Biological Assessment of Used Dental Implant Healing Abutments

BY

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THESIS

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I dedicate this work to my family.

I am extremely grateful to my parents for their love and encouragement, for educating me and giving my brother and myself the best possible life. You have taught me the value of hard work, to never give up, and to always follow my dreams.

I am very much thankful to the two most kindhearted individuals I know, my wife Lucia and my dog Curry for their unconditional love and support throughout, their understanding during stressful times and motivating me to be the best version of myself.

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LIST OF ABBREVIATIONS

GMCSF - Granulocyte-macrophage colony-stimulating factor

HA – Healing abutment

PAMPs - Pathogen associated molecular patterns

PI - Principal investigator

PrPc – Cellular PrP

PrPSc – Scrapie

mD-Mφ - Monocyte derived-macrophages

sCJD - Creutzfeldt-Jakob disease

SUMMARY

Once exposed to the oral environment, healing abutments (HA) become contaminated from several sources which may play a fundamental role in shaping the immune response in proximity to dental implants. Manufacturers recommend HA for single use. However, it is well known that clinicians re-use HA following decontamination and sterilization methods available in clinical settings. The aim of this study is to evaluate 4 decontamination strategies, available in most clinical settings, to determine the extent to which biomaterial can be removed ("decontaminated") on used HA and secondly to determine the degree to which the "decontaminated" healing abutments trigger an inflammatory response *in-vitro* compared to unused, new sterile abutments. Forty healthy adult patients with prior placement of at least 1 dental implant were recruited for this study. HA were collected following 2-4 weeks of use, placed in PBS and distributed randomly into 4 test groups (Group A-D): Group A: HA were sterilized by autoclave only, Group B: HA were placed in an ultrasonic bath plus autoclave, Group C: HA were debrided by prophy jet and glycine powder plus autoclave, and Group D: HA were debrided using a non-scratch scrub sponge plus autoclave. New, unused, sterile HA were used as controls (Group E). HA were placed in cultures containing human CD14+ monocyte derived-macrophages (mD-Mφ) for 7 days. Supernatant were collected at 4, 24, 48 hours and day 5 to analyze cytokine profiles using a multiplex bead assay. Residual protein concentration from each group was determined by a Micro BCA protein assay while HA from each group were stained with Phloxine B and macroscopically examined for the presence of debris and other contaminants. The

results indicate that test groups showed differences in the degree of "decontamination" compared to control, with group C and D showing most effectiveness in debris removal and reduced concentration of protein. A multiplex analyte assay performed revealed high levels of cytokine secretion from HA in the test groups (A-D).

A survey was distributed to Postgraduate Periodontics Programs Directors (in the United States to assess the frequency of HA re-use and to inquire about the methods of decontamination and sterilization protocol used. Data from responses of three (21.43%) out of fourteen dental schools indicated over one hundred dental implants are placed every year and healing abutments are re-used at least once in their program. Responses as to the description of the decontamination protocol varied from manual debridement, ultrasonic cleaning and rinsing prior to heat sterilization in an autoclave. IRB # 2018-1412)

In conclusion, our study demonstrates that used HA were not fully "decontaminated" using common methods available in a clinical setting relative to new, unused HA and were capable of stimulating an immune response. Regarding detoxification strategies, we show that the most effective detoxification protocol is represented by group C and D. It is recommended that HA be viewed as single-use fixtures, unless more efficient and consistent decontamination techniques can be developed and validated for use in implant dentistry. Results from the survey indicate HA which are designed for single use by manufacturers, are re-used on a large scale and no standardized method of decontamination exist prior to their re-use in an educational setting

I. INTRODUCTION

A. Background

A healing abutment (HA) is a titanium screw that is placed on the coronal aspect of the dental implant either at the time of implant insertion or after a dental implant has successfully osseointegrated. It serves two purposes; to shape the gingival tissues prior to the placement of the permanent abutment and crown, and to prevent the accumulation of debris within the internal aspect of the body of the implant. Manufacturers indicate HA are designed for single use, some of the reasons being contamination and wear affect the screw thread and may result in damage within the implant, damage of the screw access hole and abrasive impregnation into the titanium, resulting in contamination with the use of mechanical abrasion methods. However, it is well known that clinicians re-use HA following sterilization (Wadhwani et al 2016a). There has been a significant amount of debate on the safety, ethics and economic value of reusing HA. While supporters of re-sterilization of these components say manufacturers label them for single use to increase their profit margins, others say that the possibility of cross infection/contamination outweighs the economic gain.

HA may be contaminated from several sources such as plaque, gingival epithelium, blood and saliva. All of these possible sources contain proteins and free amino acids which once attached to the surface of titanium may be hard to remove (Wadhwani et al 2016b). Reusable stainless steel, titanium drills and instruments are widely used in

dentistry today. Paradoxically, according to manufacturer instructions, implant drills are re-usable after an ultrasonic bath to remove physical debris followed by autoclave sterilization to neutralize pathogens. It is likely that physical debris including proteins and other biomaterial remains that can be potentially transferred between patients. One of the hypothesized causes of peri implantitis is the presence of a micro-gap at the implant-abutment interface which allows bacteria to colonize and form a biofilm predisposing the implant to bone loss. Debris present on the HA could prevent its close adaptation to the implant, thereby causing an introduction of contaminants within the soft tissue surrounding the implant (Grecchi et al 2017a). In a study by Wadhwani et al (Wadhwani et al 2016c), one hundred HA from eight private offices were cleaned, sterilized, stained with Phloxine B, and analyzed for the presence of debris. They reported that ninety-nine percent of healing abutments were "contaminated" with proteins and peptides even after sterilization was performed. Their concern with the remnants on the surface was the "potential" transfer of biologic elements between patients. However, this has yet to be demonstrated. Similarly, a study by Popovic (Popovic et al 2010) in 2010 evaluated endodontic instruments following cleaning and sterilization procedures for the presence of debris. Eighty instruments were evaluated by staining following various decontamination procedures and sterilization. They reported that ninety six percent of the samples were still contaminated. Vassey (Vassey et al 2011) performed a quantitative assessment of residual protein concentration on instruments used regularly in general dentistry following manual, ultrasonic and automated cleaning method. They noted a wide variation of residual protein levels amongst instruments

as well as between various decontamination strategies. A similar concern was raised in a study by Al-Jandan et al (Al Jandan et al, 2015) which assessed the rate at which unused burs were contaminated compared to used burs. Out of forty test samples, two showed the presence of bacterial growth following forty-eight hours of incubation. The authors suggest adequate protocols of decontamination and sterilization should be performed to prevent any contamination. Unfortunately, standardized protocols have yet to be described in the literature which could lead to the transmission of microorganisms between patients.

Sennerby studied the soft tissue response to used titanium screws collected from humans followed by reimplantation in rats (Sennerby et al 1989). Findings demonstrated an increase in the number of macrophages in the active state and the development of a fibrous capsule surrounding the used titanium screws as compared to unused titanium screws. Although the most likely explanation was the contamination of the used screw, it was impossible to determine the exact cause for the difference in the response. Cakan (Cakan et al 2015) examined sixty used, but sterilized HA in sealed sterilization bags collected from six manufacturers. On macroscopic observation, they found dirty screw grooves in 10.5% of the samples and debris filled driver slots in 5.2% of the samples.

Another cause for concern is the biologic implication of multiple rounds of sterilization. In a study by Vezeau (Vezeau et al 1996), surface changes and its impact on *in vitro* cell attachment and spreading on titanium following multiple rounds of sterilization were assessed. Their findings indicated cell attachment levels were diminished and cell spreading was reduced in the autoclave sterilization group which had a strong correlation

with the number of sterilization cycles. This is important as avoidance of bacterial penetration into the peri-implant structures requires a strong transmucosal barrier. A recent study by Chew (Chew et al 2018), evaluated one hundred and twenty HA following autoclaving only, autoclaving and airflow polishing and after autoclaving and sodium hypochlorite treatment. Similar to a previous study by Wadhwani et al (Wadhwani et al 2016d) they stained the samples using Phloxine B and the proportion of stained areas were calculated using Image J. Although the results showed a significant accumulation of debris in the first two groups, minimal staining was seen in group three and the authors concluded that the decontamination of used HA may be achievable, thus strengthening the feasibility of reusing HA. However, the inflammatory potential of used HA was never assessed so there is uncertainty as to whether they are truly "decontaminated". Finally, a study by Smith (Smith et al 2005) examined methods of decontamination used by 220 dental endodontic files scoring visible debris and residual protein levels. The authors detected debris present on 98% of files. These results demonstrate that the cleaning of complete debris removal from instruments is not always possible and can be a potential source of cross-contamination.

