A 6-month comparison of piezo ultrasonic scaler and hand instrumentation in the maintenance of peri-implant tissues: A randomized clinical trial

Maria Castro*, Reem Atout-, Stefan Renvert+, Anastasia Kelekis-Cholakis^
* Resident of Periodontology, University of Manitoba;
- Assistant Professor Graduate Periodontology Program, University of Manitoba
+ Professor Oral Health Sciences, University of Kristianstad, Kristianstad, Sweden;
^ Graduate Director Periodontology Program, University of Manitoba

Abstract

Objectives: The aim of this study was to compare the clinical effects, presence of inflammatory cytokines, and the patients' perceived discomfort of two mechanical non-surgical methods of peri-implant maintenance: the piezo ultrasonic scaler vs hand instrumentation.

Material and Methods: A total of thirty-four (34) patients with at least one healthy or with peri-implant mucositis dental implant were randomly assigned to the piezo ultrasonic scaler or to the hand instrumentation groups. The clinical parameters analyzed at the implant level were the following: Plaque Index (PI), Bleeding on Probing (BOP), Probing Depths (PD), Keratinized tissue (KT) and Recession (REC). All these measurements were recorded at baseline, three, and six months. Full mouth plaque index (FPI) and full mouth bleeding on probing (FBOP) were also calculated. Samples of Peri-Implant Crevicular Fluid (PICF) from the four aspects of the implant were collected for analysis of cytokine levels followed by the corresponding maintenance therapy. At the end of the study patients were asked to fill in a pain questionnaire (Visual analogue scale, VAS).

Results: Thirty-one (31) subjects completed the study (Piezo=17, SRP=14). Even though, reduction on FPI and FBOP were observed, it was found that the FPI and FBOP did not have a statistically significant difference over the 6 months or at any of the checkup times (0, 3, 6 months) for any of the treatment groups. Both peri-implant therapies slightly reduced the implant plaque index and implant probing depths in healthy and peri-implant mucositis implants from 0 to 6 months; however, these differences were not statistically significant. The model results indicated that the presence of KT and REC were not statistically significant different between treatments at any given time. This study demonstrated that the anti-inflammatory cytokine IL-4 statistically significant increased for both therapies from baseline to 6 months ($P<0.05$). There were not statistically association between the implant PD, PI, and BOP and the cytokines levels (IL-2, IL-4, IL-6, IL8, IL-10, TNFα, and IFNγ) during the six-month study period. In addition, subjects from both therapies reported minimum perceived discomfort after treatment.

Conclusion: Within the limits of this study, it was demonstrated that both peri-implant therapies had a beneficial clinical effect in the reduction of all clinical parameters; however, these results were not statistically significant. In addition, there was no statistically significant difference in the clinical outcomes measurements at any given time between the two groups and the study could not demonstrated that peri-implant therapy decreases the presence of inflammatory cytokines.

Key words: hand instrumentation; ultrasonic; cytokines; peri-implant mucositis; piezo; scalers.

Nowadays, dental implants are a very attractive and affordable treatment option for patients. According to the American Society of Implant Dentistry the estimated US and European market for dental implants is expected to reach $4.2 billion by 2022 (AAID, 2016).

Despite the high success rates of dental implants, it is clear that osseointegrated implants are susceptible to diseases (De Boever, et al., 2009). The prevalence of dental implant complications is rising as the number of individuals that are receiving implant treatment is also increasing (Berglundh, et al., 2002) (Klinge & Meyle, 2012). One of these peri-implant complications is an inflammatory condition known as peri-implant mucositis that occurs in 64.6% to 80% of the implant population (Ferreira, et al., 2006) (Roos-Jansåker, et al., 2006) (Lindhe & Meyle, 2008).

The lack of preventive maintenance therapy in subjects with peri-implant mucositis is associated with a high incidence of peri-implantitis (Costa, et al., 2012), which eventually may lead to implant loss.

Conflict of interest and source of funding statement
None of the authors have a conflict of Interests or have an affiliation with the brands used in the study. Dental hygiene aids and scalers were provided by TePe and Hu-Friedy, respectively. The College of Dentistry, Faculty of Health Sciences at the University of Manitoba, provided the funding for this study.

According to Rokn et al, 2016, after a 5-year period of implant loading without any regular maintenance program, one out of five

patients could experience peri-implantitis. Inadequate oral hygiene with plaque accumulation must be considered as a major risk factor for endo-osseous implant failure (Berglundh, et al., 1992) (Lang, et al., 1993) (Lindhe, et al., 1992).
One important method in the prevention of peri-implant mucositis is the reduction in plaque accumulation, through individual oral hygiene procedures and regular peri-implant professional maintenance (Balshi, 1986) (Orton, et al., 1989). It is highly important that patients be educated about the importance of developing good oral hygiene habits and to attend regular periodontal maintenance appointments. The clinicians have to recognize the significance of monitoring and maintaining peri-implant health (Preshaw & Heasman, 2005).

Unfortunately, it is unclear which of the different maintenance regimens and treatments strategies for peri-implant mucositis and peri-implantitis are more effective (Esposito, et al., 1999). There is lack of information about which peri-implant maintenance protocol offers the best outcome in terms of reduction of inflammation and improved patient comfort. According to Grusovin et al, 2010 "there is only low quality evidence for which are the most effective interventions for maintaining or recovering health of peri-implant soft tissues and there is no reliable evidence as to which regimens are most effective for long term maintenance". Moreover, current approaches to implant maintenance are somewhat haphazard and not standardized (Wilson, et al., 2013).

It is assumed that what is appropriate for teeth is also beneficial for implants; as stated by Persson et al, 2010 "therapies proposed for the management of peri-implant diseases are currently based on the evidence available from the treatment of periodontitis". Two conventionally used methods of biofilm and calculus removal from teeth in North America are hand instruments (curettes and scalers) and ultrasonics. In teeth these two modalities of treatment have been studied extensively (Tunkel, et al., 2002); conversely, there are less studies on dental implants. One study by Renvert et al, 2008 concluded that mechanical non-surgical treatment might be effective to treat peri-implant mucositis but not peri-implantitis; however, the data supporting this literature review was scarce.

