

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-774

STATISTICAL REVIEW(S)

Statistical Review and Evaluation

NDA / Drug Class: 20-774 / 3S SEP 10 1997

Applicant: Perio Products Ltd.
7 Hamarpeh Street
POB 23590
Har Hotzvim Industrial Zone
Jerusalem 91237, Israel

Name of Drug: PerioChip™ (Chlorhexidine Gluconate)

Documents Reviewed: NDA Index and Summary sections (Vol. 1.1) and Statistical sections (Vols. 1.50- 1.78) dated December 20, 1996, and diskettes containing SAS datasets provided by the sponsor.

Type of Report: Statistical Review

Indication: PerioChip™ is indicated as a part of scaling and root planing procedures for the treatment of periodontitis.

Clinical Input: Dr. Hyman (HFD-540)

I. Introduction

The PerioChip™ is a biodegradable cross-linked, hydrolyzed gelatin matrix that releases chlorhexidine gluconate (2.5 mg) directly into the periodontal pocket over a seven to ten day period. The major focus in the development of the PerioChip™ was to discover an effective drug delivery system that would enable the dental practitioner to arrest and control chronic periodontal disease when used as an adjunct to scaling and root planing.

Antimicrobial therapy can be expected to reduce subgingival bacteria and reduce inflammation to the base of the periodontal pocket, thus reducing pocket depth (PD). An antimicrobial agent, however, is not anticipated to influence soft tissue or bone reduction, thus significant reduction of attachment level (AL) is not expected. Since AL is a major quantitative indication of destructive periodontitis, it is important to show that PerioChip™ does not cause a deterioration of AL. Therefore, the reduction of PD and the maintenance of AL were selected as the primary efficacy variables. Secondary measures of efficacy were reduction from baseline in bleeding on probing, gingival index, plaque index, and stain index.

Three Phase III studies were conducted. One study was a multicenter study in Europe and Israel and there were two multicenter studies in the United States. The European Israeli study was a split-mouth study with 172 patients that compared scaling and root planing (SRP) alone with SRP plus the PerioChip™. The data of this study are considered in the safety analysis only. The two US studies were the pivotal efficacy parallel-group studies conducted according to identical protocols, comparing the PerioChip™ plus SRP to a placebo chip plus SRP and SRP alone. A total of 447 patients were enrolled in the two US studies.

The objective of each of the US Phase III trials was to determine the effect of the placement of a PerioChip™ with chlorhexadine (as an adjunct to regular scaling and root planing treatment) on the reduction of probing pocket depth and on the maintenance of probing attachment level. Scaling and root planing alone and placebo chip plus SRP were used as controls. Each of these Phase III trials were multicenter, double-blind, randomized, parallel-group clinical studies. Patients were randomized to one of two groups. Three treatments were evaluated within the two groups.

Group 1 (PerioChip™): Two randomized target sites received a PerioChip™ after SRP (*Treatment 1/ Active chip*); two additional randomized target sites received no additional treatment following SRP for the purpose of the maintenance of the study blind but, by design, were not included in the efficacy evaluation.

Group 2 (Placebo Chip): Two randomized target sites received a placebo chip after SRP (*Treatment 2/ Placebo control*); two additional randomized target sites received no additional treatment following SRP (*Treatment 3/ SRP control*).

At each of five centers, at least 40-45 patients were to be enrolled. The duration of the study was 9 months. The clinical indices were measured at Baseline, Week 6, Month 3, Month 6, and Month 9. In order to be enrolled into the study, a patient had to have at least four target teeth with probing pocket depth of 5-8 mm. At Month 3 and Month 6, PerioChips™ or placebo chips were placed in the pockets that previously received chips and still measured a PD of 5 mm or more.

II. Efficacy Evaluation

For this review, the primary efficacy variables are the change in PD from baseline at Month 9 and the change in AL from baseline at Month 9. Statistical significance must be obtained at the 0.05 level for the change in PD variable. As discussed by the sponsor at the End of Phase II meeting, successful SRP is a 0.5 mm to 1 mm reduction in PD. Therefore, an additional 0.4 mm above SRP would be needed for the PerioChip to show clinical efficacy. Since the claim is maintenance of AL, if a statistically significant difference is not shown between the active and control treatments but the PerioChip™ treatment does not cause further AL loss then, PerioChip™ will be considered effective.

As a supportive analysis for the clinical significance of PD, an evaluation of the distribution of patients who exhibited pocket improvement of 2 mm or more relative to baseline at Month 9 will be made.

The secondary efficacy variables are Bleeding on Probing (BOP) at Month 9 (all patients had BOP at baseline) and the change from baseline at Month 9 in Gingival Index, Plaque Index, and Stain Index.

A comparison of the PerioChip™ treatment is made with either the placebo chip control or the SRP control. For each subject, the changes from baseline with respect to PD and AL are summarized for each post-baseline visit by taking the means over those target pocket sites where chips were placed and by taking means over those target sites where chips were not placed. Each comparison is made by an analysis of covariance (ANCOVA) model, in which the baseline score of the respective parameter is used as a covariate. The analyses for the treatment comparisons use a main-effects model involving four factors: treatment, study center, smoking status, and baseline score. Smoking status is included in the model because it has been documented that smokers are more resistant to periodontal treatment than nonsmokers. Patients were stratified according to smoking status by study design. Comparisons for the distribution of patients with 2 mm or more improvement and for BOP are made using the Cochran-Mantel-Haenszel statistic. Gingival Index, Plaque Index, and Stain Index comparisons are made using the ANCOVA model described above.

Reviewer's Comment: All efficacy treatment comparisons are performed on the Intent-to-Treat/ Last Observation Carried Forward (ITT/LOCF) population. This differs slightly from the sponsor performed analyses in that the sponsor did not use the ITT population defined at baseline and then use a LOCF for the remaining visits but instead defined at each monthly visit an ITT population. The conclusions drawn are similar. As determined by the sponsor, there was an insignificant difference in the number of patients included in the ITT and per protocol populations and the results for both of these analyses were similar. Therefore, the per protocol population was not analyzed for this review.

Study US 94-002

- Patient Demographics

Study US 94-002 had 107 patients randomized to the placebo group and 108 patients randomized to the PerioChip™ group. The following table contains the demographic characteristics by treatment group for all randomized patients. As can be seen from Table 1, distributions of these variables are similar across the two treatment groups ($p > 0.19$). The descriptive variables, race (white versus others), sex, and smoking status, are evaluated using Cochran-Mantel-Haenszel (CMH) tests stratified on study site. Age is evaluated using ANOVA with study site effects.

Table 1
Patient Demographics
Study US 94-002

| | Placebo | PerioChip™ | P-value |
|---------------------------|------------|------------|---------|
| # Patients | 107 | 108 | |
| Age mean (SD) | 46.5(10.6) | 46.8(9.0) | .696 |
| Race (N) | | | |
| Caucasian | 77 | 87 | .190 |
| Black | 27 | 16 | |
| Asian | 1 | 4 | |
| Hispanic | 1 | 1 | |
| Other | 1 | 0 | |
| Gender(N) | | | |
| Male | 50 | 52 | .811 |
| Female | 57 | 56 | |
| Smoking Status (N) | | | |
| Nonsmoker | 67 | 63 | .573 |
| Smoker | 40 | 45 | |

- Analysis Results**

Table 2 contains a summary of the results of the treatment comparisons for each of the primary efficacy variables. Included in the table are the mean values for the Control (Placebo Chip or SRP only) and PerioChip™ treatment groups adjusted for the parameters discussed above, the difference of the respective adjusted mean of the PerioChip™ group from the Placebo Chip and SRP only group, and the p-values from the pairwise comparison of Placebo Chip vs. PerioChip™ and SRP only vs. PerioChip™. Since separate analyses are performed to compare the PerioChip™ group to the Placebo chip group and to compare the PerioChip™ to the SRP only group, there are estimates of the adjusted mean value of the PerioChip™ group from each analysis.

Table 2
Summary of Primary Efficacy Variables
PerioChip™ vs. Control Groups at Month 9
ITT/LOCF Population
Study 94-002

| Parameter | Control | PerioChip™ | Difference | P-value |
|----------------------------------|---------|------------|------------|---------|
| Mean Reduction in PD (mm) | | | | |
| • PerioChip vs. Placebo Chip | .702 | .973 | .271 | .0034* |
| • PerioChip vs. SRP only | .764 | .988 | .224 | .0132* |
| Mean Gain in AL (mm) | | | | |
| • PerioChip vs. Placebo Chip | .541 | .751 | .210 | .0484* |
| • PerioChip vs. SRP only | .631 | .751 | .120 | .2657 |

*Indicates significance at the 0.05 level.

Note: The mean values and pairwise comparison p-values adjust for Study Site, Smoking Status, and the baseline values for the respective parameter.

Based on the results in Table 2, a statistically significant reduction of PD is achieved for PerioChip™ compared to both the Placebo chip sites and the SRP only sites. This reduction is not greater than mm. In the sponsor's statement of clinical significance, an additional mm is needed for the PerioChip™ to show efficacy. For AL, PerioChip™ only shows a statistically significant different gain when compared to the Placebo Chip sites. Even though there is not a statistically significant difference when PerioChip™ is compared to the SRP only sites, PerioChip™ does not cause further worsening in AL.

To further support the efficacy of PerioChip™, the number of sites which had at least a 2 mm reduction in PD at Month 9 are described in Table 3. From this table, it can be seen that the PerioChip™ group has a statistically significant benefit when compared to both the Placebo Chip and the SRP only sites.

Table 3
Classification of PD Reduction \geq 2mm at Month 9
Study US 94-002

| Treatment | Both sites < 2 mm | One site \geq 2 mm | Both sites \geq 2mm | P-value |
|--------------|-------------------|----------------------|-----------------------|---------|
| PerioChip™ | 71 (65.7%) | 29 (26.9%) | 8 (7.4%) | |
| Placebo Chip | 84 (78.5%) | 20 (18.7%) | 3 (2.8%) | .031 |
| SRP only | 88 (82.2%) | 14 (13.1%) | 5 (4.7%) | .018 |

Note 1: All comparisons are to the PerioChip™ group.