Significantly, while these studies investigated the surface characteristics of previously used HA, very little is known regarding the biological responses to biomaterial left on these surfaces as well as what decontamination protocol is most effective. This is important given the uncertainties as to whether used HA trigger an immune response and whether they can be successfully decontaminated to prevent inflammatory responses. The transmission of disease among individuals not only limited to used HA but can occur with any dental instrument. For instance, prions are unusually resistant to commonly used

chemical and physical decontamination methods and a potential risk of transfer of these organisms is unknown and is a cause for considerable concern. Normal prion protein, or PrPc, consists of a cell membrane glycoprotein present in all tissues, with its highest concentration within the central nervous system. In transmitted prion disorders, PrPSc the infectious agent, causes disease at a posttranslational level, resulting in conversion of PrPc to PrPSc. This pathogenic process causes a rise in protein levels leading to plaques of amyloid material and neural death (Porter, 2003).

B. Statement of the problem

HA may be contaminated from several sources such as bacterial biofilm, gingival epithelium, blood and saliva. All of these possible sources contain proteins and free amino acids which once attached to the surface of titanium may be hard to remove. Although studies have investigated the surface characteristics of previously used healing abutments, very little is known regarding the biological responses to biomaterial left on these surfaces as well as what decontamination protocol, available in most clinical settings, is most effective. This is important given the uncertainties as to whether used HA trigger an immune response and whether they can be successfully decontaminated to prevent inflammatory responses.

C. Purpose of study

The purpose of this study is to determine if HA can be truly "decontaminated" and to evaluate 4 decontamination strategies, available in most clinical settings, to determine the extent to which biomaterial can be removed. HA were examined macroscopically for the presence of debris and other contaminants using a Phloxine B stain followed by the use of a Micro BCA Assay to determine the residual protein concentration. Next, the study determined the degree to which the "decontaminated" HA trigger an inflammatory response *in vitro* using a cytokine multiplex bead assay compared to unused, new sterile HA. In addition, we queried the frequency of re-use of HA amongst post graduate Periodontics programs in the United States via a qualtrics survey and determine if there is a standardized protocol for decontamination and sterilization prior to their re-use.

D. Significance of the Problem

Manufacturers indicate HA are designed for single use. However, it is well known that clinicians re-use these HA following sterilization. Currently, there are no studies describing a standardized protocol for decontamination and sterilization of HA prior to their re-use. The clinical implications of their re-use are unknown and there is a high risk of transmission of biological material between patients. Used HA contaminated from several resources may play a fundamental role in adversely shaping an immune response via the

sensing of PAMPs thus triggering local inflammation which may lead to bone loss. Further, prions are organisms which are not easily killed using conventional sterilization techniques and are known to cause disorders of the nervous system which is of particular importance to dentists due to the possibility of cross contamination following the re-use of infected dental instruments. Although their transmission has not yet been documented to have occurred in dentistry, a study by Bourvis (Bourvis et al 2007) estimated the risk of transmission of sCJD following endodontic treatment to be very high if no deactivation procedures were performed on the instruments prior to their reuse.

E. Significance of the Study

This study will provide the clinician with information regarding the inflammatory potential of used HA and to determine if they can be re-used safely in patients following commonly used decontamination methods available in most clinical settings. Studies so far have mostly examined the qualitative accumulation of debris following the re-use of HA. This study aimed to quantify the accumulation of debris and in addition examined the biological (inflammatory) implications of these debris. The potential risk transfer of biologic debris from one individual to another due to the re-use of these fixtures is far too great compared to the economic gain which would be encountered by the clinician and patient. This will also create a foundation for further studies and research to look into this practice as a possible etiology in the development of peri-implant diseases.

F. Aim/Objectives

The aim of this study is to evaluate 4 decontamination strategies to determine the extent to which biomaterial can be removed ("decontaminated") on used HA, and secondly to determine the degree to which the "decontaminated" HA trigger an inflammatory response *in vitro* compared to unused, new sterile healing abutments. This study also aimed to determine the frequency of re-use of HA amongst post graduate Periodontics programs in the United States and methods of decontamination and sterilization utilized being prior to their re-use.

G. Hypothesis

There is no significant difference in terms of surface protein and plaque biofilm removal and biologic responses between a used HA and a new, unused HA.

There is no standardized protocol for decontamination and sterilization of HA prior to their re-use.

II. MATERIALS AND METHODS

This study was approved by the Ethics Committee at the University of Illinois at Chicago, College of Dentistry, IRB protocol # 2018-0565 and IRB protocol # 2018-1412.

Inclusion Criteria:

ASA I or II systemically healthy English-speaking subjects between the ages of 18 and 70 years of age with prior placement of at least one dental implant were recruited for this study. Individuals were otherwise in good periodontal health with probing depths ≤ 3mm, negative for bleeding on probing, no radiographic evidence of attachment loss, and ≥ 4 mm attached gingiva. In addition, all subjects were required to have dental radiographs within the past six months of good diagnostic quality.

Exclusion Criteria:

Subjects diagnosed with diabetes or uncontrolled systemic disorders such as hypertension, heart disease, bleeding disorders, autoimmune disorders, etc., that may influence cellular status were excluded from the study. Individual who take medications known to affect host immunity or periodontal tissues (ex. steroids, antibiotics, phenytoin, etc.), chronic anti-platelet/anti-coagulant therapy in the

previous 6 months or taking antibiotics within one month prior to the screening examination for any medical or dental condition were also excluded. Current smokers and non-English-speaking individuals that are unable to provide consent to participate in this study were not included in the study.

In total, 40 adult subjects were recruited for this study with only one abutment collected per subject (Straumann, Basel, Switzerland; Dentsply Astra Tech, Charlotte, North Carolina, USA). The HA were removed and collected following 2-4 weeks of use post osseo-integration at the time of final impression for restoration. A new, sterile and identical (Straumann or Astra Tech) HA was delivered to the patient at the same appointment. The "used" HA were then placed in a 50 ml conical tube containing 20 ml phosphate-buffered saline (PBS) and distributed randomly into 4 test groups (Group A-D, N=40/group) for further evaluation as follows:

Group A: HA were sterilized using a steam autoclave at 121˚C / 250˚F for 30 minutes with minimum dry time of 15 minutes.

Group B: HA were placed in an ultrasonic bath (L&R Manufacturing Company, New Jersey, USA) for 5 minutes followed by sterilization in a steam autoclave at 121˚C / 250˚F for 30 minutes with minimum dry time of 15 minutes.

Group C: HA were debrided using a prophy jet (Airflow Master, Hu-Friedy Mfg. Co., Chicago, Illinois, USA) and glycine powder (Hu-Friedy Mfg. Co., Chicago, Illinois,

USA) until there were no visible debris present followed by sterilization using a steam autoclave at 121˚C / 250˚F for 30 minutes with minimum dry time of 15 minutes.

Group D: HA were debrided using a non-scratch scrub sponge (3M, St. Paul, Minnesota, USA) until there were no visible debris present followed by sterilization using a steam autoclave at 121˚C / 250˚F for 30 minutes with minimum dry time of 15 minutes.

Group E (Control) : New, unused, sterile Dentsply Astra Tech or Straumann HA.

For standardization purposes, all decontamination and sterilization procedures were conducted by one investigator (AN). A description of the Test and Control HA is been listed in Table 1 and 2.

Macroscopic evaluation

For macroscopic evaluation, "decontaminated" abutments from each group (N=2 /group) were stained using Phloxine B (Young Dental Manufacturing, Earth City, Missouri, USA) and examined macroscopically for the presence of debris and other contaminants using a modified method described by Wadhwani et al (Wadhwani et al 2016e). Briefly, each HA was placed in individual sterile 15ml plastic tubes containing 2 mL of Phloxine B stain for 5 minutes, lightly rinsed in PBS solution and then air

dried. Following this, six photographs were taken of the body of the HA, the connection to the implant fixture, screw thread shank, and the screwdriver engagement site were taken using a Nikon D7000 D-SLR digital camera (Nikon corporation, Tokyo, Japan).

Determination of protein levels

Residual protein concentration was determined using a Micro BCA assay kit (Pierce Biotechnology, Rockford, Illinois, USA) according to manufacturer's instructions (N=3/group). Briefly, the content of one Albumin standard ampule was diluted into 7 clean vials. The working reagent was prepared by mixing 25 parts of Micro BCA reagent MA and 24 parts reagent MB with one part of reagent MC. 150 µl of each standard was pipetted into a microwell and 150 µl of working reagent was added to each well and mixed on a plate shaker for 30 seconds. The plate was covered using sealing tape and incubated at 37° C for two hours. The plate was cooled to room temperature and the absorbance was measured on a 562 nm plate reader (Versa Max, Molecular Devices, Hampton, NH, USA). A standard curve was prepared by plotting the average blank corrected 562 nm reading vs for each BSA standard vs its concentration in µg/ml.