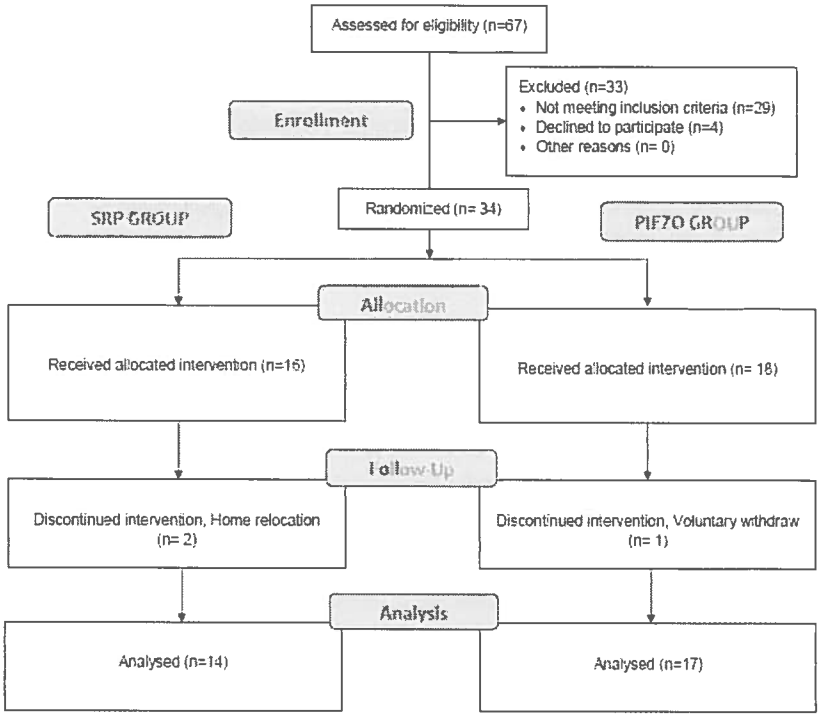

Fig. 1 CONSORT Flow Diagram

One of the main concerns for dental implants is that metal scalers and ultrasonics generate a roughened surface on the implant, which in turn facilitates plaque accumulation and therefore makes maintenance of plaque free surfaces more difficult (Rapley, et al., 1990). It was observed in a recent study that special coated scalers and ultrasonic tips have been shown *in vitro* to be compatible with implant surfaces, however this has not been confirmed *in vivo* (Ruhling, et al., 1994). The previous finding is in agreement with a current study, which demonstrated that the roughness values of the titanium surface of implants treated with piezoelectric ultrasonic scalers with a newly developed metallic tip and plastic hand curettes, are equal to the surface's roughness of untreated implants (Otgonbayar & al, 2012). Mann et al, 2012 showed in an *in vitro* study that plastic-coated scalers cause minimal damage to the implant surface but leave plastic deposits behind on the implant surface, suggesting further research is needed to evaluate the use of such plastic tips in the debridement of implants.

An additional factor, in evaluating the efficacy of different instrumentation in peri-implant maintenance, which needs to be taken into consideration, is patient perception. There is currently no data evaluating patient perception of comfort in regards to hand vs. ultrasonic instrumentation. This information is very important because should both methods of debridement be considered of equal efficacy, patient preference may play a role in the practitioner's selection of instrumentation. Knowing that patient comfort will increase the patient's compliance to the maintenance therapy, further evaluation of this factor is necessary.

The focus of recent research is being concentrated on the association of clinical parameters and biochemical markers of inflammation between implants with peri-implant diseases and healthy peri-implant tissues. Markers in Peri-implant Crevicular Fluid (PICF) including cytokines, enzymes, and proteases have been investigated. The presence of these biochemical mediators secreted into the PICF have been studied with the objective of identifying, diagnosing and monitoring peri-implant health. More recently saliva samples have been evaluated, with the

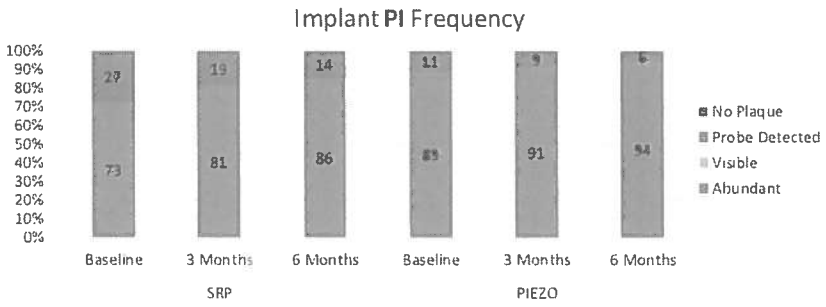

Fig 2. Frequency of plaque Index from baseline to 6 months by therapy

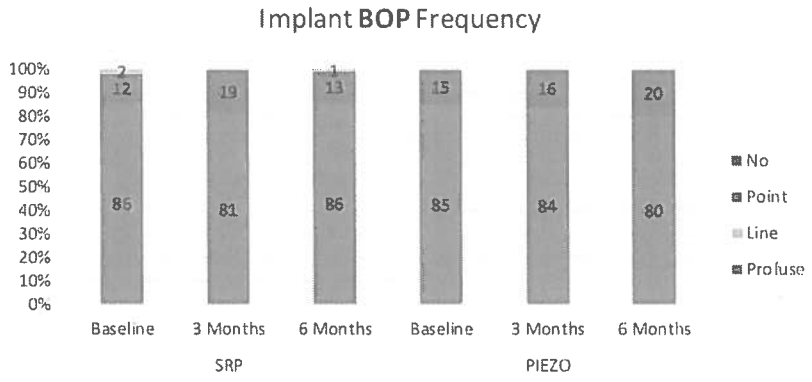

Fig. 3 Frequency of Bleeding Scores from baseline to 6 months by therapy

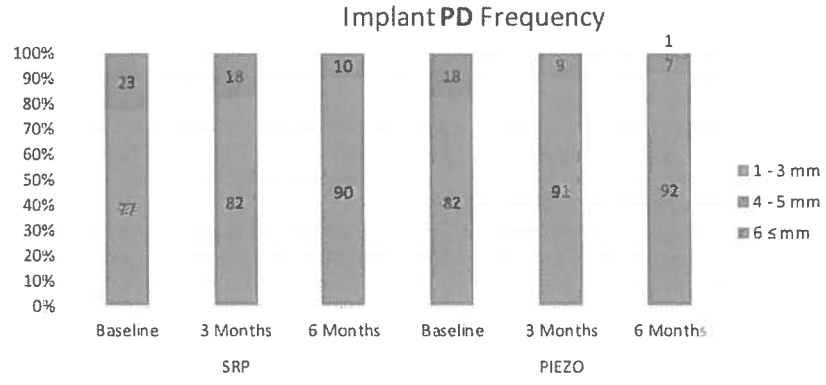

Fig 4. Frequency of probing depths from baseline to 6 months by therapy

advantage of being non-invasive and simpler to collect than PICF (Heitz-Mayfield, 2008).