Note 2: P-values for CMH test stratified by study site.

For the secondary efficacy parameters, BOP, Gingival Index, Plaque Index, and Stain Index, only BOP has statistically significant differences between PerioChip™ and either of the two control groups. Table 4 summarizes the distribution of sites with BOP at Month 9 for each of the treatment groups. This table indicates that there are significantly fewer PerioChip™ sites with BOP and more sites without BOP at Month 9 than the Placebo Chip or SRP only groups.

Table 4
BOP at Month 9
Study US 94-002

| Treatment | Neither site BOP | One site BOP | Both sites BOP | P-value |
|--------------|------------------|--------------|----------------|---------|
| PerioChip™ | 31 (28.7%) | 39 (36.1%) | 38 (35.2%) | |
| Placebo Chip | 22 (20.6%) | 35 (32.7%) | 50 (46.7%) | .046 |
| SRP only | 19 (17.7%) | 37 (34.6%) | 51 (47.7%) | .018 |

Note 1: All comparisons are to the PerioChip™ group.

Note 2: P-values for CMH test stratified by study site.

Since patients who still had pocket depths greater than 5 mm at either 3 months or 6 months could receive additional chips at these visits, the medical reviewer also requested an analysis performed by the number of chips placed. He was interested in determining if there was additional benefit in receiving the additional chip(s). Table 5 includes the adjusted mean in pocket depth at baseline and the adjusted mean reduction in pocket depth at Months 3, 6, and 9 by the number of chips placed. The category of two chips is broken down by whether the second chip was given at Month 3 or 6. As would be expected, patients who received additional chips had deeper pockets at baseline than those who did not receive additional chips. The table also shows that the mean pocket depth reduction from baseline increases the month following the placement of an additional chip for those sites which received two chips and for those which received three chips the mean pocket depth reduction from baseline is greatest at Month 9.

Table 5
Adjusted Mean Reduction in PD by Number of Chips Placed
Study US 94-002

| Treatment | N | # Chips | Baseline | Month 3 | Month 6 | Month 9 |
|-----------|----|---------|----------|---------|---------|---------|
| Placebo | 59 | 1 | 5.37 | 1.42 | 1.43 | 1.14 |
| | 14 | 2.3 | 5.82 | .57 | 1.49 | 1.01 |
| | 17 | 2.6 | 5.57 | 1.33 | .41 | .66 |
| | 57 | 3 | 6.01 | .29 | .27 | .26 |
| PerioChip | 63 | 1 | 5.44 | 1.48 | 1.62 | 1.43 |
| | 25 | 2.3 | 5.93 | .56 | 1.65 | 1.11 |
| | 18 | 2.6 | 5.70 | 1.38 | .47 | .91 |
| | 57 | 3 | 6.13 | .36 | .35 | .57 |

Note 1: N is the number of patients who received a given number of chips. The total N is more than the total number of patients because each tooth site of a patient did not necessarily receive the same number of chips.

Note 2: # Chips=2.3 means second chip given at Month 3
Chips=2.6 means second chip given at Month 6

Study US 94-003

- Patient Demographics

Study US 94-003 had 115 patients randomized to the placebo group and 117 patients randomized to the PerioChip™ group. The following table contains the demographic characteristics by treatment group for all randomized patients. As can be seen from Table 6, distributions of these variables are similar across the two treatment groups ($p > 0.27$). These variables were evaluated using the same statistics as used in Study US 94-002.

Table 6
Patient Demographics
Study US 94-003

| | Placebo | PerioChip™ | P-value |
|---------------------------|------------|------------|---------|
| # Patients | 115 | 117 | |
| Age mean (SD) | 46.8(10.0) | 47.5(10.9) | .599 |
| Race (N) | | | |
| Caucasian | 89 | 83 | .271 |
| Black | 15 | 27 | |
| Asian | 4 | 3 | |
| Hispanic | 2 | 4 | |
| Other | 5 | 0 | |
| Gender(N) | | | |
| Male | 55 | 50 | .487 |
| Female | 60 | 67 | |
| Smoking Status (N) | | | |
| Nonsmoker | 74 | 78 | .739 |
| Smoker | 41 | 39 | |

- **Analysis Results**

Table 7 contains a summary of the results of the treatment comparisons for each of the primary efficacy variables. Included in the table are the mean values for the Control (Placebo Chip or SRP only) and PerioChip™ treatment groups adjusted for the parameters discussed above, the difference of the respective adjusted mean of the PerioChip™ group from the Placebo Chip and SRP only group, and the p-values from the pairwise comparison of Placebo Chip vs. PerioChip™ and SRP only vs. PerioChip™. Since separate analyses are performed to compare the PerioChip™ group to the Placebo chip group and to compare the PerioChip™ to the SRP only group, there are estimates of the adjusted mean value of the PerioChip™ group from each analysis.

Table 7
Summary of Primary Efficacy Variables
PerioChip™ vs. Control Groups at Month 9
ITT/LOCF Population
Study 94-003

| Parameter | Control | PerioChip™ | Difference | P-value |
|----------------------------------|---------|------------|------------|---------|
| Mean Reduction in PD (mm) | | | | |
| • PerioChip vs. Placebo Chip | .617 | .800 | .183 | .0458* |
| • PerioChip vs. SRP only | .520 | .791 | .271 | .0022* |
| Mean Gain in AL (mm) | | | | |
| • PerioChip vs. Placebo Chip | .477 | .614 | .137 | .1685 |
| • PerioChip vs. SRP only | .539 | .627 | .008 | .3680 |

*Indicates significance at the 0.05 level.

Note: The mean values and pairwise comparison p-values adjust for Study Site, Smoking Status, and the baseline values for the respective parameter.

Based on the results in Table 7, a statistically significant reduction of PD is achieved for PerioChip™ compared to both the Placebo chip sites and the SRP only sites. Again, this reduction is not greater than mm which is not at least the additional mm that was needed for the PerioChip™ to show efficacy. For AL, there are no statistically significant differences between PerioChip™ and either the Placebo Chip or SRP only group. Even though there is not a statistically significant difference, PerioChip™ does not cause further worsening in AL.

To further support the efficacy of PerioChip™, the number of sites which had at least a 2 mm reduction in PD at Month 9 are described in Table 8. From this table, it can be seen that the PerioChip™ group has a statistically significant benefit only when compared to SRP only sites.

Table 8
Classification of PD Reduction \geq 2mm at Month 9
Study US 94-003

| Treatment | Both sites < 2 mm | One site \geq 2 mm | Both sites \geq 2mm | P-value |
|--------------|-------------------|----------------------|-----------------------|---------|
| PerioChip™ | 88 (75.2%) | 21 (18.0%) | 8 (6.8%) | |
| Placebo Chip | 88 (76.5%) | 22 (19.1%) | 5 (4.4%) | .458 |
| SRP only | 100 (87.0%) | 14 (12.2%) | 1 (0.8%) | .003 |

Note 1: All comparisons are to the PerioChip™ group.

Note 2: P-values for CMH test stratified by study site.

None of the secondary efficacy parameters have statistically significant differences between PerioChip™ and either of the two control groups. For completeness, Table 9 includes the distribution of sites with BOP at Month 9 for each of the treatment groups.

Table 9
BOP at Month 9
Study US 94-003

| Treatment | Neither site BOP | One site BOP | Both sites BOP | P-value |
|--------------|------------------|--------------|----------------|---------|
| PerioChip™ | 13 (11.1%) | 44 (37.6%) | 60 (51.3%) | |
| Placebo Chip | 13 (11.3%) | 36 (31.3%) | 66 (57.4%) | .443 |
| SRP only | 11 (9.6%) | 33 (28.7%) | 71 (61.7%) | .146 |

Note 1: All comparisons are to the PerioChip™ group.

Note 2: P-values for CMH test stratified by study site.

Reviewer's Comment: The claim that PerioChip™ reduces bleeding on probing is not supported by study US 94-003.

Table 10 includes the results of the analysis by the number of chips placed which was requested by the medical reviewer. As would be expected, patients who received additional chips had deeper pockets at baseline than those who did not receive additional chips. The table also shows that the mean pocket depth reduction from baseline increases the month following the placement of an additional chip for those sites which received two chips and for those which received three chips the mean pocket depth reduction from baseline is greatest at Month 9.

Table 10
Adjusted Mean Reduction in PD by Number of Chips Placed
Study US 94-003

| Treatment | N | # Chips | Baseline | Month 3 | Month 6 | Month 9 |
|-----------|----|---------|----------|---------|---------|---------|
| Placebo | 57 | 1 | 5.07 | 1.29 | 1.38 | 1.20 |
| | 25 | 2.3 | 5.61 | .34 | 1.01 | .73 |
| | 18 | 2.6 | 5.38 | 1.25 | .31 | .79 |
| | 76 | 3 | 5.99 | .21 | .13 | .26 |
| PerioChip | 60 | 1 | 5.24 | 1.30 | 1.39 | 1.26 |
| | 21 | 2.3 | 5.64 | .41 | 1.33 | 1.38 |
| | 19 | 2.6 | 5.37 | 1.17 | .37 | .75 |
| | 68 | 3 | 6.08 | .08 | .10 | .38 |

Note 1: N is the number of patients who received a given number of chips. The total N is more than the total number of patients because each tooth site of a patient did not necessarily receive the same number of chips.

Note 2: # Chips=2.3 means second chip given at Month 3

Chips=2.6 means second chip given at Month 6

Subset Analysis

To investigate possible differences among demographic subsets, subgroups of patients were formed by gender, race (white vs. others), age (< 50 years, ≥ 50 years), and smoking status. The data of the two US studies were pooled to provide a larger sample size to detect a significant interaction. For each subset, the analysis performed on PD reduction from baseline was done using ANCOVA as before. In order to test for interaction of the given subset with treatment, the given subset and its respective interaction with treatment were added to the model. At the 0.10 level for the interaction term, the only statistically significant interaction term ($p=0.0498$) was between PerioChip™ and SRP only for smoking status. For all subsets, the reduction of PD was greater for the PerioChip™ than either the Placebo Chip or SRP only groups. This reduction was more pronounced for PerioChip™ males than PerioChip™ females, PerioChip™ whites than PerioChip™ nonwhites, PerioChip™ nonsmokers than PerioChip™ smokers, and PerioChip™ patients ≥ 50 years old than PerioChip™ patients < 50 years old. Table 11 includes the results of the mean reduction from baseline at Month 9 in PD by each subset variable.

