treatment entered the facial aspect of the papilla just coronal to the alveolar crest. A second injection entered the same region on the lingual aspect. Depending on the amount of test or control material left, a third injection was made; the investigator inserted the needle from the tip of the papilla to the crest of the bone and injected

the fluid as the needle was withdrawn slowly. The investigator carefully avoided producing, during the injection, so much ischemia that the papilla would be at risk for tissue death. On average, 0.3 to 0.4 ml of test or control solution was deposited into the papilla at each of the three treatment sessions. Clinical assessments were performed at treatment visits and at 2, 3, and 4 months after the first set of injections. Periapical radiographs were taken of each study site at baseline and 4-month visits.

Maintenance Care Program

Five to 7 days prior to the first treatment, all subjects underwent plaque removal at all study sites, and the areas were root planed and scaled as needed. All subjects were advised to follow good oral hygiene habits in all non-study sites by brushing twice daily, using a soft-bristled toothbrush, and flossing once per day. All subjects were instructed to avoid brushing and the use of interdental cleaning devices following the PPP until after the first injection. In addition, all subjects were instructed to avoid chewing directly on the test and control sites and to avoid trauma to the test and control sites following the PPP until after the first injection. During that interval, the subjects were instructed to rinse twice daily after meals with 0.12% chlorhexidine gluconate oral rinse. After the first injection, the subjects were instructed to resume gentle toothbrushing and interdental cleaning with dental floss and to discontinue the 0.12% chlorhexidine gluconate oral rinse. All subjects received dental cleanings at 2 months.

Sample Size Determination

Twenty-five subjects were to be enrolled and treated in this study, but enrollment was stopped early because of manufacturing limitations; 21 subjects were enrolled, and 20 subjects were treated in this study. Each subject served as his or her own control by receiving test and placebo therapy. The sample size, although typical of an exploratory early-phase study in which the objective is to investigate the safety and efficacy of the treatment, was unable to be estimated using statistical methods because insufficient clinical experience existed about how this new procedure would effect changes in papillary height.

Image Analysis

Image analysis consisted of several steps: image identification, image scaling, calibration, and measurement. During the image identification process,

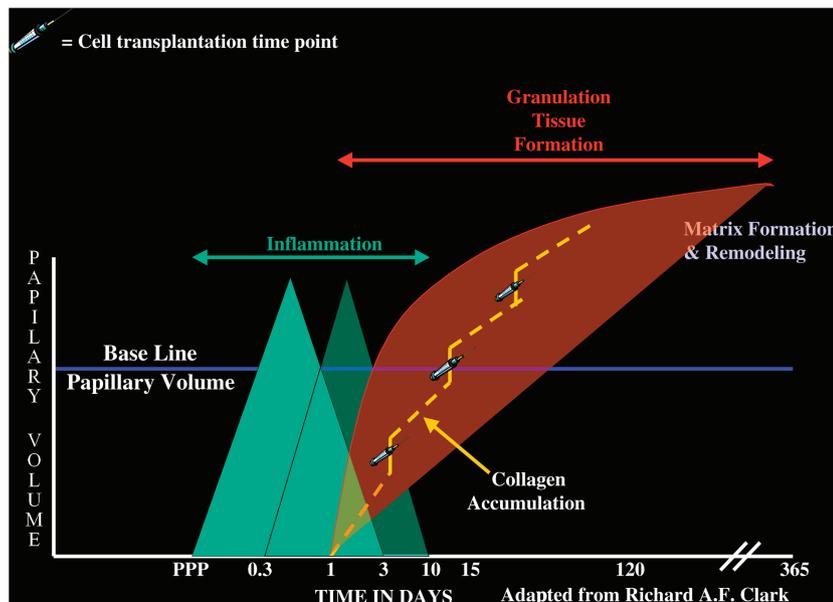


Figure 1.

The three phases of wound healing beginning with the PPP, which creates the basis for this study's experimental design of extending the granulation tissue formation phase in an effort to increase papillary volume. (Adapted with permission from Dr. Richard A.F. Clark, Biomedical Medicine, Dermatology, and Medicine, Stony Brook University, Stony Brook, New York.)

all sites were identified and labeled properly. The image analysis was conducted by a blinded third party. After a careful review of images, potential errors in the measurement data, such as rotation differences in site representation, were recognized and minimized by examiners matching teeth sizes of the same site at all time points. Images containing millimeter probes were calibrated by a process in which the number of pixels within a measured distance between the probe's millimeter markings was recorded. After determining how many pixels were contained in 1 mm, the images that did not contain such probes were considered measurable.