Currently, there is scant information in the literature to guide the clinician as to which peri-implant maintenance debridement technique will offer better results in conserving health or in decreasing inflammation long term around dental implants. The aim of this study was to determine the clinical effects, presence of inflammatory cytokines, and the patients' perceived discomfort by comparing resin implant scalers to ultrasonic piezo scalers in a patient population with healthy peri-implant tissues and implants with peri-implant mucositis during a six-month period of peri-implant maintenance.

It is postulated in this study that (1) peri-implant therapy will have a beneficial clinical effect and will be well tolerated by the patient population, (2) will decrease the presence of inflammatory cytokines, and that (3) there will be no differences in the outcomes between the two peri-implant maintenance therapies.

Materials and Methods

The Biomedical Research Ethics Board of the University of Manitoba approved this trial and it was registered with the U.S. National Institutes of Health clinical trial service (NCT02100384). The randomized clinical trial was conducted between May, 2014 and May, 2016 at the Periodontics Clinic of the University of Manitoba. The Oral Biology Laboratory of the University of Manitoba performed the analysis of samples of the PICF. The Consolidated Standards of Reporting Trials, CONSORT Statement were followed.

A total of thirty-four (34) participants with at least one single dental implant were recruited and randomly assigned to one of the two peri-implant maintenance groups (Fig 1). Subjects enrolled in the study were patients of the Graduate Periodontics Clinic at the University of Manitoba, College of Dentistry. Each participant signed a written informed consent. The randomization used was the sealed envelope system generated by a second person (MK) not involved in the study. The records for each patient and the master sheet linking the patient identifiers were stored in a safe in the clinic administrative office. Only the research coordinator (AC) had access to the patients/codes matching list and its corresponding therapy. To thank them for their participation in the study patients received a free of charge maintenance appointment.

Inclusion criteria: Subjects having at least one implant restored by a single crown or two implants restored by a three unit fixed partial denture, probing depths less than 5mm in all six aspects around each implant (mesiobuccal MB, buccal B, distobuccal DB, mesiolingual ML, lingual L, and distolingual DL), radiographic bone loss less than 2mm in the interproximal aspects of the implant confirmed with periapical radiographs with a cone paralleling technique at baseline.

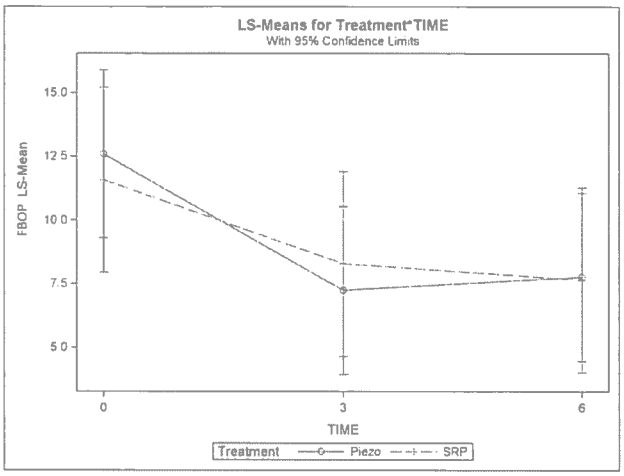

Fig. 5 FBOP reduction over time

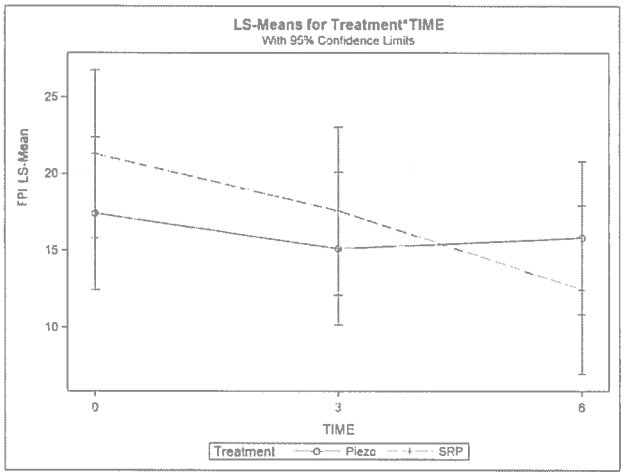

Fig. 6 FPI reduction over time

Exclusion criteria: Implant supported removable prostheses, use of antibiotics within the preceding 3 months, missing clinical examination and/or periapical radiographs at baseline.

Clinical measurements and procedures

A single blinded calibrated examiner (MC) performed the recording of clinical findings and PICF sampling before the corresponding maintenance therapy at baseline, 3, and 6 months. Intra-examiner calibration was completed previous to initiation of data collection.

The parameters assessed at implant site and patient level were the following:

- Modified plaque index (IPI) by Mombelli, score 0 = no plaque; score 1 = plaque only detected with probe; score 2 = plaque visible to the naked eye; score 3 = abundant plaque.
- Modified gingival index (IBOP) by Mombelli, score 0 = no bleeding; score 1 = isolated bleeding point; score 2 = line of blood; score 3 = profuse bleeding.
- Implant Probing Depths (PD) at six sites (MB, B, DB, ML, L, DL).
- Presence of keratinized gingiva (KT), measure from free gingival margin to mucogingival junction.
- Recession (REC), middle buccal distance between gingival margin and most occlusal point of restoration.
- Full mouth plaque index (FPI).
- Full mouth bleeding on probing (FBOP).
- Cytokine levels in PICF sample from M, D, B, and L implant aspects; each sample was taken using the technique described by Offenbacher et al, 1981.
- In addition, patients were also asked to fill in a pain questionnaire (Visual analogue scale, VAS) at the end of the study period.

Collection of Samples

To collect the PICF, the site was isolated from saliva using cotton rolls and gentle air drying before the sampling. Periopaper strips (Oraflow) were introduced at the B, M, D and L sites of the implant sulcus for 30 sec. Once the PICF was collected, each Periopaper strip was placed in a sealed Eppendorf tube previously identified with a code corresponding to the number of the patient, implant surface and time of collection. Samples were transported in a portable freezer to the laboratory where the Eppendorf tubes were stored at -86 C until further analysis.