Table 11
Adjusted Mean Reduction in PD (mm) at Month 9 by Subset
Pooled Studies US 94-002 and US 94-003

| PerioChip Comparison | Int. P-value | White | | | Female | | |
|----------------------|--------------|-------------|---------------|---------|-------------|---------------|---------|
| | | Control (N) | PerioChip (N) | P-value | Control (N) | PerioChip (N) | P-value |
| vs. Placebo Chip | .5902 | (N=105) | (N=102) | .0152* | (N=117) | (N=123) | .0669 |
| vs. SRP only | .5745 | .5968 | .8543 | .0418* | .7507 | .9306 | .0669 |
| | | | | | | | |
| | | White | | | Non-White | | |
| vs. Placebo Chip | .7225 | (N=166) | (N=170) | .0047* | (N=56) | (N=55) | .2233 |
| vs. SRP only | .1736 | .7454 | .9779 | .0001* | .4713 | .6453 | .2233 |
| | | | | | | | |
| | | Non-Smoker | | | Smoker | | |
| vs. Placebo Chip | .1063 | (N=147) | (N=149) | .1252 | (N=75) | (N=76) | .0021* |
| vs. SRP only | .3694 | .7357 | .8715 | .0151* | .5610 | .9424 | .0045* |
| | | | | | | | |
| | | Non-Smoker | | | Smoker | | |
| vs. Placebo Chip | .1948 | (N=141) | (N=141) | .0014* | (N=81) | (N=84) | .4071 |
| vs. SRP only | .0498** | .7417 | 1.03 | .0001* | .6390 | .7369 | .4071 |
| | | | | | | | |
| | | | | | | | |

*Indicates significance at the 0.05 level.

**Indicates significant interaction at the 0.10 level.

Note: The pairwise comparison p-values adjust for study, smoking status, baseline value, subset variable of interest, and the respective treatment interaction.

III. Safety Evaluation

The following is an analysis of the safety data provided by the sponsor. It is to be noted that this analysis contains all patients enrolled in the two US Phase III studies as well as the patients enrolled in the European/Israeli Phase III study. Recall that the European/Israeli study was a split-mouth study. Therefore, only a summary of the adverse events recorded for the European/Israeli study are reported

Adverse Events

Table 12 summarizes the reported adverse events for US studies 94-002 and 94-003 combined. The total number of patients, the number and percent of patients with at least one adverse event, and the total number of adverse events for PerioChip and Placebo Chip are included in the table. From the table, it can be seen that the difference between PerioChip and Placebo Chip relative to the number of patients with adverse events was not statistically significant (p=.847).

Table 12
Adverse Events
US Studies 94-002 and 94-003 Combined

| | PerioChip | Placebo Chip | P-value |
|------------------------|------------------|---------------------|----------------|
| Total # Patients | 225 | 222 | |
| # (%) Patients with AE | 193 (85.8%) | 189 (85.1%) | .847 |
| Total # AEs | 784 | 777 | |

Table 13 summarizes the adverse events in the European/Israeli study.

Table 13
Adverse Events
European/Israeli Study 92-002

| | |
|------------------------|------------|
| Total # Patients | 172 |
| # (%) Patients with AE | 95 (55.2%) |
| Total # AEs | 148 |

Adverse Events Related to Study Drug

Tables 14 and 15 present the adverse events that were considered possibly, probably, or definitely related to the study drug for US studies 94-002 and 94-003 combined and European/Israeli Study 92-002, respectively. These tables include the total number of patients, the number and percent of patients with at least one study drug related adverse event, and the total number of study drug related adverse events. From Table 14, it can be seen that the difference between treatment groups relative to the number of patients with drug related adverse events is significant ($p=.01$). The PerioChip group has more patients with drug related adverse events than the Placebo Chip group. The percentage of patients with PerioChip related adverse events are similar in the combined US studies and the European/Israeli study, 32.0% and 33.1%, respectively.

Table 14
Adverse Events Possibly, Probably, or Definitely Related to Study Drug
US Studies 94-002 and 94-003 Combined

| | PerioChip | Placebo Chip | P-value |
|------------------------|------------------|---------------------|----------------|
| Total # Patients | 225 | 222 | |
| # (%) Patients with AE | 72 (32.0%) | 47 (21.2%) | .010 |
| Total # Related AEs | 93 | 64 | |

Table 15
Adverse Events Possibly, Probably, or Definitely Related to Study Drug
European/Israeli Study 92-002

| | |
|------------------------|------------|
| Total # Patients | 172 |
| # (%) Patients with AE | 57 (33.1%) |
| Total # Related AEs | 85 |

As discussed by the sponsor, toothache was the most prevalent adverse event related to treatment. Toothache was mainly reported within the first three months and especially in the first week after initial chip placement. Therefore, the sponsor concluded that it may be suggested that toothache was associated with SRP along with initial chip placement. Even though there was an increased occurrence of toothache in the PerioChip compared to the Placebo Chip group which suggests a relationship to the presence of the active ingredient, the toothaches were generally of mild to moderate severity and of a transient nature.

**APPEARS THIS WAY
ON ORIGINAL**

Reviewer's Conclusions (which may be conveyed to the sponsor in the action letter)

1. *Two randomized, multicenter, double blind studies were provided to support the claim of efficacy of PerioChip™ versus Placebo Chip and SRP only in the treatment of periodontitis. A multicenter, blinded split-mouth study performed in Europe and Israel was provided as additional support of the safety of PerioChip™.*
2. *All efficacy treatment comparisons are performed on the Intent-to Treat/ Last Observation Carried Forward (ITT/LOCF) population. This differs slightly from the sponsor performed analyses in that the sponsor did not use the ITT population defined at baseline and then use a LOCF for the remaining visits but instead defined at each monthly visit an ITT population. The conclusions drawn are similar though. As determined by the sponsor, there was an insignificant difference in the number of patients included in the ITT and per protocol populations and the results for both of these analyses were similar. Therefore, the per protocol population was not analyzed for this review.*
3. *Based on the efficacy analyses performed, it has been demonstrated that PerioChip™ is statistically significantly more effective than Placebo Chip or SRP alone in the reduction of PD. It is up to the medical reviewer to determine whether this difference is clinically significant since the difference observed did not reach the difference stated by the sponsor at the End of Phase II meeting to claim clinical efficacy of PerioChip™. Even though statistical significance was not achieved showing a difference in the mean gain of AL between PerioChip™ and Placebo Chip or SRP only, PerioChip™ did not cause further worsening in AL. It was shown in only one of the two pivotal efficacy studies that PerioChip™ has statistically significantly fewer sites with BOP at Month 9 than either Placebo Chip or SRP only.*
4. *The safety of PerioChip™ has been demonstrated. The PerioChip™ group had significantly more treatment related adverse events than the Placebo Chip group however, most of these adverse events were mild to moderate toothaches which may be expected in any dental population.*

/S/

9/10/97

Cheryl Dixon, Ph.D.
Biostatistician, DOB IV


Sept 10, 97

Concur: R. Srinivasan
Team Leader, DOB IV

cc:

Archival NDA 20-774 PerioChip

HFD-540

HFD-540/ Dr. Wilkin

HFD-540/ Dr. Kelsey

HFD-540/ Dr. Hyman

HFD-540/ Dr. Blatt

HFD-725/ Dr. Harkins

HFD-725/ Dr. Srinivasan

HFD-725/ Dr. Dixon

Chron.

This review contains 14 pages.

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CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-774

MICROBIOLOGY REVIEW(S)

REVIEW FOR HFD-540
OFFICE OF NEW DRUG CHEMISTRY
MICROBIOLOGY STAFF, HFD-805
MICROBIOLOGIST'S REVIEW No. 1 OF NDA

540
Kozma-
Forma

OCT 3 1997

2 October 1997

A. 1. NDA 20-774

SPONSOR Oxford Research International Corp.
1425 Broad Street
Clifton, NJ 07013-4221

2. PRODUCT NAMES: PerioChip (chlorhexidine gluconate) 2.5 mg
3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Solid cross-linked gelatin matrix containing chlorhexidine gluconate for topical application into periodontal pockets for 7 to 10 days
4. METHOD(S) OF STERILIZATION: Not sterilized. Microbial limits are specified.
5. PHARMACOLOGICAL CATEGORY: Antimicrobial, periodontal product
6. DRUG PRIORITY CLASSIFICATION: 3S

B. 1. DATE OF INITIAL SUBMISSION: 20 December 1996

2. DATE OF AMENDMENT: (none)

3. RELATED DOCUMENTS: (none)

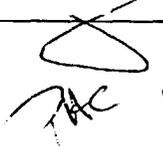
4. ASSIGNED FOR REVIEW: 9 September 1997

C. REMARKS: The review division has sent volume 1.11, volume 1.12 and copied stability studies (pages 039 - 071) from the primary stability studies.

The source of gelatin for the product is not reviewed here relative to issues and specifications for product components were not submitted. The IND for this product (IND was reviewed (submission date February 16, 1994, Microbiologist's Review #2) did address certification of the gelatin and notes the source was Croda Colloids, UK, and a UK Ministry of Agriculture, Fisheries and Food certificate for the gelatin was provided to FDA at that time. The current source of gelatin should be documented and if different from that previously accepted, a new certificate should be requested.

D. CONCLUSIONS: The application is approvable. The chemist should note remarks (above) concerning the suitability of gelatin for pharmaceutical use. Additional information requested for the NDA file by the microbiologist is provided in the "Microbiologist's List of Comments".