Cell culture

Freshly prepared buffy coats were purchased from healthy donors (n = 3, Sylvan N. Goldman Oklahoma Blood Institute, Oklahoma City, OK, USA) and CD14+

monocytes were isolated by density gradient centrifugation and magnetic bead isolation as described previously (Fordham et al., 2015; 2016; Naqvi et al., 2015). Briefly, peripheral blood mononuclear cells (PMBCs) were purified using Ficoll Paque™ (GE Healthcare, Chicago, IL, USA) based density centrifugation. PBMCs were incubated with magnetically labeled CD14 beads (Miltenyi Biotech, Bergisch Gladbach, Germany) according to manufacturer's instructions. Using this established method, we consistently obtained monocyte purity and viability >95%, as determined by flow cytometry. For monocyte derived macrophage (mD-Mφ) differentiation, monocytes were plated at a density of $2 \times 10_6$/ml in DMEM, supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml). After 2 hours, the media was substituted with media containing 10% heat-inactivated FBS (Life Technologies, Grand Island, NY, USA) and rhM-CSF (50 ng/ml; PeproTech, Rocky Hill, NJ, USA) for an additional 7 days. Media was replaced every 72 h. Confirmation of the mD-Mφ phenotype can be found in our previous publications (Fordham et al., 2016; Naqvi et al., 2015). On day 7, the "decontaminated" and autoclaved abutments (N=5/group) were placed in individual macrophage cultures.

Bacterial culture

Porphyromonas gingivalis (Pg) were grown in the Todd Hewitt Broth (THB) (Becton Dickinson, Franklin Lakes, NJ, USA) supplemented with Hemin (0.001%) and vitamin K12 (0.0001%, both from Sigma, St. Louis, Missouri, USA). *Aggregatibacter actinomycetemcomitans* (Aa) was grown in THB supplemented with yeast extract (1%, Becton Dickinson), Hemin and vitamin K12. All bacteria were grown at 37°C in an

anaerobic chamber (Thermo Fisher Scientific, Waltham, MA, USA) filled with anaerobic mixed gas (5% CO_2, 10%H_2 and 85% N_2) for 48 hours. Bacteria were harvested by centrifuge, washed, and adjusted to biomass to OD_{550nm}=1.0 in sterile PBS.

Multiplex bead assay

Supernatants were collected after 4, 24, 48 hours and five days of culture, centrifuged at 15000 rpm to remove cellular debris and stored at -70° C until further use. Human Cytokine Fluorokine MAP kit was purchased from R & D Systems (Minneapolis, MN, USA) and assays were performed according to manufacturer's instructions. Readouts included IL-10, IL-12p40, CCL22, IL-1RA, IL-1a, IL-1b, IL-6, IL-8, CXCL10, TNFα. Supernatants were assayed on a Bio-Plex 200 System (Bio-Rad, Hercules, CA, USA). Control cultures were incubated with never used, new HA for the indicated times as above.

Survey

An electronic survey (Qualtrics, Provo, UT, USA) consisting of 8 multiple choice and/or short answer questions were sent to post-doctoral Periodontics program directors at 57 accredited dental schools in the United States. The questions pertained to the re-use of healing abutments and the sterilization protocol used at the post-doctoral level. A complete list of survey questions is found in Appendix 1.

III. DATA ANALYSIS

Collected HA were randomly assigned either into “A” group, “B” group “C” group or “D” group. The power analysis identified a total sample size of 40 subjects per group (Group A-D) for a Type I error rate (α) of 0.05 and Power of 0.80 ($1-\beta$) to perform the proposed experiments. For protein determination assays and multiplex analyte assays, p-values were calculated using an unpaired t-test (GraphPad Prism, La Jolla, CA, USA). Results are presented as mean + standard deviation (SD) or ±SEM. A p-value of <0.05 was considered statistically significant. Descriptive statistics were used to summarize survey data.

IV. RESULTS

TABEL I – DESCRIPTION OF HEALING ABUTMENTS IN TEST GROUP A

Group A		
Manufacturer	**HA Diameter**	**HA Length**
STRAUMANN	5	4
STRAUMANN	4.5	4
STRAUMANN	5	4
ASTRA TECH	4	4.5
STRAUMANN	5	4
STRAUMANN	5	4
STRAUMANN	4.5	4
STRAUMANN	5	4
STRAUMANN	6.5	4
STRAUMANN	5	4

TABLE II - DESCRIPTION OF HEALING ABUTMENTS IN TEST GROUP B

Group B		
Manufacturer	**HA Diameter**	**HA Length**
ASTRA TECH	5	4.5
STRAUMANN	6.5	4
ASTRA TECH	5	3.5
STRAUMANN	4.5	4
ASTRA TECH	4	4.5
ASTRA TECH	6.5	4.5
STRAUMANN	4.5	4
STRAUMANN	5	4
STRAUMANN	4.5	4
ASTRA TECH	4	4.5

TABLE III - DESCRIPTION OF HEALING ABUTMENTS IN TEST GROUP C

Group C		
Manufacturer	**HA Diameter**	**HA Length**
ASTRA TECH	5	4.5
ASTRA TECH	6.5	4.5
ASTRA TECH	4	4.5
ASTRA TECH	5	3.5
STRAUMANN	5	4
STRAUMANN	4.5	4
ASTRA TECH	6.5	4.5
STRAUMANN	4.5	4
ASTRA TECH	6.5	2.5
ASTRA TECH	4.5	4

TABLE IV - DESCRIPTION OF HEALING ABUTMENTS IN TEST GROUP D

Group D		
Manufacturer	**HA Diameter**	**HA Length**
STRAUMANN	6.5	4
STRAUMANN	6.5	4
ASTRA TECH	6.5	4.5
STRAUMANN	5	2
STRAUMANN	6.5	4
ASTRA TECH	4	4.5
ASTRA TECH	6.5	4.5
STRAUMANN	5	4
ASTRA TECH	6.5	4.5
ASTRA TECH	5	3.5

TABLE V - DESCRIPTION OF HEALING ABUTMENTS IN CONTROL GROUP E

Group E		
Manufacturer	**HA diameter**	**HA length**
ASTRA TECH	6	4.5
STRAUMANN	5	6
ASTRA TECH	4	4.5
ASTRA TECH	5	4.5
STRAUMANN	4.5	4
STRAUMANN	4.5	6
STRAUMANN	4.5	2
ASTRA TECH	5	3.5
ASTRA TECH	6	4.5
STRAUMANN	4.5	4

1. Macroscopic evaluation

Two healing abutments from each group A-E were stained using Phloxine B and photographed using a Nikon D7000 D-SLR digital camera. The images show a significant difference in the degree of contamination within groups A-E (Figures 1-5). Group A and group B showed the highest accumulation of stain indicating the highest concentration of residual protein (Figures 1, 2). Group D had staining to a lesser degree limited to the screw access hole and threads of the healing abutment with group C showing similarity to control (Figures 3, 4). Group E (control) demonstrated no visual staining on any of the surfaces of the healing abutments.

Figure 1. Images of healing abutments in test group A stained with Phloxine B demonstrating residual protein on the surface, screw threads and in the screw access

Figure 2. Images of healing abutments in test group B stained with Phloxine B demonstrating residual protein on the surface, screw threads and in the screw access

Figure 3. Images of healing abutments in test group C stained with Phloxine B demonstrating no evidence of residual protein on the surface. screw threads and in the screw access

Figure 4. Images of healing abutments in test group D stained with Phloxine B demonstrating minimal residual protein on the surface, screw threads and in the screw access

Figure 5. Images of healing abutments in test group A stained with Phloxine B demonstrating no evidence of protein on the surface, screw threads and in the screw access

2. Determination of protein levels

Next, we determined the residual protein concentration using three HA from each group. Findings revealed significant differences and variations between groups as shown in figures 6 and 7. The average protein concentration for groups A-E were 59.5, 94.5, 16.4, 19.3 and 11.9 percent, respectively. This finding is in agreement with the macroscopic evaluation with the highest level of protein contamination being seen in group A and B followed by group D, with the lowest concentration in group C of the test groups compared to control group E. The concentration of protein in group A were significantly higher compared to control. However, protein concentrations in groups B, C and D were not statistically significant.

TABLE VI – TABLE DESCRIBING SIGNIFICANCE OF RESIDUAL PROTEIN CONCENTRATION IN TEST GROUPS A-D COMPARED TO CONTROL. GROUP A HAS A SIGNIFICANTLY HIGHER LEVEL OF PROTEIN CONCENTRATION COMPARED TO CONTROL.