Treatment Procedures

Once the patients were randomly selected and the clinical data and PICF samples were collected, the peri-implant maintenance was performed by a single experienced dental hygienist (MD).

The implants in the Piezo group were debrided using the Tigon+ Piezo Scaler with the W&H special tip 1I implant clean. All the surfaces of the implant were debrided for on average 1 minute using a circumferential and vertical motion. This ultrasonic unit has a frequency of 27-32 KHz. Tap water treated with A-dec ICX waterline treatment tablets was used.

Implants in the SRP group were debrided using the Implacare II Implant maintenance tips by Hu-Friedy. Each high grade unfilled resin tip (Plaststeel) was used a single time as per manufacturer's recommendation. Appropriate tip selection between Langer ½ and Columbia 4R/4L was made depending on the hygienist preference. A transversal motion following the circumference of the implant, debriding buccal, lingual and interproximal surfaces was performed for approximately one minute. As described above, tap water was treated with A-dec ICX treatment tablets to rinse the implant after treatment.

The oral hygiene instructions (OHI) were standardized and given by the hygienist to each patient at the end of every maintenance therapy. OHI consisted of an intraoral demonstration of the Modified Stillman brushing technique and the cross "shoe-shine" flossing motion. Patients were encouraged to brush two times per day and floss daily. In addition, at every visit all participants were provided with a three months' kit supply of dental aids for their home care. The kit included a Colgate toothpaste containing Sodium Mono-fluorophosphate 0.76% fluoride, a TePe Select toothbrush with soft end-rounded filaments and a TePe bridge and implant floss.

		SRP (N=14)						PIEZO (N=17)					
		0 MONTHS		3 MONTHS		6 MONTHS		0 MONTHS		3 MONTHS		6 MONTHS	
	VARIABLE	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
FULL MOUTH	PI	21.3	12.6	17.6	8.4	12.4	4.3	17.4	11.7	15.1	9.8	15.8	11.9
	BOP	11.6	6.2	8.3	3.1	7.6	3.8	12.6	12.5	7.2	5.3	7.8	4.3
IMPLANT	DB	2.6*	1.0	2.8	1.0	2.4*	1.1	3.2*	0.6	2.8	0.6	3.0*	0.6
	B	2.4	1.0	2.0	0.7	2.1	1.0	2.0	0.6	2.3	0.5	2.1	0.6
PD	MB	2.9	0.6	2.8	0.9	2.2*#	0.7	2.8	0.7	2.6	0.7	2.7*	0.6
	DL	3.2	0.8	2.6	0.9	2.4#	0.6	3.1	1.1	3.1	0.8	2.9	1.4
	L	2.4	0.8	2.3	0.7	2.0	0.4	2.4	0.5	2.3	0.5	2.4	0.7
	ML	3.1	0.9	3.1	1.2	2.8	1.1	2.9	0.9	2.8	0.7	2.8	0.8
KT		3.1	0.9	3.3	1.0	3.3	1.0	3.0	1.1	3.0	1.4	3.1	1.3
REC		9.6	3.0	9.3	3.0	9.4	3.0	8.4	2.0	8.5	1.8	8.5	1.9

* Statisticaly significant difference between therapies

Statisticaly significant by therapy from 0 to 6 months

Table 1. Statistics summary for clinical data

Handling and analyses of Samples

A MDS (Rockville, Maryland, USA) V-PLEX 7-plex custom panel Human Inflammatory Cytokines Kit was used in conjunction with a MSD MULTI-SPOT® 96-well 10-Spot plate for the detection and quantification of the following cytokines: Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Tumor Necrosis Factor alpha (TNFα), and Interferon gamma (IFNγ).

Periopaper samples were treated to extract the cytokines by incubating the Periopaper in 70 µl of extraction solution for one hour on ice, followed by a brief centrifugation. Then, 50 µl of the supernatant was added directly to the plate. The solution used for the extraction was PBS, 0.1% BSA, 0.05% Tween-20. Data was read using a MSD SECTOR Imager 2400, the units of the readings were expressed in pg/mL. Samples that didn't have any interleukin presence were recorded as not determine value (NaN).

Statistical analysis

Sample size: The G*Power 3.1.7 software was used to calculate the sample size with a Cohen effect size of 1.0 mm for a total number of 32 patients, 16 patients per group. In addition, an independent t-test with alpha=0.05, power (1-beta) = 0.80, 95% confidence interval and two tailed test was used. Allocation ratio, n1/n2=1. Standard deviation of the outcome in the population S=1.

Clinical and cytokine measurements were compared between treatment groups with mixed-effects repeated measures models, which account for within-subject correlation by including random effects. The group-by-time interaction term tests whether treatment groups significantly diverge over time, which would indicate a treatment effect. To meet distributional assumptions, many outcome variables were log-transformed. Models were evaluated via residual diagnostics, including histograms, QQ-plots, and scatter plots. Additionally, the least–squares means (LS-Means) plots showed good interaction for both treatments up to 6 months.

A p-value < 0.05 was considered the threshold for establishing that a significant treatment effect existed. A p-value larger than this implied we cannot reject the null hypothesis of no effect.

Implant PI and implant BOP were not included in the mixed-effects models due to a lack of variability in responses. Attempted models were not estimable. SAS PROC MIXED software was used for all analyses.

For the correlation of clinical data with laboratory data, PI and BOP were modeled as binary predictors of the cytokine outcomes, whereas PD was modeled as continuous, this was necessary to be consistent with the other models, where PD was a continuous outcome. The primary outcome was the change in PD, the secondary outcome was the change in the cytokines levels.