Measurement of Photographs

After a visual examination, an image was picked based on its focus and full site view. Two clearly visible points on the incisal edges of the teeth surrounding the site of interest were chosen. A rectangular area of interest (AOI) was placed on the image, with its lower side aligning the two points. The upper side of the rectangular AOI marked the base of the contact point between the teeth. A perpendicular distance was measured from the most coronal point of papilla to the most apical side of the AOI (Fig. 2).

The same rectangle was placed on another image of the same site at a different time point, and the same two points were aligned, matching the orientation of the teeth. This step accounted for two-dimensional site rotations from time point to time point. The width of the rectangle did not change, marking a constant location of the contact point on all images of this site. This step decreased standard deviations in the measurement data that were due to a three-dimensional site rotation from time point to time point and allowed for more precise measurement from the tip of the papilla to the base of the contact area.

To reveal any method precision issues, a designated third-party analyst measured 10% of the images a second time. There were only small standard devi-

ations in the measurement data, which suggested that the initial analysis was correct.

Statistical Methods

Standard statistical methods were used to analyze all data. The following techniques were used: descriptive statistics, Wilcoxon signed-rank test, McNemar test, repeated measures analysis, and graphic displays. Assumptions of normality and homogeneity of variance were tested with the Shapiro-Wilks test. All tests were declared statistically significant at $P \leq 0.05$. All tests appear as two-sided P values.

Summary statistics consist of numbers and percentages of responses in each category for discrete measures and of means, medians, standard deviations, 95% confidence intervals, and minimum and maximum values for continuous measures; they are presented for each treatment, where applicable. A statistical software package^{||} was used to provide all statistical analyses.

RESULTS

The primary efficacy analysis was the difference, from baseline to 4 months, in the percentage change of the distance from the tip of the papilla to the base of the contact area in the primary treatment areas, as measured by a periodontal probe. The results of this analysis failed to show a treatment effect.

The percentage change in the distance from the tip of the papilla to the base of the contact area from baseline also was examined using measurements from digital photographs. The analyses at months 3 and 4 showed no indication of efficacy. However, at month 2, the test treatment areas showed a statistically significant mean percentage increase from baseline in papillary height ($P = 0.0067$; signed-rank test). The difference between treatments was borderline statistically significant at month 2 ($P = 0.0730$; signed-rank test), suggesting that test treatment was superior to placebo.

A repeated measures analysis was done on the distance from the tip of the papilla to the base of the contact area measured using the dental probe over the acute portion (through month 4) of the study. The analysis was done with the statistical software package[¶] using the PROC MIXED procedure with the unstructured covariance type option, meaning that all variances and covariances may be unequal. This procedure is quite powerful in detecting statistical differences, so caution should be used when interpreting these results. A graph of the raw data is provided in Figure 3.

No visit by treatment interaction effect was found, but the visit and treatment factors were statistically

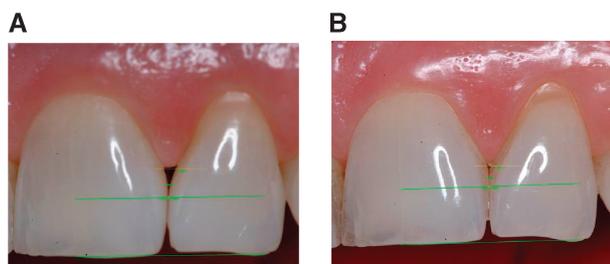


Figure 2.

A) Normalized photograph at baseline with the overlying area of interest (AOI) depicted with horizontal lines. **B)** A second image of the same site with the AOI transferred from the baseline photograph. This image shows the positive tissue formation that occurred at the 4-month follow-up visit.

^{||} SAS, version 8.0 or higher, SAS Institute, Cary, NC.

[¶] SAS, version 8.0 or higher, SAS Institute.

significant on their own (both P values <0.0001). This indicates that over time, there was some change in the distance from the tip of the papilla to the base of the contact area, and the test and placebo treatments were statistically significantly different.

A second repeated measures analysis was done on the percentage change in distance from the tip of the papilla to the base of the contact area measured using the dental probe over the acute portion of the study. The analysis was done with the statistical software package[#] using the PROC MIXED procedure with the first-order autoregressive covariance type option. A graph of the percentage change data is provided in Figure 4.

No visit by treatment interaction or treatment effect was found, but again, the visit factor was statistically significant ($P = 0.0275$). This indicates that over time, there was some difference in the percentage change in distance from the tip of the papilla to the base of the contact area, but no treatment effect was detected.