	GROUP A	GROUP B	GROUP C	GROUP D
PROTEIN	0.0201	NS	NS	NS

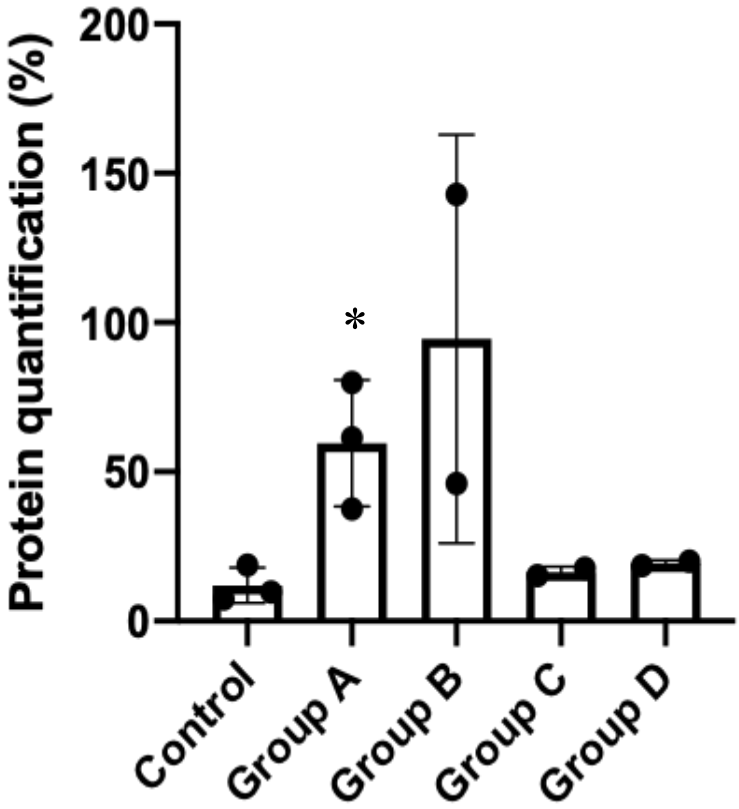

Figure 6. Bar graph demonstrating significantly higher concentrations of protein in test group A compared to control group E. Group A has a significantly higher level of protein concentration compared to control.

3. Inflammatory cytokine evaluation

Human primary mD-Mφ were co-cultured with HA from each group over the course of 5 days and supernatants assayed for secretion of cytokines and chemokines. A total of ten proinflammatory and anti-inflammatory markers, including IL-10, IL-12p40, CCL22, IL-6, IL-8, CXCL10, TNFα, IL-1RA, IL-1a, IL-1b, were tested on N=5 test (Groups A-D) and control HA (Group E) from each group. Data for cytokines and chemokines in test groups A-D and control Group E are listed in Figures 8-17.

IL-10

IL-10 levels were statistically significant in test groups A-D at 4 hours, 24 hours and 48 hours. At 5 days, levels were significant in groups A, B and D however group C levels were not significant compared to control.

IL-12p40

IL-12p40 production levels in test groups A-D showed no significance at all time points (4 hours, 24 hours, 48 hours and 5 days).

CCL22

CCL22 production levels at 4 hours were only significant in test group A. At 24 hours and 5 days, levels were raised and showed significance in test groups A and B while at 48 hours levels were significantly raised in test groups C and D.

IL-1RA

A significant increase in levels were seen in test groups A, B and D at 4h. At 24 hours, a significant rise in levels were seen in all test groups. However, the lowest levels were seen in test group C. At 5 days, none of the test groups showed any significant production of IL-1RA.

IL-1A

A significant increase in levels were seen in test groups A, B, C and D at 4h. At 24 hours, 48 hours and 5 days, a significant rise in IL-1A levels was seen in test groups A-D.

IL-1B

A significant increase in levels were seen in test groups A, B and D at 4h. At 24 hours, 48 hours and 5 days, significant levels were seen in test groups A and B. Test groups C and D showed no significant IL-1B production.

IL-6

A significant increase in levels were seen in test groups A, B and D at 4 hours. Test group C showed no significant rise in levels. At 24 hours, 48 hours and 5 days, significant levels of IL-6 production were seen in test groups A-D.

IL-8

A significant increase in levels were seen in test groups A and B at 4 hours with no significant increase in levels seen in test groups C and D. At 24 hours, 48 hours and 5 days, a significant rise in IL-8 levels were seen in test groups A-D.

CXCL10

A significant increase in levels were seen in test groups A, B and D at 4h with no significant rise in test group C. At 24 hours and 48 hours, a significant increase in levels were seen in test groups A-D. At 5 days, significant levels of CXCL10 were seen in test groups A and B only.

TNFα

A significant increase in levels were seen in test groups A-D at 4 hours and 24 hours. At 48 hours, significant TNFα levels were only detected in test group B and at 5 days significant levels were detected in test groups A, B and D. Test group C showed no statistical increase.

TABLE VII - TABLE DESCRIBING SIGNIFICANCE OF CYTOKINE SECRETION IN TEST GROUPS A-D COMPARED TO CONTROL.

CYTOKINE	TIME POINT	GROUP A	GROUP B	GROUP C	GROUP D
IL-1A	4hrs	0.0327	0.0038	NS	NS
	24hrs	0.0012	0.0002	<0.0001	0.0004
	48hrs	0.0002	0.0205	0.0010	0.0334
	5d	0.0023	<0.0001	<0.0001	0.0082
IL-1B	4hrs	0.0118	0.0045	NS	0.0075
	24hrs	0.0036	0.0411	NS	NS
	48hrs	0.0039	0.0204	NS	NS
	5d	0.0001	0.0143	NS	NS
IL-1RA	4hrs	<0.0001	<0.0001	NS	0.0019
	24hrs	<0.0001	<0.0001	0.0030	<0.0001
	48hrs	0.0008	NS	NS	0.0074
	5d	NS	NS	NS	NS
IL-6	4hrs	<0.0001	0.0002	NS	0.0016
	24hrs	0.0009	0.0021	0.0043	0.0007
	48hrs	<0.0001	0.0014	0.0217	0.0003
	5d	<0.0001	<0.0001	0.0019	<0.0001
IL-8	4hrs	0.0002	0.0011	NS	NS
	24hrs	0.0009	0.0021	0.0043	0.0007
	48hrs	<0.0001	0.0014	0.0217	0.0003
	5d	<0.0001	<0.0001	0.0019	<0.0001
IL-10	4hrs	<0.0001	0.0001	0.0029	0.0001
	24hrs	<0.0001	0.0004	<0.0001	0.0262
	48hrs	<0.0001	0.0031	0.0020	0.0006
	5d	0.0085	0.0071	NS	0.0418

TABLE VII - TABLE DESCRIBING SIGNIFICANCE OF CYTOKINE SECRETION IN TEST GROUPS A-D COMPARED TO CONTROL.

CYTOKINE	TIME POINT	GROUP A	GROUP B	GROUP C	GROUP D
IL-12P40	4hrs	NS	NS	NS	NS
	24hrs	NS	NS	NS	NS
	48hrs	NS	NS	NS	NS
	5d	NS	NS	NS	NS
CCL-22	4hrs	0.0440	NS	NS	NS
	24hrs	0.0340	0.0354	NS	NS
	48hrs	NS	NS	0.0230	0.0284
	5d	0.0023	0.0029	NS	NS
CXCL10	4hrs	<0.0001	0.0020	NS	<0.0001
	24hrs	<0.0001	<0.0001	0.0103	<0.0001
	48hrs	<0.0001	0.0002	0.0108	<0.0001
	5d	0.0046	0.0343	NS	NS
TNFα	4hrs	<0.0001	<0.0001	0.0004	<0.0001
	24hrs	0.0010	0.0087	0.0060	<0.0001
	48hrs	NS	0.0220	NS	NS
	5d	0.0008	0.0304	NS	0.0033

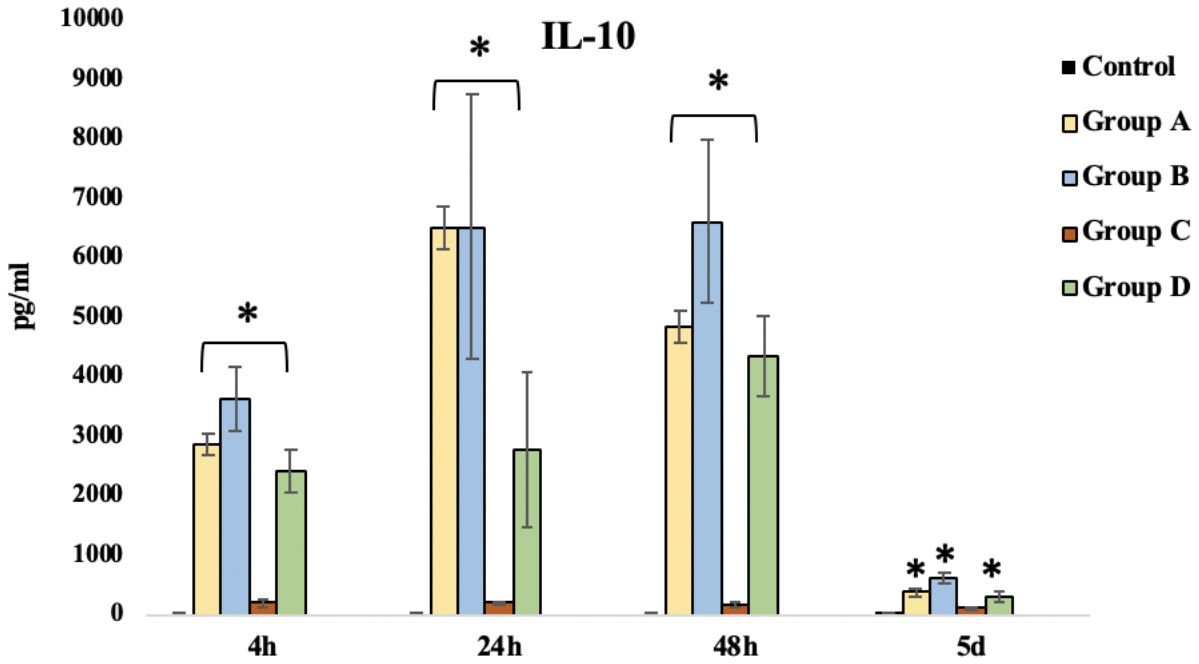

Figure 7. Bar graph demonstrating significantly higher levels of IL-10 in test groups A-D at 4 h, 24h and 48h compared to control. * = $P<0.05$.