Results

Descriptive distribution of the clinical data

34 patients were enrolled for the clinical trial, two patients from the Piezo group and one patient from the SRP group discontinued; therefore, a total of 31 patients completed the study with 14 patients for SRP and 17 patients for the

INTERLEUKIN (IL)	SPR						PIEZO					
	0 MONTHS		3 MONTHS		6 MONTHS		0 MONTHS		3 MONTHS		6 MONTHS	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
IL-2	5.1	10.2	14.5	60.4	9.4	26.0	6.7	7.4	7.7	10.7	11.2	33.6
IL-4	5.1	3.4	9.4	6.9	10.0	7.6	6.3	3.4	11.2	7.5	11.4	7.4
IL-6	50.1	134.3	14.0	17.3	22.5	29.2	64.3	121.3	1298.8	9241.9	57.5	90.0
IL-8	9519.8	5557.6	29985.0	24999.0	22884.6	16871.0	7775.4	5208.7	29966.0	19353.9	27247.4	20374.0
IL-10	57.2	57.7	56.8	76.9	49.7	67.9	43.0	47.0	64.4	79.5	50.9	62.8
TNFα	53.3	60.2	65.0	132.1	57.2	63.4	101.5	198.5	138.6	367.8	177.3	345.6
IFNγ	80.5	69.0	143.8	126.1	183.6	238.8	148.7	264.2	156.1	120.4	156.1	172.8

Table 2. Cytokines statistics summary

		IMPLANT SURFACE					
		B	DB	DL	L	MB	ML
IL-2	PI	0.575	0.044*	0.792	0.543	0.214	0.141
	PD	0.292	0.305	0.184	0.501	0.950	0.449
	BOP	0.610	0.114	1.000	0.974	0.529	0.733
IL-4	PI	0.876	0.949	0.584	0.244	0.626	0.179
	PD	0.941	0.330	0.447	0.290	0.294	0.641
	BOP	0.993	0.123	0.495	0.152	0.101	0.285
IL-6	PI	0.229	0.164	0.082	0.943	0.159	0.254
	PD	0.406	0.315	0.348	0.702	0.082	0.405
	BOP	0.412	0.181	0.089	0.868	0.848	0.380
IL-8	PI	0.664	0.163	0.157	0.660	0.952	0.042*
	PD	0.077	0.359	0.952	0.390	0.100	0.526
	BOP	0.808	0.507	0.405	0.572	0.638	0.759
IL-10	PI	0.563	0.646	0.105	0.026	0.687	0.208
	PD	0.019*	0.222	0.420	0.482	0.087	0.512
	BOP	0.904	0.948	0.469	0.803	0.260	0.641
TNFα	PI	0.191	0.077	0.198	0.929	0.353	0.280
	PD	0.273	0.795	0.444	0.323	0.218	0.814
	BOP	0.263	0.354	0.513	0.511	0.667	0.179
IFNγ	PI	0.159	0.262	0.002*	0.573	0.924	0.227
	PD	0.464	0.172	0.768	0.168	0.947	0.988
	BOP	0.108	0.459	0.405	0.935	0.996	0.604

Table 3. P values for Implant surface association between cytokines and clinical parameters *Stadistically significant P<0.05

Piezo group. The mean age of the participants was 60.42 years (SD±10.29).

Within the total of patients, two were smokers with a pack years mean of 8. Smokers were equally distributed among the groups. Four and seven formers smokers were reported in the SRP and Piezo groups, respectively. A total of four patients, one for the Piezo group and three for the SRP group stated taking antibiotic treatment during the study period for approximately one week.

Descriptive results showed that implant PI frequency in both groups decreased consistently from baseline to six months (Fig. 2). Presence of plaque was more noticeable in the lingual aspects of the implant through the study time for the two groups.

Implant BOP frequency in the SRP group showed an increase at three months and returned to baseline levels at six months. In the piezo group, an increase in the frequency of implant BOP was observed at three and six months (Fig. 3). For both groups, the implant surfaces with higher presence of BOP during all the study time were the L, DL and ML.

This study demonstrated that both peri-implant therapies reduced the frequency of peri-implant PD from baseline to six months (Fig 4). One patient in the piezo group had a probing depth increased from 5 to 8 mm in the DL aspect of the implant. Additionally, in both groups the implant surfaces with higher PD during all the study time were the ML, DL and DB.

Patients in the SRP group perceived equal satisfaction as patients in the piezo group after therapy. Both groups reported maximum comfort in the VAS questionnaire after therapy.

Clinical Data Repeated Measure Models Results

Mean FBOP and mean FPI values improved for both groups from baseline to six months (Fig. 5 & 6); however, after running the models, these differences were not statistically significant over the 6 months or at any of the checkup times (0, 3, 6 months) for any of the treatment groups.

The implant probing depth repeated measures models showed that only the SRP therapy demonstrated a statistically significant reduction for implant probing depths at the MB (2.9 to 2.2mm) and DL (3.2 to 2.4mm) aspects from 0 to 6 months (P<0.05). Although, both therapies reduced the rest of implant probing depths from baseline to 6 months, these differences only account for 0.1-0.2mm, which are not considered statistically or clinically significant (P>0.05) (Table 1).

The data for KT and REC was treated as normally distributed (Table1). The model results indicated that any of the treatments had statistically significant effect over KT and REC at any given point (0, 3, 6 months) (P>0.05); however, during the study REC was statistically significant different between therapies due to an initial recession mean difference at baseline.

Cytokines Repeated Measures Models Results

The mean values from baseline to six months for the IL-2, IL-4, IL-8, and TNFα levels increased for both therapies, SRP and piezo. In contrast, mean values of IL-6 decreased over the 6 months for both treatments and mean values for IL-10 decreased only in the SRP group (Table 2). The cytokines repeated measures model found that only the IL-6 was significantly different between therapies over the 6 months (P<0.05).

Analysis of cytokines repeated measurements by group showed that in the piezo group, only IL-4 level was statistically significant increased between 0 to 3 months and IL-4, IL-8, and TNFα levels were statistically significant higher from 0 to 6 months (P<0.05). In the SRP group, IL-4, IL-8 and IFNγ levels statistically significantly increased and IL-10 level was statistically significant reduced at 6 months (P<0.05).

While analyzing the cytokines in relation with implant surface, only IL-2, IL-4 and IFNγ levels were statistically significant different within surfaces; specially, when comparing interproximal aspects against B and L sites.