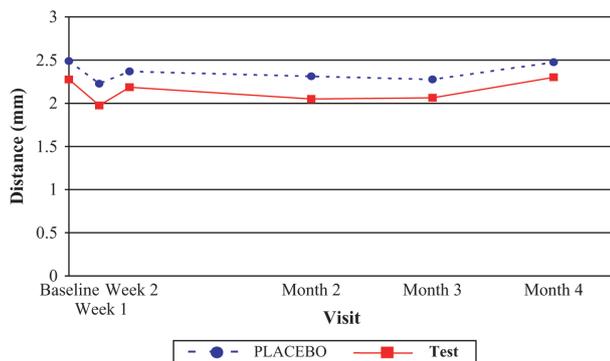


Figure 3.

The mean distance from the tip of papilla to the base of contact area, from baseline to the 4-month follow up.

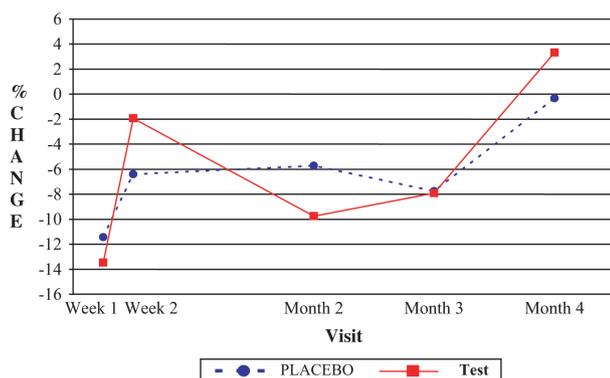


Figure 4.

The mean percentage change in the distance from the tip of papilla to base of the contact, from baseline to 4 months.

The following endpoints failed to show evidence of a treatment effect: the distance from the tip of the papilla to the base of the contact area using measurement from the dental arch molds, the distance from the tip of the papilla or the base of the contact area to the alveolar crest, the width of the interproximal spaces, and the assessment of papilla treatment on probing depth (all assessed using Wilcoxon signed-rank test); the investigator's assessment of papilla treatment using Nordland Class scores (assessed using Wilcoxon signed-rank test); and color and texture assessments and inflammation scores (all assessed using McNemar test).

The VAS evaluation ranged from 0 to 100, where 0 indicates "worst imaginable defect" and 100 indicates "no defect." Using the VAS, the blinded examiner used the subjects' current appearance to make an assessment at baseline and each visit. The percentage change from baseline in the VAS evaluation was calculated for each treatment area at each visit (Table 1). The difference in percentage change also was calculated. It should be noted that the VAS scores have high variations (SDs); therefore, the median scores are more appropriate to report.

The analysis of the investigator's assessment showed improvement in the test and placebo treatment areas at month 2. The improvement effect was greater in the areas treated with the test therapy, resulting in the difference being statistically significantly different from zero ($P = 0.0192$; signed-rank test).

At nearly all post-treatment visits, the areas treated with the test therapy showed improvement, whereas the placebo-treated areas showed worsening. The difference between treatments was statistically significantly different from zero at months 3 and 4 ($P = 0.0153$ and $P = 0.0064$, respectively; signed-rank test), indicating that the test therapy was superior to placebo. A graph of the median percentage change from baseline in investigator VAS scores is provided in Figure 5.

The subject VAS was evaluated and scored in the same manner as the investigator VAS. Using the VAS, the subject used his or her current appearance to make an assessment at baseline and at each visit. The percentage change from baseline in the VAS evaluation was calculated for each treatment area at each visit (Table 2). The difference in percentage change also was calculated. As before, the VAS scores had high variations (SDs); therefore, the median scores were more appropriate to report. At month 4, the test treatment areas showed improvement, whereas the placebo-treated areas showed worsening. The difference between treatments was statistically significantly different from zero at month 4 ($P = 0.0153$;

[#] SAS, version 8.0 or higher, SAS Institute.

Table 1.