ISI
10-3-97

David Hussong, Ph.D. 
PAC 10/3/97

cc:

HFD 540/Consult File
HFD 540/ Kozma-Fornaro
HFD 830/ Vidra
HFD 805/D. Hussong

Drafted by: D. Hussong, 10/02/97
R/D initialed by: P. Cooney

Filename, c:\nda\20-774r1.wpd

**CONSULT
DIVISION OF ANTI-INFECTIVE DRUG PRODUCTS
CLINICAL MICROBIOLOGY REVIEW**

NDA#: 20-774 REVIEW: #1 REVIEW DATE: Initial 6/27/97 Final 10/7/97

| SUBMISSION TYPE | DOCUMENT DATE | CDER DATE | ASSIGNED DATE |
|------------------------|----------------------|------------------|----------------------|
| ORIGINAL NDA | 12/20/96 | 12/27/96 | 1/26/97 |

NAME & ADDRESS OF APPLICANT: PERIO PRODUCTS LTD.
7 HAMARPEH STREET
HAR HOTZVIM INDUSTRIAL ZONE
JERUSALEM 91237, ISRAEL

CONTACT PERSON: Dr. Robert J McCormack
Oxford Research International Corp.
1425 Broad Street
Clifton, New Jersey 07013-4221
Phone Number: 201-777-2800

DRUG PRODUCT NAME:

| | |
|------------------------|-------------------------|
| Proprietary: | PerioChip |
| Nonproprietary: | None |
| Code Names/#'s: | None |
| Chemical Name: | Chlorhexidine Gluconate |
| Molecular Description: | See USP(1) |
| Therapeutic Class: | 3S |

INDICATION(S): Treatment of Periodontal Disease

DOSAGE FORM: Chip
STRENGTH: 2.5mg/chip
ROUTE OF ADMINISTRATION: Insertion into gingival crevice
DISPENSED: Rx

RELATED DOCUMENTS: IND

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CLINICAL MICROBIOLOGY REVIEW

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REMARKS/COMMENTS:

INTRODUCTION:

This review is of the application for the product PerioChip, which is a hydrolyzed gelatin chip impregnated with the antibacterial compound chlorhexidine gluconate. The chip is used along with root scaling and planing for the treatment of periodontal disease. The chip is inserted by a dental professional into the diseased gingival crevice. The chip releases the chlorhexidine gradually over a period of days with the chip eventually dissolving thus alleviating its removal.

PRE-CLINICAL EFFICACY

IN-VITRO

SPECTRUM OF ACTIVITY:

Chlorhexidine is 1,6-di(4-chlorophenol-diguanide) hexane, a cationic bisbiguanide. Chlorhexidine itself is a strong base practically insoluble in water. Solubility is dependent on the salt form. Chlorhexidine digluconate is the most soluble form of chlorhexidine(2).

The antimicrobial spectrum of activity of chlorhexidine includes vegetative gram-positive and gram negative bacteria inclusive of vegetative anaerobes(2). It is inactive against bacterial spores except at elevated temperatures(3). Chlorhexidine has antifungal activity with this activity being greater against the yeast forms than the mold forms. The level of activity varies with the species of the fungi. As is the case with bacterial spores chlorhexidine is inactive against fungal spores(4). Chlorhexidine has been shown to have clinically relevant activity against those bacteria which have been associated with periodontal disease(5,6,7,8).

MECHANISM(S) OF ACTION:

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At low concentrations (approximately $<100\mu\text{g/mL}$) chlorhexidine tends to be bacteriostatic while at higher concentrations it is bactericidal. The mechanism of bacteriostasis is not well understood(2). The bactericidal concentrations vary from genus to genus of microorganisms and within the genus from species to species(2).

The main site of action of chlorhexidine is the cellular membrane of bacteria and fungi and the lipophilic envelope of viruses(9). This activity against the cellular membrane results in dissolution of the membrane with resulting leakage of the cytoplasmic content. In the case of chlorhexidine-induced leakage of intracellular material from *Escherichia coli* and *Staphylococcus aureus* a diphasic leakage/concentration pattern is found. The first part of the pattern(kill curve) shows increasing leakage of cytoplasm as the concentration of chlorhexidine increases. The second part of the curve shows that at higher concentrations the leakage actually slows. This is due to the fact that the chlorhexidine causes a coagulation of the cytoplasmic protein and this coagulation tends to slow down the flow of the cytoplasmic content from the affected cell(10). Bacteriostatic concentrations of the compound do not cause leakage of cytoplasmic material(10). At bacteriostatic concentrations enzyme activity associated with transport activities across the cell membrane are believed to be inhibited(2). The rapid activity of chlorhexidine against bacteria is partially attributed to the fact that chlorhexidine is a positively charged molecule which is readily attracted to the negatively charged bacterial cell(2).

A "depathogenizing" effect of chlorhexidine has been described in the literature. The term relates to the phenomenon that sublethal levels of chemicals alter or damage bacterial cells in such a way as to reduce their ability to initiate the disease process. Holloway(11) showed this effect with chlorhexidine in a mouse peritonitis model. Pathogenic strains of *Escherichia coli* and *Klebsiella aerogenes* treated with sublethal concentrations of chlorhexidine were shown to be less capable of causing infection in the mouse. This work was later confirmed by Rotter(12). Minhas et al(13) has shown that sub-lethal concentrations of

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chlorhexidine significantly inhibit the production of trypsin-like proteases in *Porphyromonas (Bacteriodes) gingivalis*. The significance of these findings to the periodontal disease process is not specifically known. However, the potential exists that while organisms may be culturable from diseased sites their ability to cause disease is reduced. A possible measure of this would be the return to health of the diseased area and not the absence or the presence of periodontal pathogens.

MECHANISM(S) OF RESISTANCE:

Some bacteria, notably strains of *Proteus* and *Providencia* may be highly resistant to chlorhexidine(14,15). This resistance is believed to be due to membrane impermeability to chlorhexidine(14,15)and is intrinsic in nature(16,17).Laboratory tests have failed to demonstrate conclusively whether it is, or is not possible to "train" organisms to become resistant to chlorhexidine(17). The possible relationship between chlorhexidine resistance or susceptibility and the response to other, chemically unrelated antiseptics and antibiotics has left many questions unanswered(17). To this time the issue remains unresolved. As to this date no conclusive evidence exists to show that resistance to chlorhexidine is due to the presence of plasmids. Grenier, et al(18) have recently reported that the periodontal pathogen *Porphyromonas gingivalis* may protect itself from the action of chlorhexidine by producing vesicles. These vesicles may protect the organism by binding the chlorhexidine.

EPIDEMIOLOGY:

Chlorhexidine was first synthesized in 1950 and shown at that time to have antibacterial and antifungal properties, a strong affinity for skin and mucous membranes, and minimal toxicity. Shortly thereafter it was introduced into the market as an antiseptic for application to skin, wounds, and mucous membranes. In addition, it is used as a preservative for ophthalmic solutions and as a disinfectant(2). Despite the use of chlorhexidine in a variety of products for over 40 years no conclusive evidence exists in the literature that microorganisms have developed resistance to it(16).

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In the dental profession chlorhexidine has been advocated to be used to prevent caries, inhibit the development of plaque and gingivitis and treat dental infections for over 25 years(19). The effects of chlorhexidine on the development of plaque has been studied extensively(20). Many studies have looked for the development of resistance to chlorhexidine in plaque bacteria after use of chlorhexidine for as long as two years and while there were slight sporadic changes in the oral flora susceptibility to chlorhexidine long term resistance was not found(21,22,23). The bacteria isolated from the plaque were also shown to maintain there susceptibility to antibiotics after prolonged use of chlorhexidine(8). Studies using chlorhexidine to treat periodontal disease that have looked at the development of chlorhexidine resistant bacteria or bacteria resistant to unrelated chemicals or antibiotics have not conclusively identified this as a matter of concern(24,25,26). The applicant has provided data to show that the use of PerioChip for as long as 6 months does not result in the development of bacterial populations resistant to chlorhexidine. This is consistent with the published literature for this type of chlorhexidine use.

IN-VIVO**BIOAVAILABILITY:**

Each PerioChip contains 2.5mg chlorhexidine bound in a hydrolyzed gelatin matrix. The PerioChip releases chlorhexidine into the gingival crevicular fluid in a sustained-release manner over 7-10 days while simultaneously biodegrading. The applicant has provided information indicating that there is a burst of release of the chlorhexidine of from $\mu\text{g/mL}$ of crevicular fluid in the first four hours post insertion. This initial peak is followed by sustained release for 7-10 days. The range of the mean chlorhexidine concentration maintained in the gingival crevicular fluid at nine days is 55 to 57 $\mu\text{g/mL}$ of crevicular fluid. These concentrations of chlorhexidine are above the levels of chlorhexidine needed to inhibit the growth of the periodontopathic organisms *Porphyromonas (Bacteriodes) gingivalis* and *Prevotella (Bacteriodes) intermedia*(6).

PHARMOKINETICS:

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The applicant has provided data to indicate that the level of chlorhexidine in the plasma and urine of subjects treated with PerioChip is not detectable. It can only be surmised that since the level of chlorhexidine is at best extremely low that transient microorganisms in the blood or urine would have none or minimal opportunity to develop resistance to chlorhexidine. The amount of chlorhexidine that could be ingested and find its way to the intestines is exceedingly small even if the maximum number of chips(8) allowable were placed in the patients mouth at one time. Thus the opportunity for disruption of the patients intestinal flora to the point of causing gastrointestinal upset(diarrhea, etc), or the development of resistant intestinal flora is highly unlikely.

CLINICAL EFFICACY

CLINICAL MICROBIOLOGY:

Isolates/relevance to proposed indications:

The organisms which the applicant is claiming chlorhexidine has activity against and those which were monitored during clinical trials are recognized as being associated with periodontal disease(5,6). In addition, there was no indication in the data of colonization or overgrowth of the treated site or oral cavity with specific microorganisms. This data is consistent with the published literature as it relates to the use of chlorhexidine in dental practice(23,24,25,26).