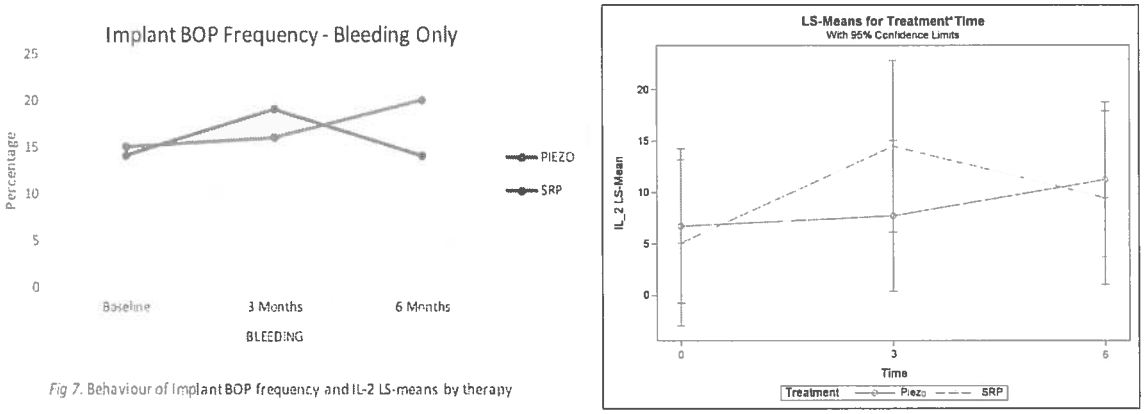

Fig 7. Behaviour of Implant BOP frequency and IL-2 LS-means by therapy

Association Between Plaque, PD, BOP, and Cytokine Levels

In general, in this clinical trial there were no statistically significant associations between the implant PI, PD, and BOP in any surfaces of the studied implant and the cytokines levels (IL-2, IL-4, IL-6, IL8, IL-10, TNFα, and IFNγ) during the six-month study period; however, few (3) PI values on the DB, ML, and DL surfaces of the observed implants were statistically significant associated with IL-2, IL-8 and IFNγ correspondingly (P=0.049, P=0.042 and P=0.002). In addition, a statistically significant association between PD in the B aspect of the studied implants and the inflammatory marker IL-10 was found (P=0.019) (Table 3).

A similarity in the behavior of implant BOP frequency and the behavior of IL-2, IL-8 and TNFα means was observed at 0, 3 and 6 months for both therapies (Fig 7).

Discussion:

Three patients discontinued the study after completion of the base line therapy. Two patients from the SRP group dropped out of the study due to relocation and one patient from the piezo group voluntarily withdrew. Baseline data for these patients was similar and within the ranges of the rest of the patients' baseline data; therefore, data for these discontinued patients was included in the statistical analysis.

Patients that ingested antibiotics during the study and smokers were included for statistical analysis. As a sensitivity test, the repeated measured models were run excluding the antibiotic users and smokers. The test demonstrated no changes in the results while including or excluding the data. This finding is in accordance with the result found by Hallstrom et al, 2012, where systemic antibiotics did not affect the treatment outcome.

Clinical parameters have been used as a way to identify, diagnose and monitor peri-implant health. Heitz-Mayfield, 2008 reported that in regards to the parameters used to diagnose peri-implant disease, BOP was considered a valuable parameter, and the absence of BOP was an indicator of a stable peri-implant condition; even though, this study showed a slight trend in the increase of BOP at the implant site frequency, this result was not statistically significant at any point in time.

Karring, et al., 2005, reported a reduction below 10% in the FBOP in a similar study using mechanical therapy only to treat peri-implantitis. Similarly, in this trial FBOP at 3 months was reduced to less than 10% and this percentage was maintained at 6 months.

According to Renvert, et al., 2008, mechanical non-surgical therapy can have positive effects in the treatment of peri-implant mucositis. In our study a trend of general improvement of clinical parameters was observed for both treatments from 0 to 6 months; however, these findings were not statistically significant. Furthermore, another recent study by the same author considered that a limited evidence exists for the clinical improvement of peri-implantitis after mechanical therapy alone with ultrasonics, carbon fiber and titanium curettes (Renvert & Polyzois, 2015).

In one study that compared ultrasonic instrumentation with specific-implant tips to titanium curettes in the treatment of peri-implantitis, both methods failed to eliminate or reduce bacterial counts and no group differences were found in the ability to reduce the microbiota in a six-month period. In addition, the authors reported that there was no difference in the treatment outcomes between therapies: the plaque and bleeding scores improved but no effects on probing depths were observed (Renvert, et al., 2009). In contrast, our study found no statistically significant improvement in IPI, IBOP, FPI and FBOP for any therapy in the maintenance and treatment of healthy implants and implants with peri-implant mucositis. Likewise, no difference in the clinical parameters between therapies was also observed in our study.

Another important parameter to consider is the increase in PD over time and its association with peri-implant attachment and bone loss as reported by Lang et al. 1993. In accordance, our study showed that both therapies had a reduction in the frequency of 4-5mm IPD category by approximately 11-13% with a corresponding increase in the frequency of IPD in the 1-3mm category at 3 and 6 months.

According to Heitz-Mayfield, et al., 2008 there is no association between the absence of keratinized peri-implant mucosa and peri-implant disease. Similarly, Wennstrom etal, 2012 stated that there is limited evidence to support the need for KT around implants to maintain health. In our study, all implants had at least 1 mm band of KT and the treatment therapies did not have any effect over time in the presence or absence of KT; however, narrow KT

might influence greater plaque accumulation and possible inflammation and bone loss (Bouri, et al., 2008).

The results of a recent systematic review indicated moderate evidence in the literature to support that implants with peri-implantitis had higher levels of pro-inflammatory cytokines (IL-1β, IL-6, IL-12 and TNF-α) in the PICF when compared to healthy implants. This review also reports that the evidence regarding the PICF levels as possible predictors of peri-implantitis is very limited for anti-inflammatory cytokines (IL-4 and IL-10), RANKL and chemokines (IL-8) (Duarte, et al., 2016). Our study demonstrated that the anti-inflammatory cytokine IL-4 significantly increased for both therapies at 6 months. On the other hand, our study also found a statistically significant increase in some of the pro-inflammatory cytokines (IL-8, TNFα, and IFNγ). However, it is uncertain if this increase is still within the normal concentrations for these specific cytokines. To our knowledge, there is scarce literature reporting specific concentrations in pg/ml for cytokines in PICF. From all our cytokines profiles only the mean concentration of IL-6 (14-64 pg/ml) was in agreement with the IL-6 mean value (13-53 pg/ml) reported by Nowzari, 2010. In the same manner, IL-6 median values reported by Renvert et al, 2015, were similar to our IL-6 median values.