Investigator VAS Evaluation of Primary Treatment Areas: Percentage Change From Baseline and Difference in Percentage Change

	Visit	Treatment	N	Mean ± SD (%)	Median	Signed-Rank Test P value
Percentage change in investigator VAS score	Month 2	Test	20	25.3 ± 82.4	12.9	0.2774
		Placebo	20	1.7 ± 50.1	-11.7	0.9563
Diff. in % change in investigator VAS score		Test	20	23.6 ± 74.5	13.8	0.0192*
		Placebo				
Percentage change in investigator VAS score	Month 3	Test	20	17.1 ± 85.1	9.2	0.8124
		Placebo	20	-13.3 ± 34.9	-19.6	0.1140
Diff. in % change in investigator VAS score		Test	20	30.4 ± 82.5	8.6	0.0153*
		Placebo				
Percentage change in investigator VAS score	Month 4	Test	20	21.0 ± 78.5	7.5	0.3884
		Placebo	20	-9.3 ± 41.0	-16.3	0.1054
Diff. in % change in investigator VAS score		Test	20	30.2 ± 79.3	19.1	0.0064*
		Placebo				

A positive percentage change indicates improvement.
 A positive difference in percentage change indicates test therapy efficacy.
 Diff. = difference.
 * Statistically significant ($P < 0.05$; signed-rank test).

signed-rank test), indicating that test therapy was superior to placebo. A graph of the median percentage change from baseline in subject VAS scores is provided in Figure 6.

Two adverse events were reported (tinnitus and gingivitis); neither was related to study treatment. These adverse events were not considered to be serious and did not require termination of the study treatment. Based on this safety data, the test treatment was deemed safe for the treatment of papillary insufficiency. The entire cell transplantation therapy, including the PPP and injections, was relatively pain-

free. Only two patients took acetaminophen for discomfort.

The lack of statistically significant differences of some of the secondary endpoints is a positive finding. The fact that there were no significant changes in inflammation, tissue texture and color, and probing depth following treatment indicates that the therapy was well tolerated and yielded no adverse effect.

DISCUSSION

Traditional approaches of natural tooth papillary augmentation typically involve invasive surgical intervention associated with significant morbidity.^{4,5,11-15,17} In addition, many of the originators of these techniques suggest that the surgery be repeated multiple times to obtain optimal results,^{4,15} and there is no statistical evidence of success with these methods in the periodontal literature. Conversely, an ideal autologous injectable material should require negligible surgery for initial tissue harvest and provide long-term correction and unlimited yield, without need for additional harvest. Cell transplantation by injection into the defect should be simple and relatively pain-free. The technique evaluated in this study met all of these criteria. Fibroblasts were extracted from the biopsy, reproduced, and reintroduced by injection into the papilla to expand the papillary tissue through the ongoing production of the patient's own collagen. This paper represents the first randomized, double-blind, placebo-controlled study to use tissue engineering to treat papillary insufficiency. The primary objective

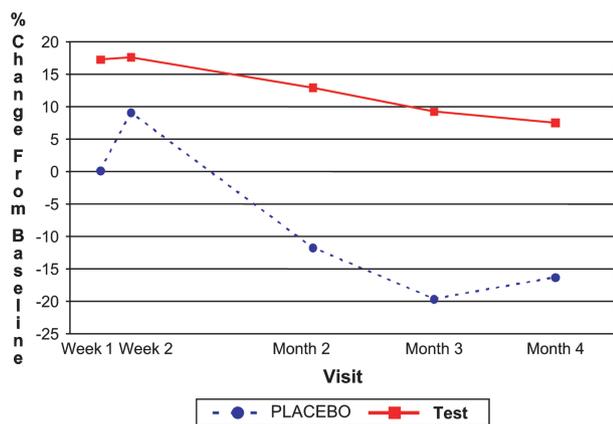


Figure 5. The median percent change from baseline to 4 months using the VAS scored by the blinded investigator.

Table 2.**Subject VAS Evaluation of Primary Treatment Areas: Percentage Change From Baseline and Difference in Percentage Change**

	Visit	Treatment	N	Mean (%) ± SD	Median	Signed-Rank Test P value
Percentage change in subject VAS score	Month 2	Test	20	22.8 ± 84.3	6.1	0.4980
		Placebo	20	5.6 ± 81.8	-15.9	0.8195
Diff. in % change in subject VAS score		Test	20	17.1 ± 106.7	16.0	0.3321
		Placebo				
Percentage change in subject VAS score	Month 3	Test	20	30.4 ± 88.6	7.3	0.2753
		Placebo	20	3.4 ± 78.1	-17.2	0.7086
Diff. in % change in subject VAS score		Test	20	27.0 ± 104.5	20.8	0.0583*
		Placebo				
Percentage change in subject VAS score	Month 4	Test	20	39.7 ± 89.8	16.2	0.1231
		Placebo	20	-0.7 ± 70.6	-33.6	0.7012
Diff. in % change in subject VAS score		Test	20	40.4 ± 87.8	29.7	0.0153†
		Placebo				

A positive percentage change indicates improvement.