Microbiological Efficacy:

DNA probes were used as a tool to determine the colony forming units of the periodontotrophic organisms *Porphyromonas(Bacteriodes) gingivalis*, *Prevotella(Bacteriodes) intermedia*, *Bacteroides forsythus*, *Campylobacter rectus(Wolinella recta)*, *Eikenella corrodens*, and *Actinobacillus actinomycetemcomitans* prior to and after insertion of PerioChips. Significant levels of reduction in the colony forming units of *B. forsythus* and *C. rectus* in relation to controls were noted after six months in patients receiving one 2.5mg chlorhexidine chip. In those patients receiving two 2.5mg chips significant

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reductions in colony forming units of *P. gingivalis*, *P. intermedia* and *B. forsythus* in relation to controls were shown after one month post insertion of the chips.

The DNA probe and MIC data submitted by the applicant suggests activity of the chlorhexidine delivered by a chip against putative periodontal pathogens. This data is consistent with published data. The submitted data indicates a correlation between elimination or reduction in the population of potential periodontal pathogens by PerioChip and elimination of gingival bleeding and reduction in probing pocket depth. Elimination of gingival bleeding and reduction in probing pocket depth suggests resolution of the periodontal disease state (27,28,29,30).

Package Insert:

Those microorganisms which are correlated with periodontal disease and for which the applicant has shown significant reductions in the number of colony forming units found in PerioChip treated patients and control patients are:

The applicant has also provided data which indicates that the use of chlorhexidine has a very low potential of causing a shift in the residing micro flora so as to cause colonization or overgrowth in the oral cavity of undesirable organisms. This data is consistent with the published literature (26).

CONCLUSIONS AND RECOMMENDATIONS:

Additional information provided by the applicant on 7/30/97 and 10/2/97 at the request of the reviewer were reviewed in addition to the initial submission.

The microbiology data submitted supports the request of the applicant to market this product for the treatment of periodontal disease. The microbiology portion of this NDA is acceptable

approved

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contingent on the following changes being made to the "Microbiology" portion of the labeling(package insert).

PACKAGE INSERT:

Based on the review of the information provided by the applicant and the literature reviewed the following changes are made to the package insert submitted by the applicant. The reference cited in the the proposed insert by the applicant has been deleted because it does not make reference to the following organisms

CLINICAL PHARMACOLOGY

Microbiology: Chlorhexidine gluconate is active against a broad spectrum of microbes. The chlorhexidine molecule, due to its positive charge, reacts with the microbial cell surface, destroys the integrity of the cell membrane, penetrates into the cell, precipitates the cytoplasm, and the cell dies. Studies with PerioChip showed reductions in the numbers of the putative periodontopathic organisms

after placement of the chip. No overgrowth of opportunistic organisms or other adverse changes in the oral microbial ecosystem were noted. The clinical significance of these findings, however, is not known.

NOTE: DELETE REFERENCE -

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/S/

Frederic J. Marsik, Ph.D.
Review Microbiologist

cc: Original 20-774
HFD-540 Division File
HFD-540/MO/C. Gilkes
HFD-540/DO/F. Hyman

Concurrence Only

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HFD-540/DO/J. Kelsey
HFD-540/Chem/J. Vidra
HFD-540/Pharm/Tox/N. See
HFD-540/Stat/C. Dixon
HFD-540/Bioparm/D. Bashaw
HFD-540/CSO/H. Blatt
HFD-520/Micro/F. Marsik

HFD-520/Dep/Dir./L. Gavrilovich
HFD-520/GLMicro/A. T. Sheldon

TS 10/12/97
up 10/16/97

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-774

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

Clinical Pharmacology/Biopharmaceutics Review

Chlorhexidine Gluconate
NDA 20-774
PerioChip® 2.5mg
Reviewer: E.D. Bashaw, Pharm.D.
APW

Perio Products, Ltd.
Clifton, NJ 07013

Submission Date:
20-Dec-96
6-Oct-97

Review of an NDA

I. Background

PerioChip® (PC) is an intra-oral delivery system intended for insertion into the periodontal pocket that is formed between the tooth and the gum in cases of mild to moderate chronic adult periodontitis where standard therapy (root planing and scaling) may be insufficient (see Fig. 1, next page for a schematic on insertion). Each PC contains 2.5mg of chlorhexidine gluconate (CHX) in hydrolyzed gelatin matrix cross-linked with glutaraldehyde to form an in-soluble compound that can only be dissolved by the action of proteolytic enzymes. This matrix is designed to be degraded in the periodontal pocket by enzymes in the gingival crevicular fluid (GCF) such that there is a "continuous" release of drug over 7-10 days.

Adult periodontitis is a microbe-mediated gingival disease that causes progressive loss of the connective tissue supporting the teeth and may lead to tooth loss. The main cause of periodontitis is bacterial plaque resulting from the colonization of bacteria on the tooth surface and under the gingival margin. The micro-organisms in plaque produce toxins, metabolic end products, and enzymes that invade the gums causing inflammation, which is characterized by swollen, bleeding gums. This process eventually leads to loss of gingival-tooth attachment with the formation of periodontal pockets. These pockets can, without treatment, lead to the progressive loss of periodontal ligaments, bone resorption in the jaw and eventually tooth loss due to the loss of bone support for the tooth. Chlorhexidine and other broad spectrum antibiotics have been used as part of a treatment regimen that includes manual removal of the bacterial plaques.

The PC has been developed for use in cases of chronic periodontitis where the periodontal pocket depth has reached such an extent that root planing and scaling are not as effective. Chlorhexidine was chosen as the antibiotic of choice for inclusion in to the PC due to the lack of bacterial resistance to it.

II. Recommendation

Chlorhexidine is currently approved for use orally as a 0.12% oral rinse (Peridex®, others) and as a topical antibiotic (Hibiclens®, others). In this NDA the applicant is proposing a maximal exposure of 20mg of chlorhexidine (8 PC's x 2.5mg). As the dose of drug is an integral component of the matrix, the delivery of this amount of drug over 7-10 days would and did result in undetectable plasma levels of chlorhexidine in both plasma and urine. While they are

requesting the use of 8 PC's at any one time, the applicant only provided in vivo pharmacokinetic information for the use of 4 PC's. In response to a request from this reviewer, the applicant provided additional information from the literature as to the absorption of chlorhexidine following oral administration. This information in combination with the in vivo data presented by the applicant are sufficient to support the use of up to 8 PC's at any one time in a patient. From a biopharmaceutic standpoint the applicant has met the requirements under 21 CFR 320 as they relate to the approvability of the application.

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III. NDA Overview

This NDA consists of three in vivo pharmacokinetic studies. Two of these studies looked at the resulting plasma levels of chlorhexidine following PC insertion and two studies followed GCF levels over the nine days following PC insertion. In all of the studies involving plasma, no plasma samples had quantifiable levels of chlorhexidine present at any time point while all subjects in the GCF studies had quantifiable levels in their GCF.

Dosage Form Description

The PC is composed of a cross-linked gelatin matrix that is intended for insertion into the periodontal pocket in subjects with chronic adult periodontitis. It is 5mm long, 4mm wide, and 270 µm thick. It is "designed" to deliver a burst of chlorhexidine into the periodontal pocket over the first 24-48hrs. The remainder of the drug will then be slowly liberated via enzymatic degradation of the gelatin matrix crosslinks over 7-10days.

IV. Analytical

V. Summary of Pivotal Studies

A. Plasma Pharmacokinetics

Study 89-001

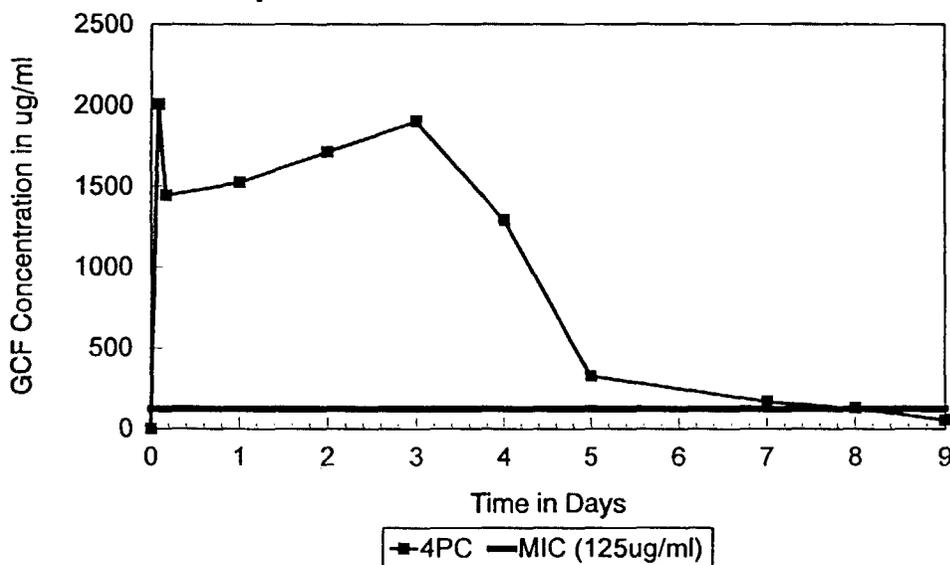
This study was conducted in 20 healthy male and female volunteers with chronic adult periodontitis. Each subject was required to have at least 4 periodontal pockets from 6-9mm in depth in two quadrants (two pockets per quadrant) with each pocket separated by at least 1 tooth. The subjects were divided into two groups (active and placebo). In the active treatment group ten subjects had two PC's placed in two of four periodontal pockets. In the placebo group ten subjects had placebo PC's containing no CHX placed in two of the four periodontal pockets. The other two pockets in each group went untreated as control pockets. During the trial plasma and urine was collected for both CHX and p-chloroaniline (a metabolite of CHX) levels at screening (14 days prior to dosing), 1 day after dosing, and 14 days after dosing. No detectable plasma or urine levels of either CHX or p-chloroaniline were seen at any timepoint during this trial.

