doses as they tried to minimize side effects, not realizing this allowed the organisms most resistant to the drug to survive and re-populate the pockets.

10) When TRAC Research tested the accuracy of organism identification by the 2 commercial DNA test kit labs used in this study (See pages 4-5), by sending organisms obtained from ATCC, they were often mis-identified. This presented a major problem since DNA identification was expected to be predictably accurate. Also these tests were a key component directing treatment by some of the clinicians. These failures forced TRAC researchers to work for many months with the commercial labs to attempt to correct the problems.

**After considerable effort to eliminate, control, and/or find a way to monitor the above problems, a second smaller highly controlled study was initiated with the same goals as the original study.**

**Study #2** involved 10 patients, 4 clinicians, and 2 of the 4 lasers included in study #1. In order to be included in study #2, the laser had to have a specific treatment protocol for periodontitis patients in general dentistry practices, be cooperative and willing to participate in the study, and select 2 fully trained and experienced clinicians from within their user base to participate. Deka $CO_2$ laser and Lares Er and Nd:YAG laser companies met all of these criteria.

The treatment procedures were performed by trained clinicians, that were selected and trained by the laser company. The laser companies were asked to check and certify that each clinician's laser was performing properly prior to beginning the study. All elements of the patient treatment were conducted according to each laser company's specific protocol (See pages 5-6). Two clinicians were selected to operate each of the lasers. Patients were selected by the clinicians from their patient pool and approved by TRAC Research. Patients with periodontits involving pockets depths of 4-6mm in all four quadrants were sought. Below are the criteria set for patient selection:

Patient <u>inclusion</u> criteria:

1. 4-6mm pockets present in all 4 quadrants
2. Willing to participate in a 1-year clinical trial including seven data gathering appointments, plus the frequency of maintenance appointments stipulated in each laser's maintenance protocol (See pages 5-6)
3. Willing to commit to directions provided for home care
4. Stable in the area for at least one year
5. General good health
6. No current endodontic needs
7. No active caries
8. Willing to sign an informed consent

Patient exclusion criteria:

1. Generalized pocket depths ≥7 mm
2. Poor overall health
3. Unwillingness or inability to keep all appointments required
4. Habitual cigarette smoker
5. Uncontrolled diabetic
6. Received any type of periodontal treatment within the preceding 12 months

For each patient, one quadrant was chosen to receive SRP alone (the control) while all remaining quadrants received SRP-plus-laser treatment. Due to the small number of patients (n=10), control quadrants were chosen such that they were representative of the whole mouth—neither better nor worse than all other quadrants. Five of the patients received treatment using the Deka $CO_2$ laser, while the other 5 received treatment using the Lares Er-Nd:YAG laser. One patient was lost at three months. This patient belonged to the Er-Nd:YAG group.

Each patient received several Periodontal Susceptibility Tests (PerioID PST, Oral DNA Laboratories, Brentwood, TN 37027) administered at random different times throughout the study. It has been claimed that patients with a positive PST result may be more prone to inflammatory diseases and may respond less successfully to periodontal treatment. Of the 9 patients who completed the 1-year outcome study, 4 had positive results consistently and 4 had negative results consistently. Of the 4 with positive results, 2 were treated with the $CO_2$ laser and 2 were treated with the Nd:YAG laser. One $CO_2$ laser patient was excluded from the PST data analysis because both a positive and a negative PST result was received. Although only one PST test in a lifetime is recommended by the test directions, it was of interest to determine if the results were consistent over time during treatment.

Pocket depth measurements were taken independently by each clinician and by one TRAC researcher before treatment, 6 months, and 1-year post-treatment. The same TRAC Researcher took all measurements throughout the study and always worked using 3.5x magnification and a headlamp. Pocket depths were recorded for 6 locations on each tooth along with information about whether or not there was bleeding and/or suppuration upon probing. The periodontal probe used was the PCP-12 by Hu-Friedy. This was chosen because it was the one used routinely by most of the clinicians in the study.

To assess the effect of the lasers on microorganisms, microbial data was collected using the following 3 different methods:

1. Site-specific sterile paper points (microIDent *plus*, Hain Diagnostics, Nehren, Germany) were used to collect DNA samples to measure presence and concentrations of the 11 periodontal pathogens listed below. Samples were taken for each of the four quadrants separately using 5 to 7

sterile paper points per quadrant. Using this method results were separable by SRP alone and SRP-plus-laser for statistical analyses.

1. Aa = Aggregatibacter actinomycetemcomitans
2. Pg = Porphyromonas gingivalis
3. Tf = Tannerella forsythia
4. Td = Treponema denticola
5. Pi = Prevotella intermedia
6. Pm = Peptostreptococcus micros
7. Fn = Fusobacterium nucleatum/periodonticum
8. Cr = Campylobacter rectus
9. En = Eubacterium nodatum
10. Ec = Eikenella corrodens
11. Cs = Capnocytophaga spec. (gingivalis, ochracea, sputigena)

2. A saliva sample (My Perio Path, Oral DNA Laboratories, Brentwood, TN 37027) was collected to analyze for the presence and concentrations of the same 11 periodontal pathogens listed above. Results of the saliva sample apply to the whole mouth, so results were not separable by SRP alone and SRP-plus-laser for statistical analyses.