A positive difference in percentage change indicates test therapy efficacy.

Diff. = difference.

* Borderline statistically significant ($0.05 < P < 0.10$; signed-rank test).

† Statistically significant ($P < 0.05$; signed-rank test).

of this study was to evaluate the efficacy of cultured and expanded autologous fibroblast injections in the treatment of interdental papillary recession defects. The secondary objectives of the study were to optimize a “PPP” intended to increase the volume that could be injected into the gingival tissue and to assess the safety of the cell therapy when fibroblasts are injected into the interdental gingival papillae.

Prior to this paper, a number of options for treatment of dermal deficiencies was reported in the literature. Autologous cultured fibroblasts have been injected to correct rhytids, acne, and scars. Usually, treatment is directed toward restoring the population of fibroblasts that is reduced as the result of photo-damage, aging, and scarring. Typically, the therapy results in early moderate fill, with a continuing gradual correction for a period of 12 to 24 months.³⁰ This therapy differs from commonly used soft tissue fillers, which usually are non-living protein-based or synthetic materials. Other autologous injectable materials include a suspension of intact autologous collagen fibers derived from the patient’s dermal layer.** At 1 year, a study using this material reported a 75% correction of the dermal depression following three injections.³⁷ Unfortunately, because there is a finite amount of dermal tissue, this technique has limited use. The approved dermal fillers, such as bovine collagen and cross-linked human collagen, are acellular and resorb within 3 to 6 months.^{38,39} In addition, 1% to 6% of patients receiving bovine collagen injections develop a localized hypersensitivity reaction.^{40,41} Syn-

thetic dermal fillers, such as silicone, have not been approved in the United States. Subcutaneous fillers, such as acellular dermal matrix,†† fat, and deepithelialized dermis, are used for the treatment of subcutaneous depressions and should not be confused with the treatment of dermal deficiencies. Subcutaneous fillers require surgical insertion and typically are not used for fine dermal defects. Acellular dermal matrix has been used for dental applications,^{42,43} but no published evidence exists where this material has been used in the treatment of papillary insufficiencies.

The study was designed to have an acute endpoint at 4 months and a long-term endpoint at 12 months. The results at 4 months following the first injection would indicate short-term change from baseline, and the 12-month endpoint would allow investigators to determine whether a change in outcome was maintained over time and to assess improvement. Although cell transplantation was not performed after the third treatment session, the papillary volume possibly could continue to expand through the production of collagen until the papilla had remodeled to meet the functional demands of the embrasure.

Several case reports investigating autologous cultured fibroblasts have been published in the medical literature and served as the foundation of the study design. A study conducted at the University of Medicine and Dentistry of New Jersey⁴⁴ asked 94 patients to

** Collagenesis, Beverly, MA.

†† Life Cell, Branchburg, NJ.

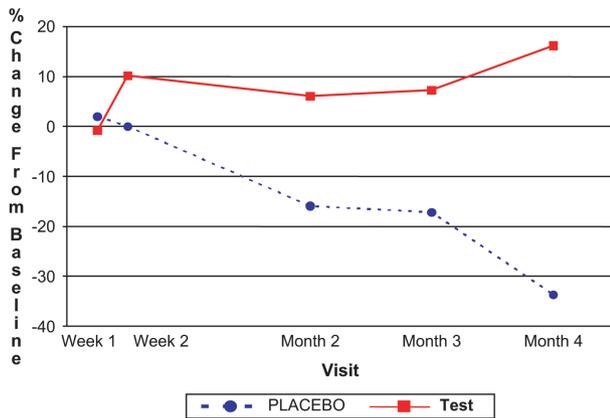


Figure 6.
VAS percentage change scored by the subject.

grade their perceived degree of correction of facial rhytids and dermal depressions, and grade their degree of satisfaction with results, and report their perception of continuing noticeable improvements in the treated areas. The average patient grading response with regard to the degree of correction was 7.8 (0-10 with 10 being total and complete correction), and 92% of the patients were pleased with the results of the overall program. Of the patients reported, 78% perceived an ongoing and continuing improvement of the treated area for periods up to 24 months. There were no reports of infection, rejection, granuloma formation, keloid formation, or overcorrection of the defects in the study. Patients' perceptions of the extent of correction of facial rhytids and dermal depressions through injection of autologous fibroblasts correlated with the objective improvement that was documented by laser profilometry on silicone