Study 95-000A

This study was designed to look at plasma, urine, and GCF levels in subjects receiving multiple PC's. A total of 19 subjects (7M/12F) between the ages of 32 to 62 with chronic adult periodontitis were enrolled in the trial. Each subject was required to have at least four periodontal pockets between 5-8mm deep with bleeding present on probing. On day 1, four target periodontal pockets were selected randomly by a computer from the available pockets present in each subject. Using standard techniques the individual pocket depths and crevicular fluid volume was determined using a Periotron® measuring device. After this was done, the PC

was implaced in each pocket with a forceps such that it was totally submerged below the gingival margin. Over the next 10 days the subjects returned for determination of both crevicular fluid turnover and CHX concentration in each pocket. This was done by inserting a periopaper strip into the affected periodontal pocket and leaving it there until 2/3 of it was saturated with fluid or 30s was reached. When this occurred, it was removed and the time was recorded. The paper strip was then placed in the Periotron® to determine the rate of fluid production. After this, the strip was placed in a labeled glass vial for shipping to the applicant for CHX concentrations. Reproduced below are the results from the crevicular fluid analysis for CHX presented graphically:

PerioChip Mean GCF Concentrations



4PC data from Study 95-000A

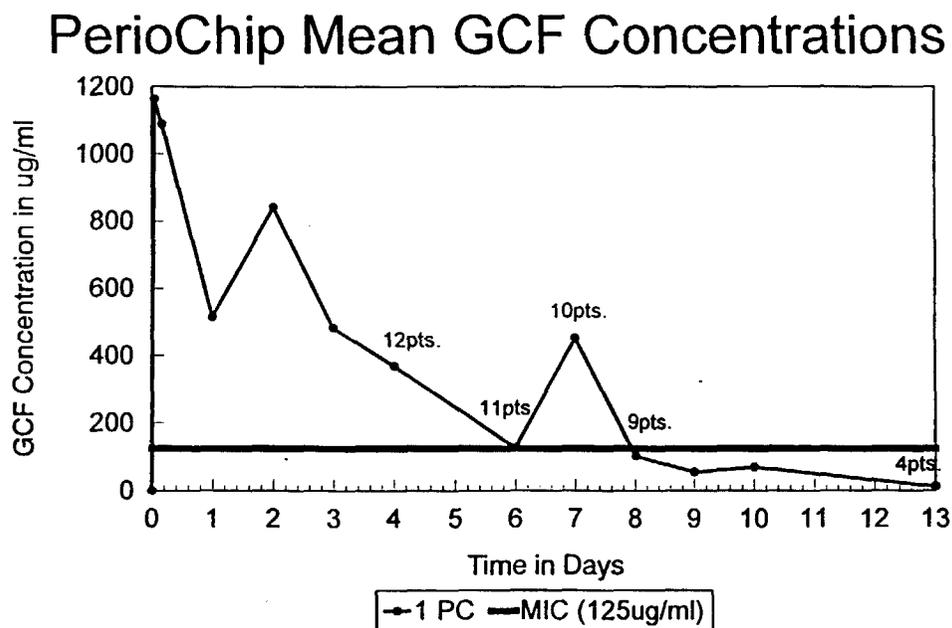
The data from this trial demonstrate that over a 10 day period the PC is capable of delivering CHX into the periodontal pocket. The rate of delivery is quite variable and the resulting amounts are also variable (the raw data supporting this graph are attached in Appendix I). Of the 19 subjects enrolled in this trial, one subject was removed from the analysis due to protocol violation (use of a chlorhexidine containing oral rinse). Of the 18 subjects available for analysis the PC produced GCF levels throughout the observation interval. The MIC indicated above is the MIC as reported by the applicant for the majority of intra-oral microbes that are thought to be involved in pocket formation. It is only provided for some perspective and is NOT intended to imply an acceptance of the PC clinical efficacy via this mechanism. What can be said is that in the majority of patients with periodontal pockets, the PC maintained levels of CHX above 100ug/ml in the GCF over 1 week.

As for the plasma and urine analysis, no detectable CHX or p-chloroaniline were detected in any of the subjects during this trial.

B. Gingival Crevicular Fluid

Study 95-000

This study was done to assess the actual pocket (crevicular fluid) concentrations of CHX in vivo. A total of 12 healthy subjects (7M/5F) between the ages of 32 to 63 with chronic adult periodontitis were screened and enrolled in this trial. Each subject had to have no more than 5 periodontal pockets (5-8mm in depth) present in their mouth. On day 1, a target periodontal pocket was selected and the pocket depth and crevicular fluid volume was determined using a Periotron® measuring device. After this was done, the PC was implaced via a forceps such that it was totally submerged below the gingival margin. Over the next 14 days, the subjects returned for determination of both crevicular fluid turnover and CHX concentration. This was done by inserting a periopaper strip into the affected periodontal pocket and leaving it there until 2/3 of it was saturated with fluid or 30s was reached. When this occurred, it was removed and the time was recorded. The paper strip was then placed in the Periotron® to determine the rate of fluid production. After this, the strip was placed in a labeled glass vial for shipping to the applicant for CHX concentrations. Reproduced below are the results from the crevicular fluid analysis for CHX presented graphically:



The data from this trial demonstrate that over a 14 day period the PC is capable of delivering CHX into the periodontal pocket. The rate of delivery is quite variable and the resulting amounts are also variable (the raw data supporting this graph are attached in Appendix I). The numbers associated with selected points in this figure correspond to the number of patients with detectable GCF levels at those timepoints. The MIC indicated above is the MIC as reported by the applicant for the majority of intra-oral microbes that are thought to be involved in pocket formation. It is only provided for some perspective and is NOT intended to imply an acceptance of the PC clinical efficacy via this mechanism. What can be said is that in the

majority of patients with periodontal pockets, the PC maintained levels of CHX above 100ug/ml in the GCF over 1 week.

In Vivo Data Summary

Examination of the data from the three in vivo trials performed by the applicant indicates that the PC is capable of producing prolonged levels of CHX in the periodontal pocket for at least a week. The in vivo bioavailability of the CHX released from the product is incalculable due to the lack of detectable levels using analytical methods with reasonable limits of detection (ng/ml). One point of interest is the rate of adverse events present in this study. It is the rare patient in any of these trials that does not either report bleeding or pain at the PC application pocket. While it is true that periodontitis causes both pain and bleeding to occur, the rate seen here is almost unity and should be reflected in the patient information sheet and labeling for this product.

VI. Supportive Studies

A. In Vitro Release

As part of this NDA the applicant presented two in vitro methods demonstrating release of CHX from the PC matrix. The proposed regulatory release method involves

method involves The second
second test was done to test the feasibility of the matrix to release drug This
method will be presented in turn. days. Each

Agar Plate Method

As noted above this is the proposed regulatory release method.

Representative data from three lots of PC were provided in this NDA and are presented below along with a proposed specification.

| Batch # | 1hr Release | 7hr release | 15hr release |
|----------------|-------------|-------------|--------------|
| R-369 | 17.5% | 44.3% | 55% |
| R-370 | 18.9% | 43.9% | 56% |
| R-371 | 20.2% | 47.5% | 58% |
| Average | 18.7% | 45.1% | 56.3% |
| Proposed Spec. | 1% | % | % |

There are many problems with this proposed methodology. First of all it is only a measure of the migration of CHX from the PC into an Agar sink. This method of drug release is not the in vivo method of release designed by the applicant for this product so it is NOT, in fact,

a measure of product quality/release. Secondly, this method only assesses product performance over 24 hours. Adequate for a once-a-day dosing form, but in this reviewer's opinion totally inadequate for a product designed to release drug over 8-10 days.

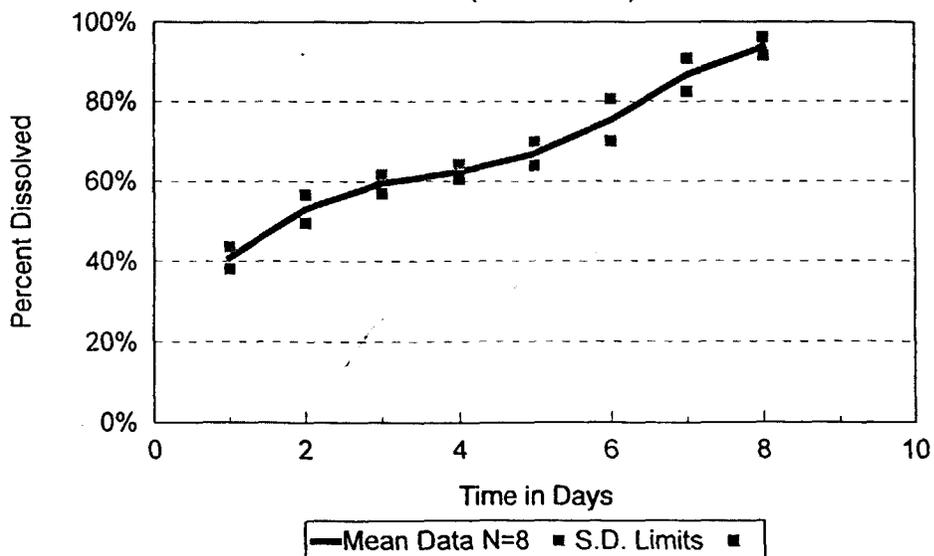
Collagenase Method

This method was designed initially to demonstrate the in vitro mechanism of release via matrix digestion via naturally occurring collagenase.

Reproduced below is a tabular and graphical representation of the data provided in the application.

| Time (days) | Mean +/-S.D |
|-------------|-------------|
| 1 | 40.9%+/-2.8 |
| 2 | 53.1%+/-3.5 |
| 3 | 59.5%+/-2.6 |
| 4 | 62.4%+/-1.8 |
| 5 | 66.9%+/-3 |
| 6 | 75.3%+/-5.3 |
| 7 | 86.7%+/-4.2 |
| 8 | 93.8%+/-2.3 |

Release of Chlorhexidine From PerioChip Over 8 Days
Mean Data (+/- 1 S.D.)