Nogueira-Filho et al, 2014 assumed that a comparable immunological response exists between one-year follow-up implants and healthy teeth as determined by similar cytokines levels between PICF and gingival crevicular fluid (GCF) after 12-month monitoring period. On the contrary, another study found a higher profile of cytokines in PICF from healthy implants when compared to teeth (Nowzari, et al., 2012). Our study reported increase levels of cytokines over time for both therapies; only pro-inflammatory IL-6 showed a tendency to decrease for both therapies and anti-inflammatory IL-10 statistically significant decrease for SRP at 6 months. A future recommendation will be to observe if there are any changes in the cytokines concentrations while comparing healthy implants and implants with peri-implant mucositis.

As described above, this clinical trial showed that IL-6 and IL-10 mean levels tend to decrease at 6 months. This finding is in line with other studies that reported decreased levels of IL-6, IL-8 and IL-10 after implant placement, correlating the reduced presence of these cytokines with osseointegration (Schierano, et al., 2003) (Schierano, et al., 2000). Likewise, a literature review by Candel-Marti et al., 2011, showed that four different studies reported statistically significant increased levels of IL-6 in patients that developed or already had peri-implantitis.

In addition, Yaghobee, et al., 2014, noted higher presence of IL-6 levels in implants with peri-implantitis followed by healthy implants and last by healthy teeth. Similarly, Wohlfahrt et al., 2014 found a positive correlation between the reduction in IL-6 concentrations and probing pocket depths reduction. In this trial a similar pattern was observed for both treatments at 6 months.

Duarte, et al., 2009 found a statistically significant correlation among pro- and anti-inflammatory cytokines and clinical parameter on soft tissue biopsies of healthy implants, peri-implant mucositis and initial and severe peri-implantitis. PI, PD and BOP were positively and negatively correlated with corresponding pro- and anti-inflammatory cytokines (IL-12, TNF-α and IL-4, and IL-10, respectively). In this clinical trial the possibility of an association between the implant PI, PD, and BOP and the cytokines levels (IL-2, IL-4, IL-6, IL8, IL-10, TNFα, and IFNγ) in each of the implant surfaces was investigated; however, the results could not establish an association of the implant clinical parameters and the presence of cytokines.

Conclusions

- This study indicates that the two non-surgical peri-implant maintenance therapies have an overall beneficial clinical effect with reduction of clinical parameters from baseline to 6 months; however, this improvement was NO statistically significant.

- This study demonstrated that there are NO statistically significant differences in the clinical outcomes between the two peri-implant maintenance therapies for the management of healthy implants and implants with peri-implant mucositis.

- This study demonstrated that the anti-inflammatory cytokine IL-4 statistically significant increased for both therapies from baseline to 6 months.

- This study failed to demonstrate that peri-implant therapy decreases the presence of pro-inflammatory cytokines (IL-8, TNFα and IFNγ) in healthy implants and implants with peri-implant mucositis.

References

AAID, 2016. *American Academy of Implant Dentistry.* [Online] Available at: http://www.aaid.com/index.html [Accessed 12 April 2016].

Balshi, J., 1986. Hygiene maintenance procedures for patients treated with the tissue integrated prosthesis (osseointegration). *Quintessence Int.* , 17(2), pp. 95-102..

Berglundh, T. et al., 1992 . Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. *Clin Oral Implants Res.* , 3(1), pp. 1-+8.

Berglundh, T., Persson, L. & Klinge, B., 2002. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. *J Clin Periodontol,* pp. 29(suppl 3):197-212.

Bouri, A. J. et al., 2008. Width of keratinized gingiva and the health status of the supporting tissues around dental implants. *Int J Oral Maxillofac Implants,* 23(2), pp. 323-326.

Candel-Martí, Flichy-Fernández, Alegre-Domingo & Ata-Ali, 2011. Interleukins IL-6, IL-8, IL-10, IL-12 and periimplant disease. An update. *Med Oral Patol Oral Cir Bucal,* 16(4), pp. 518-521.

Costa, F. et al., 2012. Peri-implant disease in subjects with and without preventive maintenance: a 5-year follow-up.. *Journal of Clinical Periodontology,* Volume 39, p. 173–181.

De Boever, L. et al., 2009. Clinical and radiographic study of implant tratment outcome in periodontally susceptible and non- susceptible patients: a prospective long-term study. Clinical Oral Implants Rese. *Clinical Oral Implants Reserch,* pp. 1341-1350.

Duarte, P., Cutrim, A. & Braz, M., 2009. Differential cytokine expressions affect the severity of peri-implant disease. *Clin. Oral Impl. Res.,* Volume 20, p. 514–520.

Duarte, P. et al., 2009. Effect of Anti-Infective Mechanical Therapy on Clinical Parameters and Cytokine Levels in Human Peri-Implant Diseases. *Journal of Periodontology* , 80(2), pp. 234-243.

Duarte, P. et al., 2016. Could cytokine levels in the peri-implant crevicular fluid be used to distinguish between healthy implants and implants with peri-implantitis? A systematic review.. *Journal Periodontal Research*.

Esposito, M., Hirsch, J., Lekholm, U. & Thomsen, P., 1999. Differential diagnosis and treatment strategies for biologic complications and failing oral implants: a review of the literature. *The International Journal of Oral and Maxillofacial Implants,* 14(4), p. 473–90.

Ferreira, S. et al., 2006. Prevalence and risk variables for peri-implant disease in Brazilian subjects. *Journal of Clinical Periodontology,* Volume 33, p. 929–935.

Grusovin, M. et al., 2010. Interventions for replacing missing teeth: maintaining and recovering soft tissue health around dental implants. *Cochrane Database of Systematic Reviews,* Issue 8.

Hallstrom, H. P. G., Lindgren, S., Olofsson, M. & Renvert, S., 2012. Systemic antibiotics and debridement of peri-implant mucositis. A randomized clinical trial. *J Clin Periodontol* , Volume 39, p. 574–581.

Heitz-Mayfield, L., 2008. Peri-implant diseases: diagnosis and risk indicators.. *Journal of Clinical Periodontology,* 35(Suppl. 8), p. 292–304.

Karring, E., Stavropoulos, A., Ellegaard, B. & Karring, T., 2005. Treatment of peri-implantitis by the Vectors system A pilot study. *Clin. Oral Impl. Res,* Volume 16, p. 288–293.

Klinge, B. & Meyle, J., 2012. EAO Consensus Report: Peri-implanttissue destruction. The Third EAO Consensus Conference. *Clin. Oral Impl. Res.,* 23(Suppl. 6), p. 108–110.