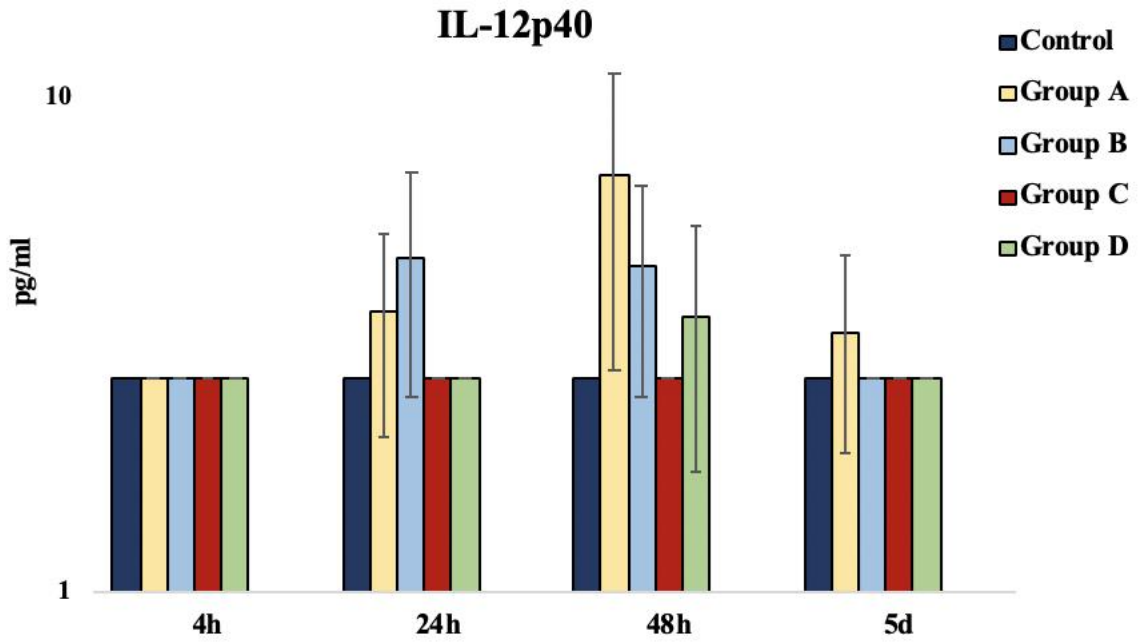

Figure 8. Bar graph demonstrating IL-12p40 levels were not significantly higher in test groups A-D at all time points compared to control

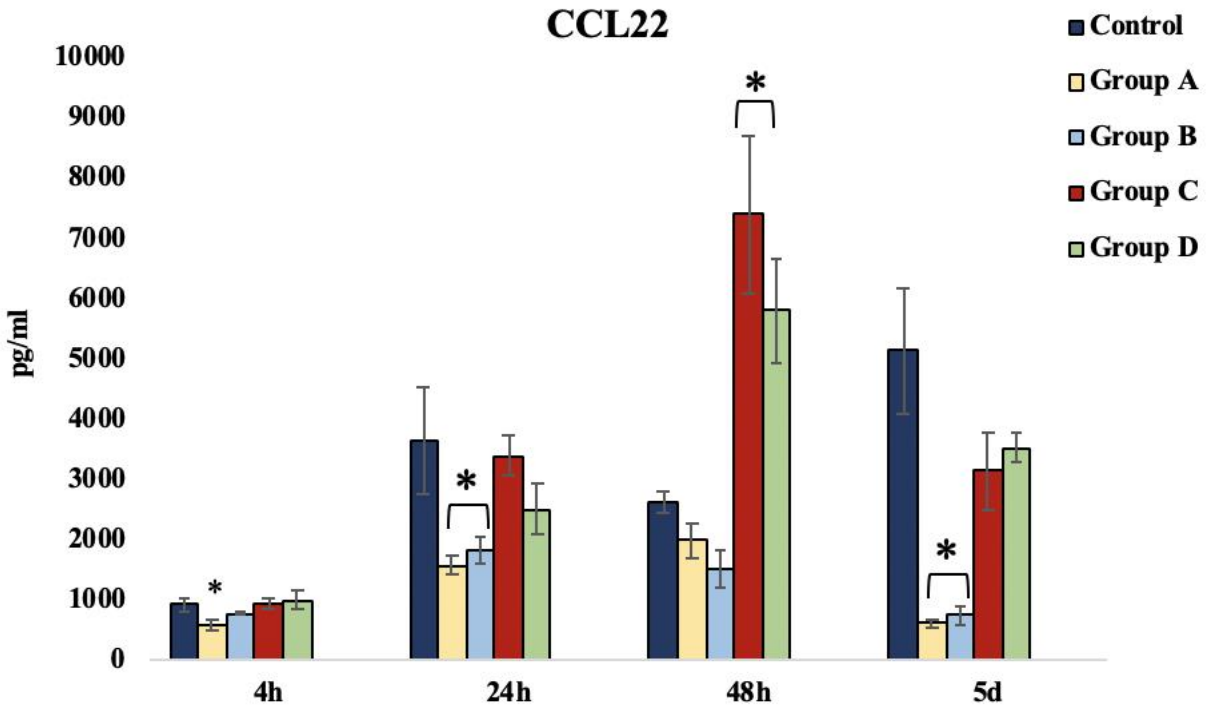

Figure 9. Bar graph demonstrating significantly higher levels of CCL22 in test group A at 4 h and test groups A and B at 24 h, 48h and 5 d compared to control

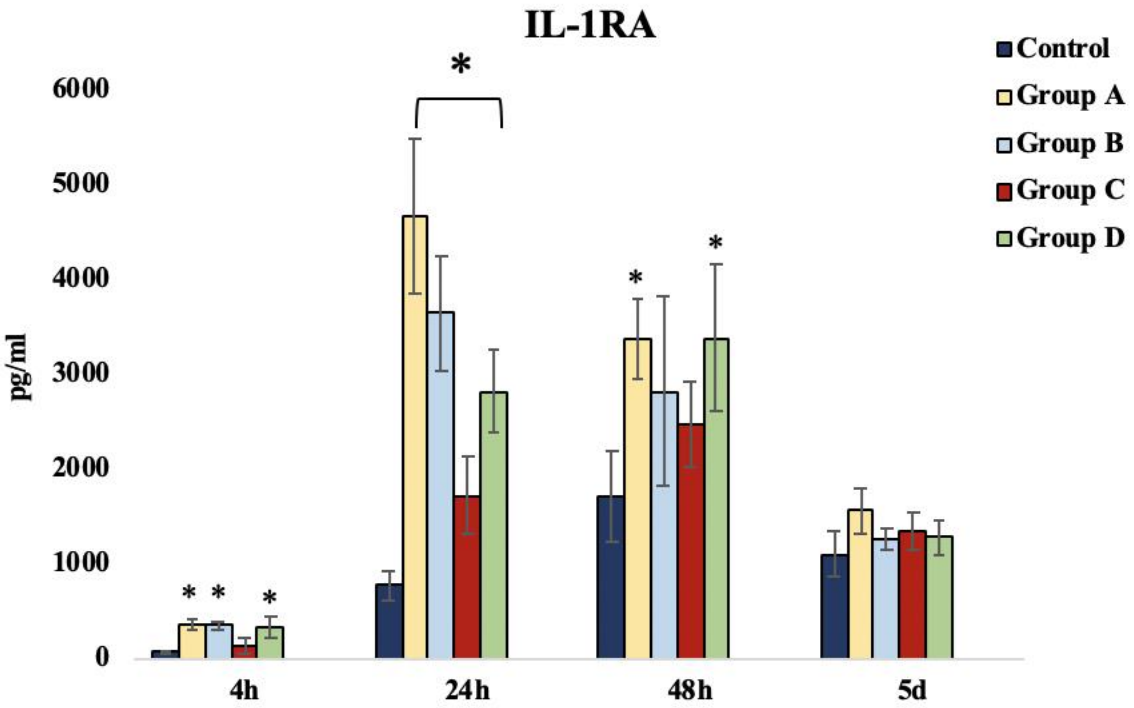

Figure 10. Bar graph demonstrating significantly higher levels of IL-1RA in test groups A-C at 4h, test groups A-D at 24h and test groups A and D at 48h compared to control