Examination of these data reveals that there is an initial burst of CHX release over the first 24 hours followed by a much slower, almost linear rate of CHX release. In an effort to capture this first release period the applicant repeated the methodology using two PC's over multiple time points in a 24hr period. These data are reproduced below.

| | Sample 1 | Sample 2 | Mean |
|-------|----------|----------|------|
| 1hr. | 20.2 | 26.2 | 23.2 |
| 2hrs. | 29.1 | 30.5 | 29.8 |
| 4hrs. | 37 | 33.2 | 35.1 |
| 8hrs. | 33.5 | 37 | 35.2 |
| 24hrs | 36.5 | 40.5 | 38.5 |

Examination of these data reveals that the rapid initial release of CHX is over by 4hrs and that the slow release of CHX continues on in a similar manner to that seen over 8 days. In comparison to the Agar plate method the collagenase method, while requiring 8 days to perform, does give reproducible results that are more in keeping with the proposed product performance characteristics. In addition, the method allows for drug release via enzymatic degradation, the same method of release proposed in vivo for this product. It is for these reasons that this reviewer prefers the collagenase release method as an in vitro control method for product release (i.e., CMC) testing. This opinion was shared with the reviewing chemist (Dr. Vidra) who concurred and has indicated in the chemistry review that the collagenase method should be adopted as the CMC release test.

At this time it is not possible to set release specifications for this test as the data provided by the applicant were only mean data from one lot of product. The applicant should provide additional information from other lots of product using this method prior to the finalization of a release specification.

B. Proposed Maximal Use Data

As noted earlier, the applicant would like permission to use upto 8 PC's at any one time. From an in vivo standpoint, sufficient clinical information has been presented by the applicant to approve this dose clinically, but no pharmacokinetic information was submitted by the applicant using more than 4 PC's at any one time. In order to evaluate this request thoroughly the applicant was asked to provide additional supportive material that could be evaluated to allow this dosing regimen. In a submission dated Oct. 6th, 1997 the applicant provided additional material consisting of literature articles on the biodisposition of CHX and arguments based upon the administered dose and in vitro release data.

Literature Data

Support for the applicant's position is provided in the published literature from an article by Wintrow¹. In this study using a radiolabeled source of CHX the disposition of CHX following oral dosing was determined in marmoset, rat, mouse, dog, Rhesus monkey, and man.

¹ Wintrow, MJ "Metabolic Studies With Radiolabeled Chlorhexidine in Animals and Man" J. Periodontal Research, 1973;8 Suppl.12:45-48.

In all species except the mouse the amount of radioactivity in the urine was <1.5%. In the single human subject in the trial less than 0.5% of the radioactivity was found in the urine and ~82% was found in the feces. Analysis of the urine by thin layer chromatography did not detect the presence of p-chloroaniline (a metabolite of CHX). While extremely limited in the number of subjects used, the study does suggest that the oral bioavailability was very low in all species. A second phase of this study involved the use of an oral rinse with expectoration. In this study after rinsing for 1 minute with a 0.2% CHX radiolabeled mouthrinse only 68% was recovered by expectoration and only an additional 5% by an additional clear water rinse. Subsequently oral plaque and saliva levels were collected over the following 24 hrs. These samples demonstrated a rapid drop in saliva levels coupled with steady concentrations in plaque suggesting a differential uptake of drug. From this data the author concludes that the bioavailability of chlorhexidine is very low and that chlorhexidine binds to oral mucosa in a differential manner.

While not disagreeing with the data presented, the small N of 1 is very unconvincing in its nature. Had the author elected to follow in vivo radioactivity the data would have been more convincing in nature. While it is true that they did repeat part of the study using a "T" tube arrangement in the hepatic duct of rats to look for biliary excretion (none was seen) the extrapolation of this type from rats to man is inherently unreliable.

The second piece of evidence put forward by the applicant is a copy of the Peridex® (0.12% Chlorhexidine gluconate oral rinse, Proctor & Gamble) package insert. In the insert they cite the following excerpts from pharmacokinetic section:

"...approximately 30% of the active ingredient, chlorhexidine gluconate is retained in the oral cavity following rinsing."

"...The mean plasma level of chlorhexidine gluconate reached a peak of 0.206µg/gm in humans 30min after they ingested a 300mg dose."

"...studies on human subjects and animals demonstrate that any ingested chlorhexidine gluconate is poorly absorbed..."

"...Less than 1% of the chlorhexidine gluconate ingested by these subjects was excreted in the urine."

It is interesting when an applicant uses another product's labeling to support the biopharmaceutic claims of another. While accepting the data here from an oral dosing perspective, the applicant in this portion of their presentation ignored the fact that the primary route of CHX absorption with their product might not be oral but might indeed be a buccal or intradental route. This possibility of an extra-hepatic route is certainly possible here as the PC matrix will be abutted to gingival tissue (see Fig. 1, page 2) that by usage definition is inflamed and subject to bleeding (implying relatively easy vascular access). The mere fact that the drug is localizing in the tissues is another possible mechanism by which CHX can obtain access to the systemic circulation.

The strongest argument put forth by the applicant is in fact their last argument, that being their in vitro release data. Using the collagenase 24hr data, it is apparent that the majority of the

dose released over the first 24hr. occurs by the second hour. At that time ~30% of the total dose has been released by the PC or approximately 0.75mg per PC. This would be equal to a total dose of 6mg (assuming 8PC's were emplaced). Of this 6mg released undoubtedly some would be swallowed and some would be available for intradental absorption over the two hour time period. The rest of the dose, being released over 6 days, would have such a slow input rate as to be extremely unlikely to contribute to any detectable circulating plasma levels of CHX and their contribution to absorption can be discounted.

With regards to the "initial burst" of CHX, this 6mg dose if absorbed quickly might yield a detectable plasma level. Unfortunately the volume of distribution of CHX is unknown. On the other hand, from the Peridex® label we know that a 300mg oral dose yielded only levels that were at the limit of detection 30min after dosing. Given that there appears to be little metabolic conversion of CHX and that following large oral doses the oral bioavailability was very small, it is highly unlikely that significant plasma levels are produced by PC system following the administration of 8 PC's even if the "initial burst" of CHX was totally absorbed. While willing to accept the extrapolation in this case, the applicant should be informed that any request to modify their labeling to use additional PC's above 8 may require an in vivo pharmacokinetic study depending on the total number of PC's used as it may be possible to detect plasma CHX levels with increased use (i.e., a larger number of PC's.)

VII. Conclusions

A review of the pharmacokinetic information provided by the applicant has yielded the following conclusions.

1.)After application of either 2 or 4 PerioChips in the mouths of subjects with chronic oral periodontitis, chlorhexidine is undetectable in either the plasma or the urine of these subjects.
2.)The PerioChip system is capable of maintaining gingival crevicular fluid level concentrations above the $\mu\text{g/ml}$ level for approximately 1 week. The levels are, however, highly variable and erratic in nature.
3.)There is no evidence of dose proportionality in gingival crevicular fluid levels following the insertion of multiple chips in a subject.
4.)The use of PerioChip is very irritating to the gingival mucosa with reports of gingival tenderness, bleeding, and pain in almost every subject.
5.)The agar plate in vitro method proposed by the applicant is inadequate. The applicant needs to develop their collagenase method as a regulatory release test.
6.)Sufficient in vitro and literature data has been provided to support the use of upto 8 PerioChips in a subject at one time.

VIII. Comments

1.)In future submissions the applicant should refrain from using the convention of ppm/ppb when referring to plasma and/or urine concentrations. Such concentrations are more appropriately provided in the corresponding ng/ml and $\mu\text{g/ml}$ convention.

2.)The agar plate method is inadequate as a quality control test as it does not follow the release of drug out far enough and the system used is not the same mechanism of drug release in vivo. The collagenase method should be developed as a regulatory release test.
3.)Should the applicant desire a modification of the labeling to allow for the use of more than 8 PerioChip's per subject, then the applicant may have to submit new in vivo pharmacokinetic trials depending on the total number of chips per subject.

IX. Labeling

The applicant has proposed the following labeling for this product. It has been modified the this reviewer. FDA changes are noted by strikethroughs for deletions and underlining for new text:

/S/

E. Dennis Bashaw, Pharm.D.
Senior Pharmacokineticist (HFD-550)
Division of Pharmaceutical Evaluation-III

Secondary Review, John Lazor, Pharm.D.

/S/

11/12/97

CC: NDA 20-774 (ORIG),
HFD-540/DIV File
HFD-540/CSO/Blay
HFD-880(Bashaw)
HFD-880(Lazor)
CDR. ATTN: B. Murphy
HFD-344(Viswanathan)

Appendix I

| <u>Study 95-000A</u> | <u>Page #</u> |
|--|---------------|
| Mean CHX Concentrations (GCF) | 1 |
| Mean CHX AUC's (GCF) Over 14 Days | 2 |
| Graphic of Mean CHX (GCF) Concentrations w/S.E. | 3 |
| | |
| <u>Study 95-000</u> | |
| Individual CHX Concentrations (GCF) & Production | 4 |
| Individual CHX AUC's (GCF) Over 14 Days | 7 |
| Graphic of Mean CHX (GCF) Concentrations w/S.E. | 8 |

Appendix 5.1.2.1 GCF sample collection - geometric mean over 4 pockets (ug/ml)