molds. This study also documented continuing clinical improvement over time following the final injection; significant improvement was noted at 3 months postinjection, and an even greater degree of correction was seen after 6 months. Nine out of 10 patients reported 60% to 100% improvement, and laser profilometry demonstrated a 10% to 85% reduction in the scar or rhytids. All 10 patients were biopsied, and there was microscopic evidence of increased thickness and density of dermal layer collagen. With respect to the histologic findings, the investigators found that the fibroblasts appeared to be incorporated into the dermal architecture. They speculated that the fibroblasts began nascent production of collagen or stimulated synthesis from the native cells, subsequently creating the thicker dermal layer. This *de novo* production of collagen helps to sustain, and, perhaps even enhance, the corrective effect over time. Injections in this study were repeated at 2- to 3-week intervals for a total of three injection sessions for each participant. As stated earlier, this study resulted in early moderate fill of the defect and gradual, continuing correction in most patients for 12 to 24 months following injection.^{30,44}

Little information is reported in the literature regarding the fate of the fibroblasts following injection. In one study,⁴⁵ rabbit fibroblast cells were cultured, and some were labeled with ³H-tritiated thymidine (TdR). The labeled and unlabeled cells, with a concentration of 8×10^7 cells/ml, were injected into superficial and deep dermal junctions of the ears of 10 New Zealand white rabbits. Each site was injected three times at weekly intervals. The animals were sacrificed at 5 months, and labeled, transplanted, active fibroblasts were identified. The transplanted fibroblasts were found to secrete collagen I and III and other extracellular matrix proteins, such as elastin, fibronectin,

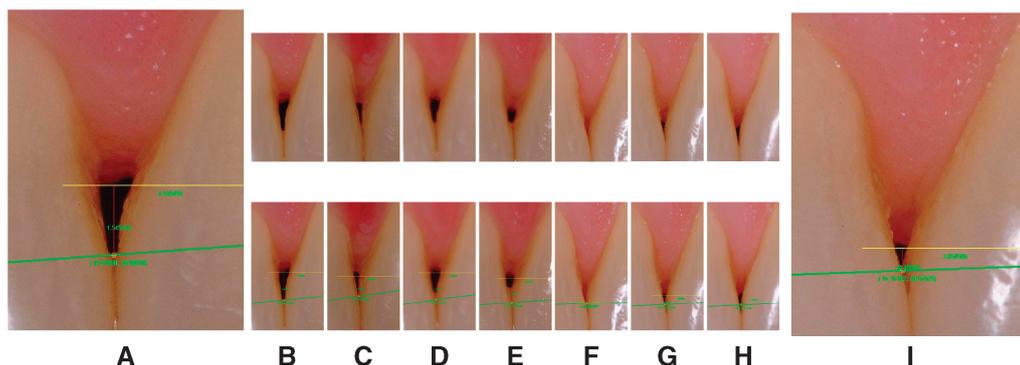


Figure 7.
This digital analysis of the images shows the progression from baseline (A) to 4-month follow up (I). The smaller images begin with the baseline view (B) prior to the PPP and then progress with the first (C), second (D), and third (E) injection visits and the 2-month (F), 3-month (G), and 4-month (H) follow-up visits. The horizontal and vertical lines were used for the digital image analysis and can be seen better in Figure 8.

and glycosaminoglycan. Biopsies were taken, and the treated sites were found to have a thicker dermal layer. It was speculated that the production of these extracellular matrix proteins might have been the reason for the increase in thickness found in the dermal layer after injections.

Any clinician who administers local anesthesia encounters difficulty in inserting much volume into the papilla because of its dense, tightly bound-down connective tissue. The phase I study³³ also found that injection of an adequate volume of cell suspension to obtain satisfactory therapeutic results was problematic because of the density of the gingival tissue. Practitioners also know that surgically manipulated papilla are swollen 7 to 10 days postoperatively and gradually return to preoperative dimension (assuming no tissue loss) by 1 month. The challenge for the investigators in this study was how to capitalize on this transient postoperative swelling, which would permit the injection of a greater volume of test and control solution into the swollen papilla than would be possible under normal circumstances, and take advantage of the increasing native fibroblast production and collagen accumulation (as described below). The PPP was conceived and developed around this concept.