3. Site-specific sterile brushes (Black Handle Short 40-45, NanoBrush, Denbur, Oak Brook, IL 60522) were used to collect biofilm samples from pockets for culturing and computing total numbers of microorganisms present within pockets. Five to eight brushes were used per quadrant. Aliquots of the total count samples were cultured aerobically and anaerobically for each quadrant separately, so results were separable by SRP alone and SRP-plus-laser for statistical analyses.

Microbial samples were taken before treatment; after each phase of the treatment on treatment day (ultrasonic scaling, hand scaling, laser treatment); and at five post-treatment times: (1 month, 2 months, 3 months, 6 months and 12 months).

## 2. Treatment protocols used in this study as specified by the two laser companies:

### A. Deka PerioPulse $CO_2$ laser

Treatment Technique

- 2.0-2.32 watts; 50 Hertz (Level 5)
- 1-2 mm crest epithelium removed buccal and lingual
- Insert tip into pocket 1-2mm and trace slowly using 16 seconds for molars; 8 seconds for smaller teeth
- Perform the above treatment four times at 10-14 day intervals

Post-op Follow-up Technique

- Recall at 3 month intervals and remove deposits using ultrasonic and hand instruments, and standard tooth polishing procedures

- Trace pockets at moderate speed, with attention to unresolved sites using 1.8 watts; 50 Hertz (Level 4)

**B. Lares PowerLase AT and Nd:YAG laser**

Treatment Technique

- Use Nd:YAG laser at 2.5-3.0 Watts; 20 Hertz
- Insert into pocket to 1mm of pocket base (to remove epithelium and improve access)
- Use Er:YAG laser with the 600µm straight quartz tip at 40 Hertz; 20 millijoules; 50µs; level 4 $H_20$; level 1 air (to remove calculus to 1mm of pocket base)
- Use Er:YAG laser with the 600µm radial quartz tip at the same settings as above (to detoxify and biostumulate root surface and dissect periodontal attachment to bone)
- Corticate with endodontic explorer (to release growth factors)

Post-op Follow-up Technique

- Use Nd:YAG laser at 2.0 watts; 20 Hertz for biostimulation.
- Move tip ~2 minutes over the area to be treated at a 2-3 inch distance from the tissue (patient feels slight warmth). Perform on days 2-4-7-14 post-op
- Recall at 2 month intervals and remove deposits using ultrasonic and hand instruments , and standard tooth polishing procedures to 3mm depth

## 3. Alphabetical listing of clinicians who advised and/or treated patients in the two studies

- Robert Barr, DDS
- Mark Colona, DDS
- Enrico DiVito, DDS
- Rob DiVito, DDS
- Laurie King, RDH
- Edison Louie, DDS
- Michael Skinner, DDS
- Gail Smith, RDH
- Mary Lynn Smith, RDH
- Kalie Wagner, RDH
- Jen Walker, RDH

## 4. Appointment intervals and Procedures performed at each of 7 TRAC Research Data Gathering Appointments

**Included 7 TRAC researcher visits**

- Appt 1 = Data Gathering
- Appt 2 = Treatment Day
- Appt 3 = 1 month Post Treat (Antibiotic-use check)
- Appt 4 = 2 month Post-Treat
- Appt 5 = 3 month Post-Treat
- Appt 6 = 6 month Post-Treat
- Appt 7 = 1 year Post-Treat

**Procedures performed at Appointments 1& 7**

1. Explanation of periodontitis and study
2. Patient signed consent
3. Health history recorded
4. Radiographs – FM periapicals, and panographic
5. Thorough examination and recording of pocket depths, bleeding and suppuration, and clinical observations (color, texture, architecture, recession, lesions, etc.). Full mouth clinical photographs made using a single lens reflex camera.
6. Saliva sample for DNA analysis
7. Sterile paper point samples for DNA analysis
8. Sterile brush samples for total counts assays
9. Homecare directions and demonstration of products provided for post treatment use

**Procedures performed at Appointments 2 through 6**

1. Post-treatment day survey of patient's rating of pain and treatment experience
2. Saliva sample for DNA analysis
3. Sterile paper point samples for DNA analysis
4. Sterile brush samples for total counts assays
5. Thorough examination and recording of clinical observations (color, texture, architecture, recession, lesions, etc.), but pocket depths, bleeding, and suppuration performed at appointment 6 and not at appointments 2 through 5. Clinical photographs were made of sites with particular interest.

## 5. Statistical analyses performed by Brigham Young University statistical team.