| Patient | 0 hours | 2 hours | 4 hours | 24 hours | 48 hours | 72 hours | 96 hours | 120 hours | 168 hours | 192 hours | 216 hours |
|---------|---------|---------|---------|----------|----------|----------|----------|-----------|-----------|-----------|-----------|
| 0.01 | 671.13 | 1433.05 | 1039.57 | 805.85 | 2330.89 | 853.92 | 278.46 | 8.92 | 14.38 | 0.19 | |
| 0.01 | 6473.50 | 3022.82 | 1817.32 | 3411.04 | 3307.87 | 1898.64 | 459.58 | 286.30 | 208.18 | 140.93 | |
| 0.01 | 3559.03 | 1581.00 | 2625.56 | 2818.64 | 1821.53 | 1981.77 | 293.01 | 93.24 | 251.32 | 144.64 | |
| 0.01 | 1120.28 | 987.22 | 292.70 | 680.06 | 979.51 | 1023.41 | 821.88 | 162.28 | 17.62 | 10.99 | |
| 0.01 | 5876.97 | 2161.83 | 1089.60 | 725.93 | 1706.60 | 637.89 | 74.05 | 7.68 | 57.72 | 5.58 | |
| 0.01 | 938.89 | 757.46 | 950.30 | 224.87 | 841.55 | 220.13 | 4.03 | 1.06 | 7.76 | 0.08 | |
| 0.01 | 2496.21 | 1552.96 | 1373.90 | 1435.46 | 3312.38 | 668.96 | 127.19 | 318.57 | 13.28 | 38.86 | |
| 0.01 | 2650.56 | 1158.88 | 2122.88 | 2448.84 | 1483.15 | 1646.94 | 388.18 | 0.21 | 67.38 | 46.97 | |
| 0.01 | 1321.78 | 1767.72 | 1512.85 | 1720.02 | 1252.41 | 750.24 | 17.31 | 11.91 | 11.42 | 1.02 | |
| 0.76 | 1572.56 | 503.60 | 2203.13 | 5025.46 | 1880.39 | 754.86 | 714.87 | 22.16 | 34.65 | 16.72 | |
| 0.01 | 3161.75 | 2112.73 | 2326.28 | 2575.18 | 1250.62 | 1342.13 | 488.57 | 1278.10 | 208.14 | 130.81 | |
| 0.01 | 388.31 | 294.61 | 692.93 | 1359.95 | 1190.00 | 953.77 | 120.36 | 110.49 | 285.63 | 73.64 | |
| 0.01 | 1578.40 | 1171.41 | 2260.33 | 2146.63 | 2164.09 | 2551.66 | 588.45 | 11.09 | 405.90 | 4.09 | |
| 0.01 | 779.83 | 918.51 | 1106.25 | 787.33 | 475.87 | 484.43 | 303.19 | 44.11 | 33.48 | 15.32 | |
| 0.01 | 1900.11 | 3020.46 | 1611.79 | 1419.91 | 2488.13 | 1187.33 | 384.22 | 0.73 | 51.00 | 63.58 | |
| 0.01 | 336.23 | 270.76 | 794.56 | 1575.10 | 638.76 | 579.57 | 134.09 | 104.48 | | 149.81 | |
| 0.01 | 1564.94 | 1544.65 | 1916.18 | 2733.61 | 1335.51 | 2973.56 | 448.24 | 273.86 | 219.89 | 80.07 | |
| 0.01 | 821.84 | 1513.83 | 2203.94 | 1820.81 | 3590.22 | 2058.11 | 217.32 | 181.34 | 227.12 | 109.34 | |
| 0.01 | 483.32 | 713.80 | 1741.99 | 2166.57 | 4059.11 | 1444.80 | 765.55 | 153.51 | 102.85 | 7.39 | |

Note: Values defined as <LLQ were set to zero. For the purposes of calculating the geometric mean, zero values were assigned the nominal value of 0.01

PE40

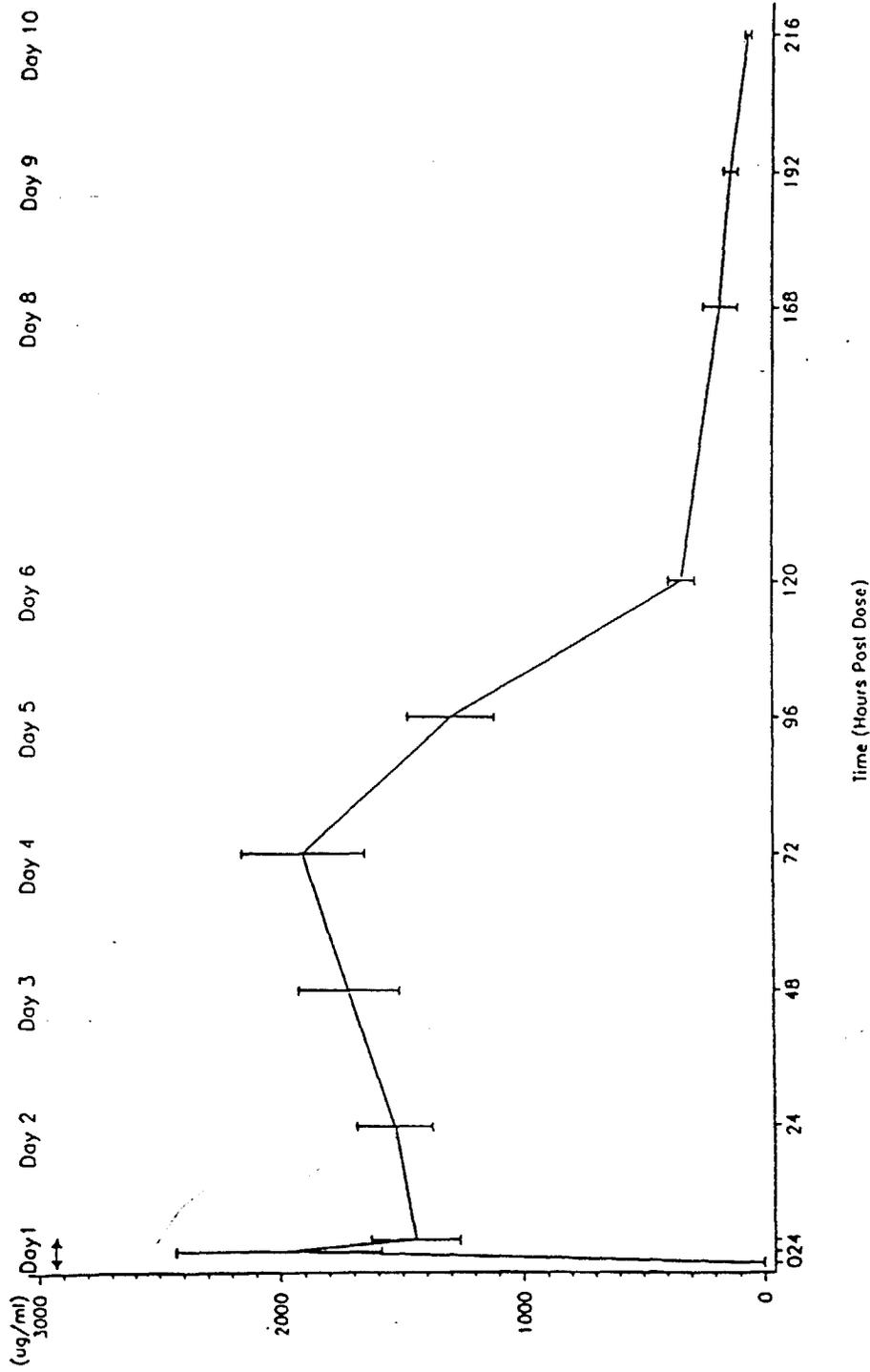
Appendix 5.1.2.2 GCF sample collection - AUC of the geometric mean chlorhexidine concentrations over 4 pockets

| Patient | AUC |
|---------|-----------|
| | 142996.93 |
| | 317588.94 |
| | 258235.10 |
| | 108105.64 |
| | 136609.42 |
| | 62302.64 |
| | 193787.39 |
| | 210148.53 |
| | 145702.58 |
| | 259732.38 |
| | 253737.24 |
| | 116630.46 |
| | 254646.68 |
| | 87266.53 |
| | 205999.40 |
| | 100652.88 |
| | 254869.79 |
| | 262372.64 |
| | 255848.42 |

Note: Values defined as <LLQ were set to zero. For the purposes of calculating the geometric mean, zero values were assigned the nominal value of 0.01

PE40

Figure 19
 Chlorhexidine Concentration (ug/ml) in Gingival Crevicular Fluid
 Geometric Mean \pm 95% Confidence Interval
 All Patients (Excluding Patient 10)



Note: Calculation of geometric mean in figure is based on the data for the 4 pockets/patient
 PE40

Appendix 5.1 Gingival crevicular fluid sample collection and analysis

| Patient | Time | Crevicular fluid time (secs) | Periotron reading | Crevicular fluid volume (μ l) | Chlorohexidine concentrations (μ g/ml) | Fluid flow rate (μ l/sec) |
|---------|-----------|------------------------------|-------------------|------------------------------------|---|--------------------------------|
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 72 (rpt) | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 24 (rpt) | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 216 (rpt) | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |

PE41 Lower Limit of Quantitation (LLQ) = .20 μ g/ml assuming the volume to be 1 μ l.

Appendix S.1 Gingival crevicular fluid sample collection and analysis

| Patient | Time | Crevicular fluid time (secs) | Periotron reading | Crevicular fluid volume (μ l) | Chlorhexidine concentrations (μ g/ml) | Fluid flow rate (μ l/Sec) |
|---------|-----------|------------------------------|-------------------|------------------------------------|--|--------------------------------|
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 96 (rpt) | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 168 (rpt) | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 4 (rpt) | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 216 (rpt) | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 144 (rpt) | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 312 (rpt) | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |

Lower Limit of Quantitation (LLQ) = 20 μ g/ml assuming the volume to be 1 μ l.

Appendix S.1 Gingival crevicular fluid sample collection and analysis

| Patient | Time | Crevicular fluid time (secs) | Periotron reading | Crevicular fluid volume (μ l) | Chlorohexidine concentrations (μ g/ml) | Fluid flow rate (μ l/sec) |
|---------|------|------------------------------|-------------------|------------------------------------|---|--------------------------------|
| | 96 | | | | | |
| | 96 | (rpt) | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |
| | 0 | | | | | |
| | 1 | | | | | |
| | 4 | | | | | |
| | 24 | | | | | |
| | 48 | | | | | |
| | 72 | | | | | |
| | 96 | | | | | |
| | 144 | | | | | |
| | 168 | | | | | |
| | 168 | (rpt) | | | | |
| | 192 | | | | | |
| | 216 | | | | | |
| | 240 | | | | | |
| | 312 | | | | | |

Lower Limit of Quantitation (LLQ) = .20 μ g/ml assuming the volume to be 1 μ l.

Appendix S.2 GCF sample collection - AUC of the chlorohexidine concentrations

| Patient | AUC |
|---------|-----------|
| | 132043.00 |
| | 53611.00 |
| | 19881.00 |
| | 55897.00 |
| | 85887.00 |
| | 88632.00 |
| | 242422.00 |
| | 109598.50 |
| | 118480.50 |
| | 61655.00 |
| | 62171.50 |
| | 77183.00 |

Figure 13
Chlorhexidine Concentration ($\mu\text{g/ml}$) in Gingival Crevicular Fluid
Mean \pm SE
All Patients