Three sequential phases distinguish wound healing: early and late inflammation, granulation tissue formation, and matrix formation and remodeling (Fig. 1).⁴⁶ The system is not organized as rigidly as this description suggests because a number of factors affect the time required for the completion of each phase, resulting in considerable overlap. Wikesjö and Selvig⁴⁷ described the following sequence in periodontal wound healing. In the first seconds, fibrinogen and other plasma proteins adhere to the surface of the wound, resulting in the production of a fibrin clot. Within 1 hour, neutrophils infiltrate the clot to phagocytize injured and necrotic tissue, initiating the early inflammatory phase of healing. By 3 days, the wound has entered the late inflammatory phase, with the influx of macrophages and decrease in neutrophil infiltrates. Macrophages, which continue the work of wound debridement, also release growth factors that encourage fibroblast proliferation, matrix production, endothelial cell proliferation, and angiogenesis. By day 7, the wound enters the third phase, as granulation tissue undergoes maturation and remodeling to meet functional needs.

This concept of wound healing was used to establish the experimental time line and possible mechanism of action of the autologous cell transplantation into the deficient papilla. The PPP, a controlled surgical insult to the papilla, begins the wound healing cycle. If left undisturbed, the tissue goes through all three phases, swelling as it goes through the inflammatory phase and gradually returning to the preoperative di-

mension as it completes the granulation tissue phase and enters matrix formation and remodeling. The experimental design takes advantage of this process, and attempts to increase papillary height and/or volume by prolonging the granulation tissue phase. Approximately 1 hour after the PPP, the tissue begins to swell as neutrophils fill the wounded papilla. Within 12 hours, the production of native fibroblasts begins to increase and is associated with collagen accumulation. Approximately 3 days after the PPP, the papilla enters the late stage of the inflammatory phase. The tissue remains swollen over baseline; macrophages begin to replace the neutrophils, and they produce growth factors supporting native fibroblast proliferation, matrix production, and angiogenesis. At ~7 days, the papilla enters the granulation tissue phase. The production of natural fibroblasts peaks, and collagen accumulation plateaus. It is at this point, ~7 days after the PPP, that the first cell transplantation takes place. The papilla is still swollen, allowing for more volume of the test or control vehicle to be injected into it, and the influx of autologous fibroblasts theoretically causes collagen accumulation in the papilla to continue, rather than plateau. This treatment session is repeated for three sessions, ~7 to 14 days apart, in an attempt to prolong the granulation tissue phase and increase collagen accumulation, resulting in a thicker dermal layer, and, thus, increasing papillary height and/or volume. Although no further cell transplantation takes place after the third treatment session, it is possible that papillary volume will continue to increase over time through *de novo* production of collagen.

The driving force behind the treatment of OISs is patient desire. Dermatology studies²⁶ have revealed a very high level of patient satisfaction following the treatment of facial defects with autologous fibroblasts. This study also revealed a high level of patient satisfaction. The analysis of the investigator and subject VAS assessments of interproximal tissue fill produced statistically significant results.

The analysis of the subjective VAS assessments by the investigator and the subjects produced statistically significant results, indicating test treatment efficacy. Other objective assessments did not produce statistically significant results to support test efficacy. It is believed that objective measurements did not correlate with the significant findings of the VAS assessments because of measurement error and inadequate power.

From the results of the acute phase of the study, statistical powers have been calculated with assumptions coming from the primary efficacy analysis, distance from the tip of the papilla to the base of the contact area. The month 2 image analysis on the primary treatment areas, with unclear images excluded,

yielded a difference in raw change between test and placebo of 0.27 ± 0.6 mm. With the sample size being 15, the study had only 34% power to detect a treatment effect. A sample size of 41 would be needed to detect a statistically significant difference assuming the same results.

The month 2 analysis of the primary treatment areas using the dental probe measurements yielded a difference in percentage change between test and placebo of $4.0\% \pm 27.8\%$. With the sample size being 20, the study had only 8% power to detect a treatment effect. A sample size of 382 would be needed to detect a statistically significant difference assuming the same results. High variability of this imprecise measurement increases the sample size and decreases the power.

Measurement of the distance from the base of the contact area to the tip of the papilla was problematic throughout the study, regardless of how it was measured. The periodontal literature suggests that probing measurement reproducibility between examiners can predictably (90% of the time) be achieved within 1 mm.⁴⁸ Most of the defects were ≤ 2 mm, which means that the defect could have a 50% change that could not be measured based on standard of error of 1 mm. In addition to that, great difficulty was encountered in creating accurate dental models of the OIS to measure with digital calipers. Because of the OISs, impression material had to be torn when removed from the mouth, making the models less accurate. There also were problems in image analysis of the digital photographs with such a minute area of interest and varying papilla anatomy (Figs. 7 and 8). Because the angulation for the digital photographs was not standardized, measurement error was introduced. A device to standardize photographs is being developed for future studies.