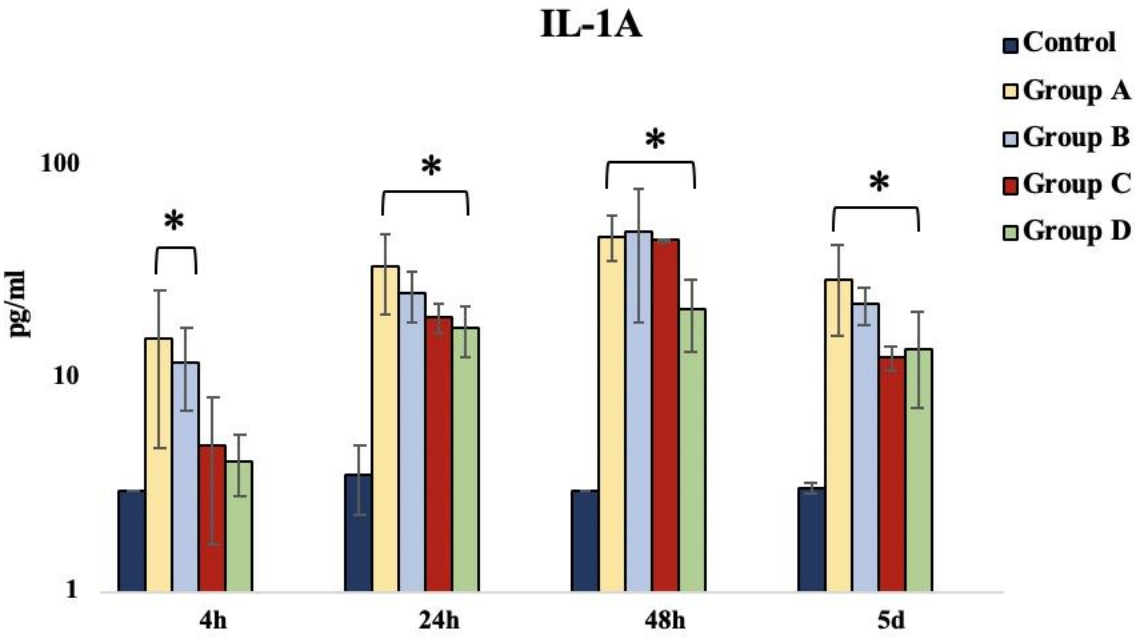

Figure 11. Bar graph demonstrating significantly higher levels of IL-1A in test groups A and B at 4h, test groups A-D at 24h, 48h and 5d compared to control

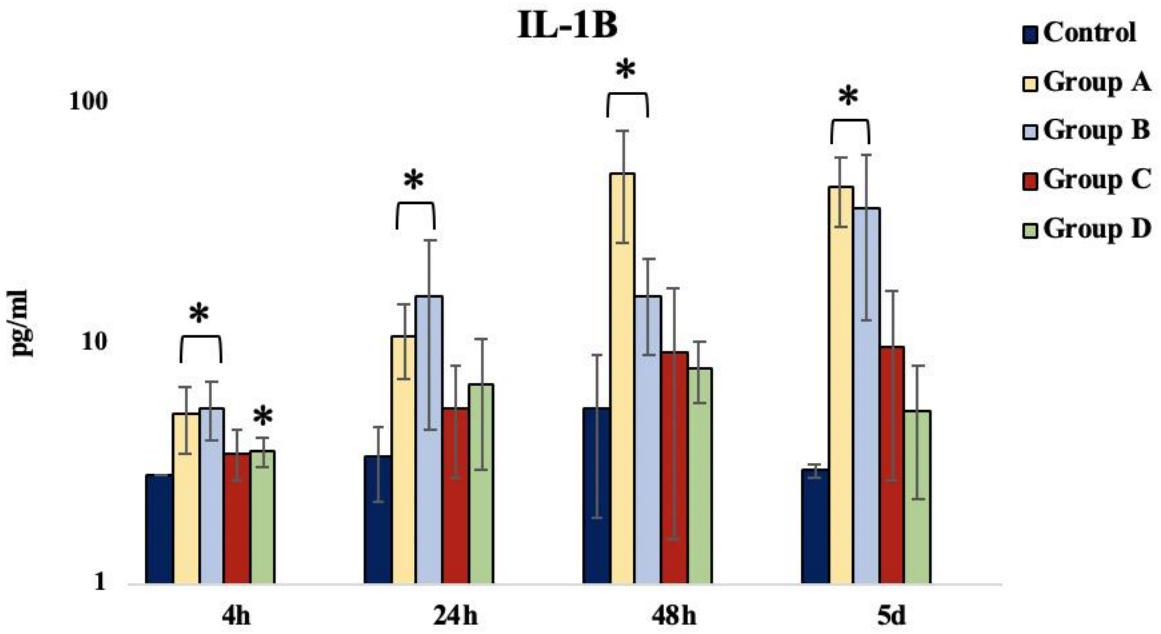

Figure 12. Bar graph demonstrating significantly higher levels of IL-1B in test groups A and B at 4h and 24h, 48h and 5d

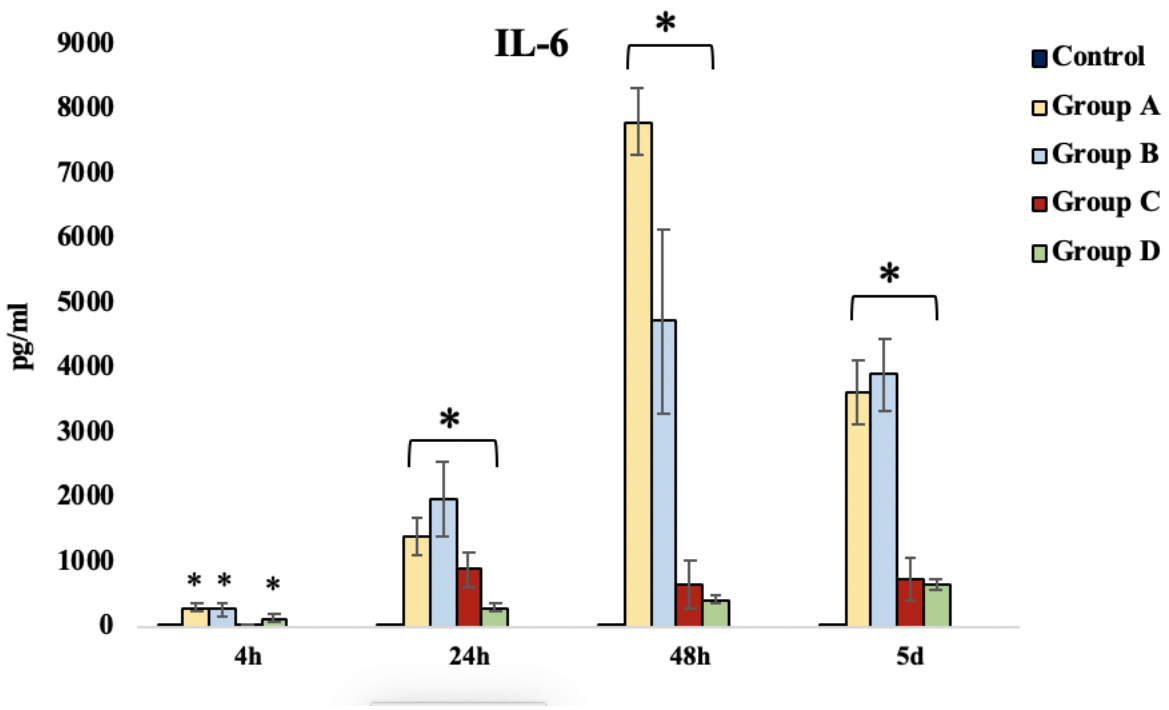

Figure 13. Bar graph demonstrating significantly higher levels of IL-6 in test groups A, B and D at 4h, test groups A-D at 24h, 48h and 5d compared to control