Lang, N. et al., 1993 . Ligature-induced peri-implant infection in cynomolgus monkeys. I. Clinical and radiographic findings. *Clin Oral Implants Res.* , 4(1), pp. 2-11.

Lang, N. et al., 1993. Ligature-induced peri-implant infection in cynomolgus monkeys. I. Clinical and radiographic findings.. *Clin Oral Implants Res. 1993 Mar;4(1):2-11,* 4(1), pp. 2-11.

Lindhe, J. et al., 1992. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog.. *Clin Oral Implants Res,* 3(1), pp. 9-16.

Lindhe, J. & Meyle, J., 2008. Peri-implant diseases: consensus report of the sixth European workshop on periodontology. *Journal of Clinical Periodontology,* 35(Suppl 8), p. 282–285.

Mann, M., Parmar, D., Walmsley, A. & Lea, S., 2012. Effect of plastic-covered ultrasonic scalers on titanium implant surfaces.. *Clin Oral Implants Res. 2012. Jan; 23 (1):76-82.* , 23(1), pp. 76-82.

Nogueira-Filho, et al., 2014. Longitudinal Comparison of Cytokines in Peri-Implant Fluid and Gingival Crevicular Fluid in Healthy Mouths. *J Periodontol,* 85(11), pp. 1582-1588.

Nowzari, et al., 2012. The Profile of Inflammatory Cytokines in Gingival Crevicular Fluid around Healthy Osseointegrated Implants. *Clinical Implant Dentistry and Related Research,* 14(4).

Offenbacher, S., Farr, D. & Goodson, J., 1981. Measurement of prostaglandin E in crevicular fluid. *J ClinPeriodontol.* , Volume 8, p. 359–67.

Orton, G., Steele, D. & Wolinsky, L., 1989. Dental professional's role in monitoring and maintenance of tissue-integrated prostheses. *Int J Oral Maxillofac Implants.* , 4(4), pp. 305-10.

Otgonbayar, U. & al, e., 2012. Comparative evaluation of roughness of titanium surfaces treated by different hygiene instruments. *J Periodontal Implant Sci,* Volume 42, pp. 88-94.

Persson, G., Samuelsson, E., Lindahl, C. & Renvert, S., 2010. Mechanical non-surgical treatment of peri-implantitis: a single-blinded randomized longitudinal clinical study.. *Journal of Clinical Periodontology,* Volume 37, pp. 563-573.

Preshaw, P. & Heasman, P., 2005. Periodontal maintenance in a specialist periodontal clinic and in general dental practice. *Journal of Clinical Periodontology,* 32(3), pp. 280-286.

Rapley, J., Swan, R., Hallmon, W. & Mills, M., 1990. The Surface Caracteristics Prodiced by Various Hygiene Instruments and Materials on Titanium Implant Abutments. *Int J Oral Maxillofac Implants,* Volume 5, pp. 47-52.

Renvert, S. & Polyzois, I., 2015. Clinical approaches to treat peri-implant mucositis and peri-implantitis. *Periodontology 2000,* Volume 68, p. 369–404.

Renvert, S., Roos-Jansa ker, A. & Claffey, N., 2008. Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review. *Journal of Clinical Periodontology* , Volume 35, p. 305–315.

Renvert, S., Samuelsson, E., Lindahl, C. & Persson, G., 2009. Mechanical non-surgical treatment of peri-implantitis: a double-blind randomized longitudinal clinical study. I: Clinical results.. *Journal of Clinical Periodontology,* Volume 36, p. 604–609.

Renvert, S., Widen, C. & Persson, G., 2015. Cytokine expression in peri-implant crevicular fluid in relation to bacterial presence.. *Journal of Clinical Periodontology* , Volume 42, p. 697–702.

Rokn, A. et al., 2016. Prevalence of peri-implantitis in patients not participating in well-designed supportive periodontal treatments: a crosssectional study. *Clin. Oral Impl. Res.*, pp. 1-6.

Roos-Jansåker, A., Lindahl, C., Renvert, H. & Renvert, S., 2006. Nine- to fourteen-year follow-up of implant treatment. Part II: presence of peri-implant lesions. *Journal of Clinical Periodontology,* 33(4), p. 290–5..

Ruhling, A., Kocher, T., Kreusch, J. & Plagmann, H., 1994. Treatment of subgingival implant surfaces with Teflon-coated sonic and ultrasonic scalers tips and various implant curettes. An in vitro study.. *Clin Oral Implants Res.* , 5(1), pp. 19-29.

Schierano, G. et al., 2000. Cytokine production and bone remodeling in patients wearing overdentures on oral implants.. *J Dent Res.* , Volume 79, pp. 1675-82.

Schierano, G. et al., 2003. Transforming growth factor-beta and interleukin 10 in oral implant sites in humans. *J Dent Res,* Volume 82, pp. 428-32.

Tunkel, J., Heinecke, A. & Flemmig, T., 2002. A systematic review of efficacy of machine-driven and manual subgingival debridement in the treatment of chronic periodontitis. *Journal of Clinical Periodontology,* 29(Suppl. 3), pp. 72-81.

Wennström, J. & Derks, J., 2012. Is there a need for keratinized mucosa around implants to maintain health and tissue stability?. *Clin Oral Implants Res.,* Volume Suppl 6, pp. 136-46.

Wilson, T., Valderrama, P. & Rodrigues, D., 2013. The Case for Routine Maintenance of Dental Implants. *Journal of Periodontology.*

Wohlfahrt, J. et al., 2014. Sulcus fluid bone marker levels and the outcome of surgical treatment of peri-implantitis.. *J Clin Periodontol* , Volume 41, p. 424–431.

Yaghobee, S. et al., 2014. Assessment of interleukin-1beta and interleukin-6 in the crevicular fluid around healthy implants, implants with peri-implantitis, and healthy teeth: a cross-sectional study. *J Korean Assoc Oral Maxillofac Surg* , Volume 40, pp. 220-224.

Clinical Relevance of the study

Scientific rationale for the study: Regular peri-implant maintenance is necessary to prevent peri-implant diseases.
Principal findings: No significance differences in the outcomes between the two peri-implant maintenance therapies. Both therapies have an overall beneficial clinical effect. The peri-implant therapies increased the presence of anti-inflammatory cytokine IL-4 for both therapies at 6 months.
Practical implications: The study showed that hand instrumentation or piezo ultrasonic scaler devices around healthy implants and implants with peri-implant mucositis can maintain and/or improve clinical parameters.