Another possible explanation for the lack of correlation between the objective measurements and the VAS assessments is that the study may be measuring the wrong variable. It is possible that the patients and examiner are seeing plumper (not taller) papilla, which they find more esthetic. Volumetric increase in the papilla following treatment was not measured.

Even with the lack of correlation with the objective measurements in the study, one should not discount the importance of the VAS assessment. The VAS is a well-accepted endpoint in the pharmaceutical industry. There are numerous studies where the VAS is used as an endpoint for pain. It also is a major component in many quality of life assessments, such as the Western Ontario and McMaster Osteoarthritis Index (WOMAC) for osteoarthritis. Over the last few years, there has been a push by the United States Food and Drug Administration, and, thus, the pharmaceutical industry, toward more patient-reported outcomes. The VAS is an important instrument to

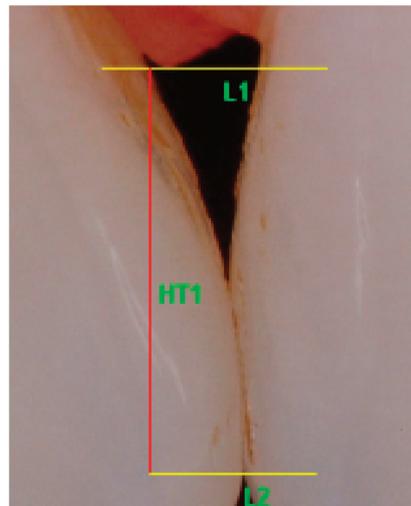


Figure 8.

The varying anatomy of the interproximal region and the horizontal and vertical references used to analyze the papillary insufficiency. L1 = line 1 at the most coronal extent of the interproximal papilla; L2 = line 2 at the most coronal point of the contact area; HT1 = height 1: the distance between L1 and L2. These lines (points) were used to determine changes in papillary height over time.

quantify patient-reported outcomes. In fact, recently approved cosmetic treatments^{†‡} used the VAS or VAS-like assessments as an efficacy endpoint in their summary basis for approval. In addition, VASs are used commonly to assess primary endpoints in tooth whitening studies and fluorosis.⁴⁹⁻⁵¹

In this study, ~ 0.3 to 0.4 ml was injected into each papilla, which translates to 6 to 8 million fibroblasts transplanted into each papilla per injection. More research is necessary to determine what effect the injection of fibroblasts into the deficient papillae may have. Cell survival or viability was not confirmed in this study, and it is not known if the autologous fibroblast survived in the wound environment. Further studies are necessary to confirm what the injected fibroblasts are producing and if the production is in therapeutic range. Low-level erbium-doped:yttrium, aluminum, and garnet (Er:YAG) laser irradiation stimulates proliferation and secretion of the gingival fibroblast,^{52,53} and it may be beneficial in wound healing. Case reports⁵⁴ in the dermatology literature demonstrated improved results after the use of laser therapy following autologous cellular transplantation. Further studies are necessary to determine whether laser therapy might improve the results of autologous cellular therapy in the papilla. Finally, other studies should evaluate the injection of healthy fibroblasts along with an agent that would direct them into a desired activity.

†‡ Sculptra, Aventis Pharma Bridgewater, NJ; Botox, Allergan, Irvine, CA.

CONCLUSIONS

The results of this exploratory, early-phase study suggest that injection of cultured and expanded autologous fibroblasts is safe, and may be efficacious, for the treatment of papillary insufficiency. The analysis by the investigators and subject VAS assessments indicated that the test treatment was superior to placebo.

The following endpoints failed to show evidence of treatment effect: distance from the tip of the interproximal papilla to the base of the contact area as measured by a periodontal probe, using measurement from the dental arch molds or the digital photographs; distance from the tip of the papilla or the base of the contact area to the alveolar crest; and width of the interproximal spaces. However, it may be that the methods used to measure effect were not sufficiently sensitive.

Further research is needed using methods that may be more sensitive and better able to measure the treatment effect that the subjects and investigators experienced.

ACKNOWLEDGMENTS

The authors thank one of our senior clinical research coordinators, Gloria Zacek, Perio Health Professionals, Houston, Texas, for her excellent work in conducting the study. The authors also thank Dr. Jacquelyn Campbell and Cindy Wainscott, Perio Health Professionals, for their help with the manuscript. Acknowledgment also is given to Synergos, Woodlands, Texas, for their assistance with the statistical portion of this paper. This study was sponsored by Isolagen Technologies, Exton, Pennsylvania.

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- Accepted for publication August 25, 2006.