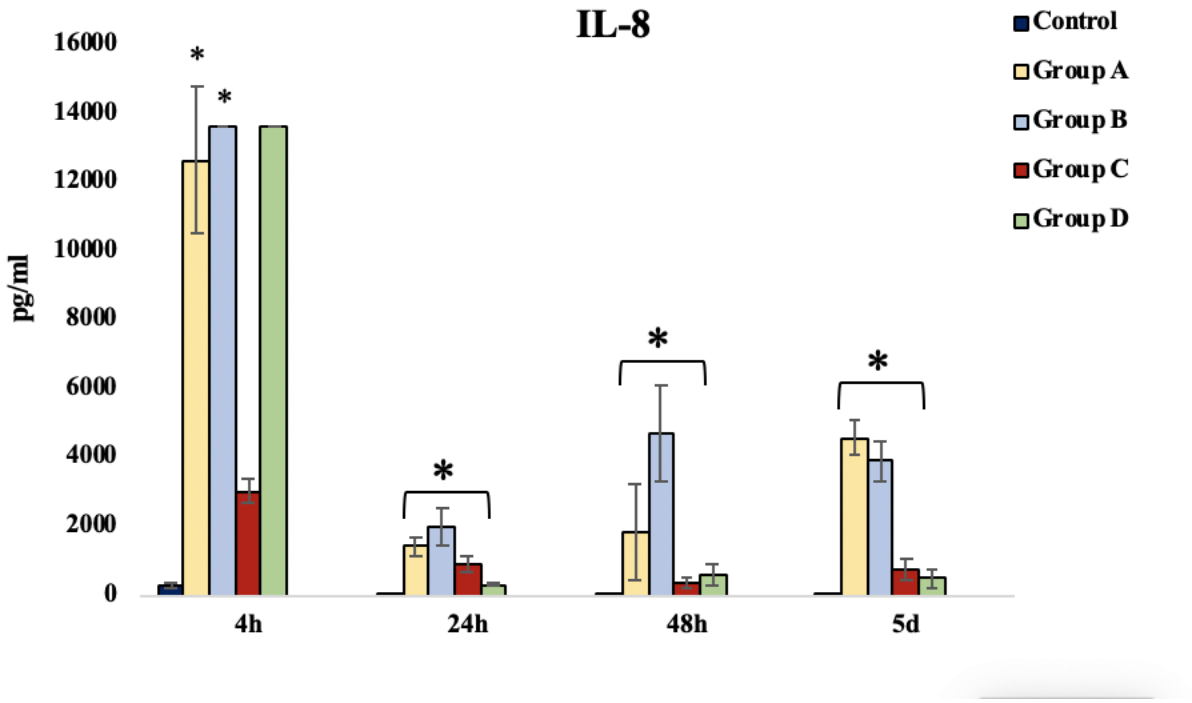

Figure 14. Bar graph demonstrating significantly higher levels of IL-8 in test groups A and B at 4h and test groups A-D at 24h, 48 h and 5d compared to control

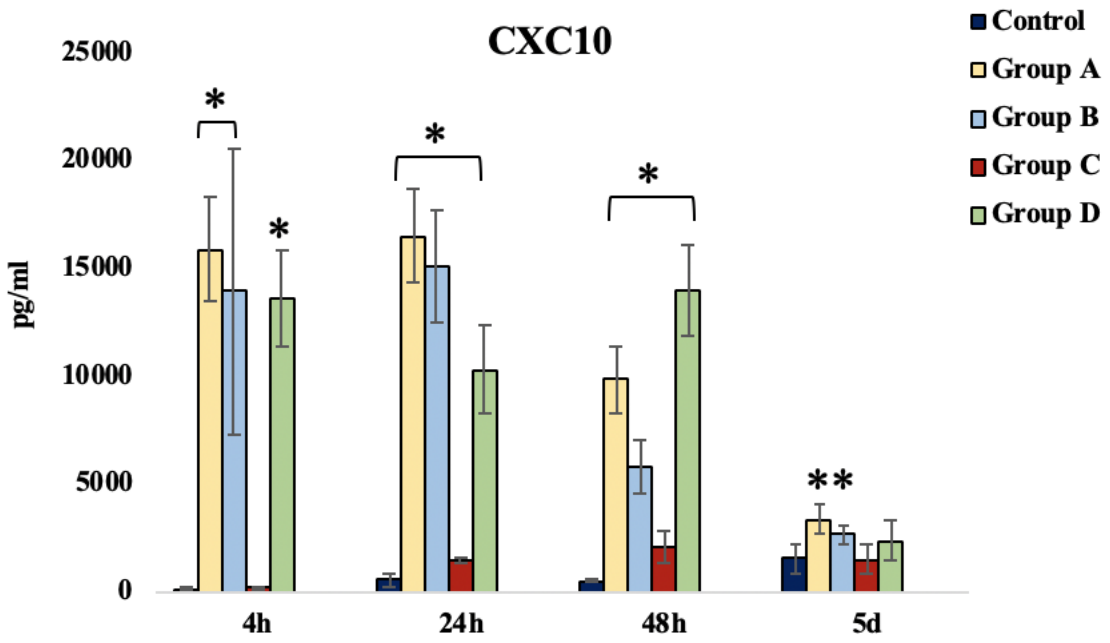

Figure 15. Bar graph demonstrating significantly higher levels of CXC10 in test groups A-d at 4h, 24h and 48h compared to control

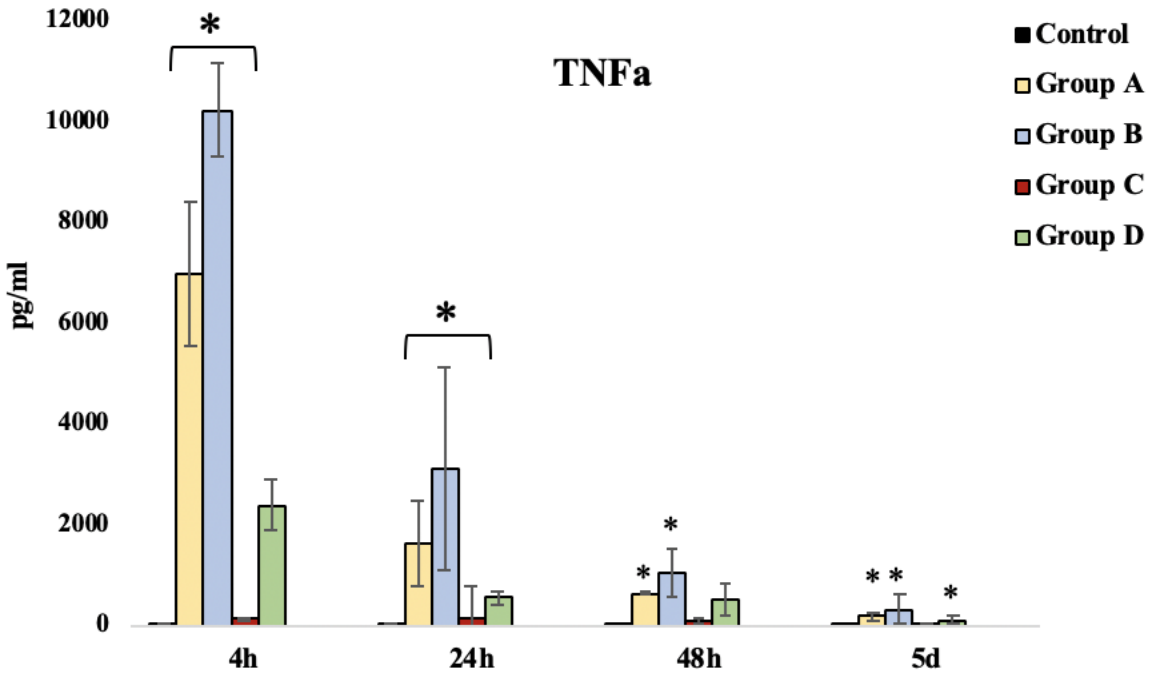

Figure 16. Bar graph demonstrating significantly higher levels of TNFα in test groups A-C at 4h, test groups A-D at 4h and 24h, test group B at 48h and test groups A, B and D at 5d compared to control

4. Survey

A total of 57 electronic surveys were sent to U.S. post-doctoral periodontics program directors in which 16 surveys were started (28%) and 14 completed (25%). Of those completed, 8 questions were answered completely. All participants (100%) responded 'yes' to dental implants being placed in their residency program. When asked how many implants were placed in their residency program per year, all the participants (100%) responded "greater than a hundred". In response to whether these implants are placed one stage or two stage 7 participants (53.85%) responded "one stage" and 6 participants (46.15%) responded "two stage". When asked how long they wait before the final restoration is placed following one stage implant surgeries, 1 participant (7.14%) responded "2 months", 6 participants (42.86%) responded "3 months", 2 participants (14.29%) "6 months" and 5 participants (35.71%) responded "other". Three of the participants who responded other specified "3-4 months". One specified "4 months depending on age, systemic picture and whether a bone graft was placed" and other specified "3-5 months depending on the case and systemic needs of the patient". When questions how long they wait between implant uncover and final restoration in two stage implant placement surgeries, 3 participants (21.43%) chose "4 weeks", 6 participants (42.86%) chose "6 weeks" and 5 participants (35.71%) chose "other". Of the participants who chose other, one said "it depends on the soft tissue healing and plan for temporization", another mentioned "4 months depending on age, systemic picture, and whether a bone graft was placed", the third participant said "it varies based on availability of restorative partners", the fourth and fifth participant said "depending on the case and they would wait longer in esthetic cases". When asked if healing abutments are

being re-used between patients in their residency program, 3 participants (21.43%) said “yes” while 11 participants (78.57%) said “no”. On questioning the respondents on the decontamination protocol being used one participant specified “manual cleaning, ultrasonic cleaning, inspection and heat sterilization”. Another respondent specified “rinse, ultrasonic followed by autoclave” and a third respondent specified “by manual wiping followed by heat sterilization in a heat autoclave”. When asked how many times a healing abutment is re-used in their residency programs all 3 participants said yes to the previous question said “1”.

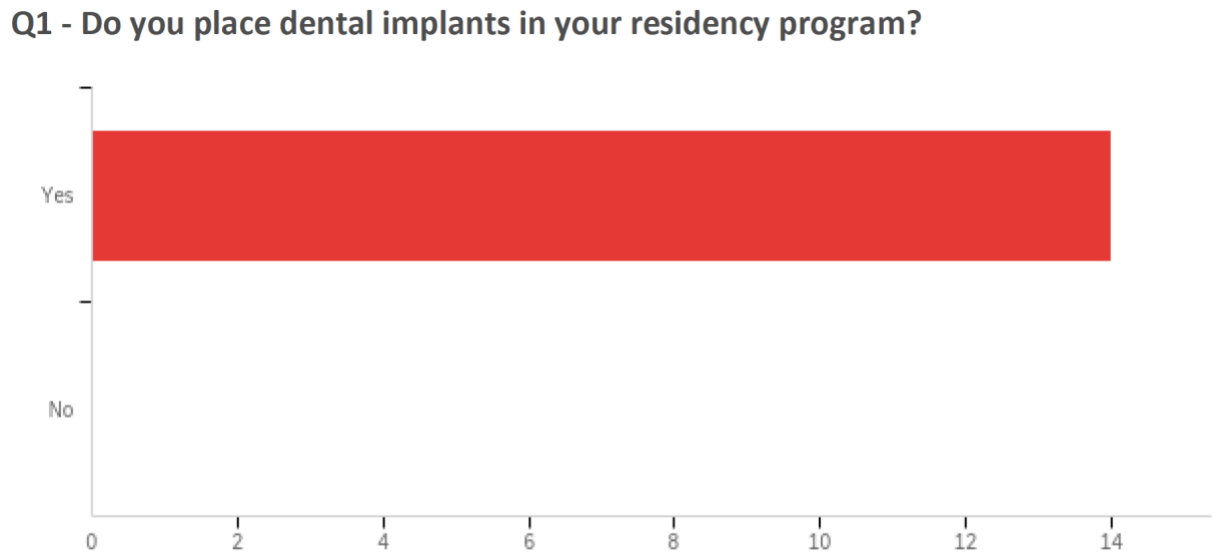

Figure 17. Survey response based on number of dental implants placed in residency program per year. Fourteen respondents chose “yes” to the survey question.

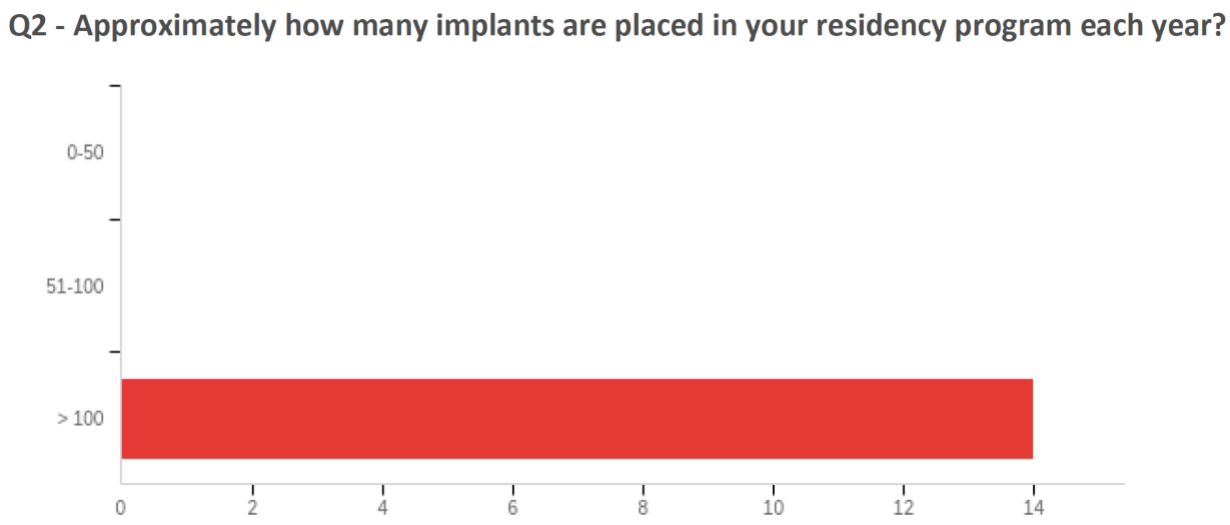

Figure 18. Survey response based on number of dental implants placed in residency program per year. Fourteen respondents chose >100 to the survey question.

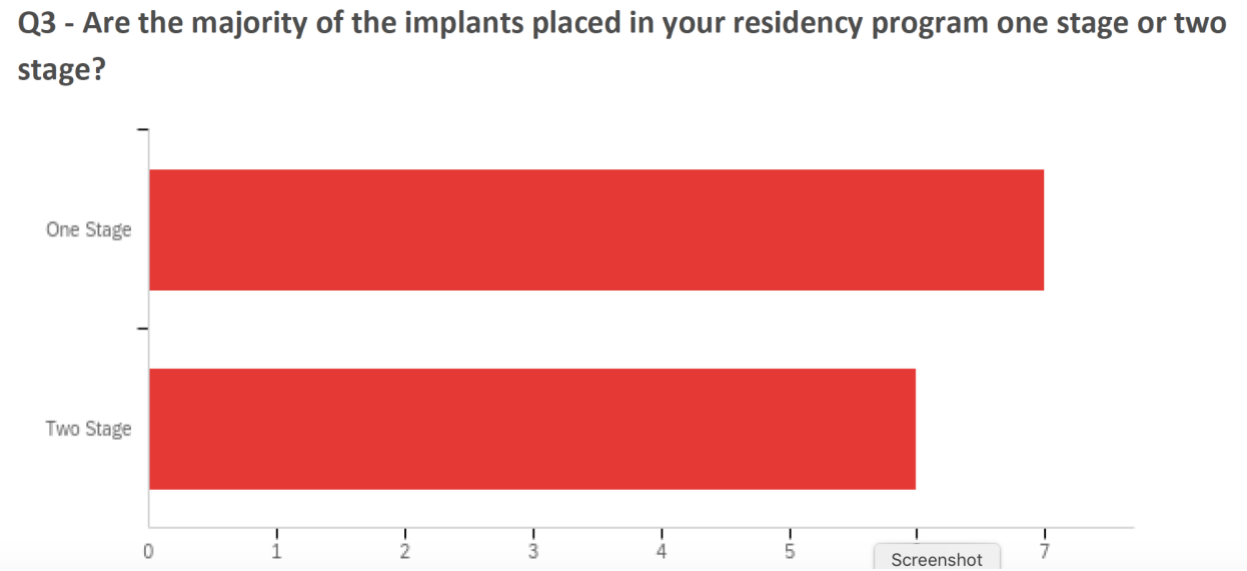

Figure 19. Survey response based on whether dental implants placed in residency program are one stage or two stage. Seven respondents chose “one stage” and six respondents chose “two stage”.

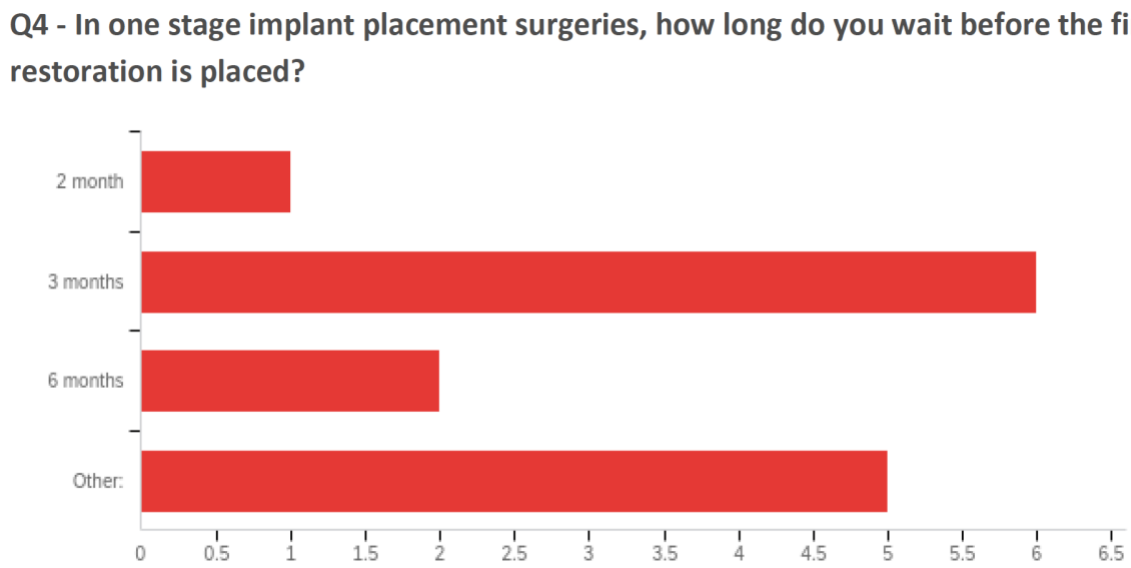

Figure 20. Survey response based on duration of time from day of implant placement to final restoration in one stage implant placement surgeries. The mean was 2.79 months in response to the survey question.

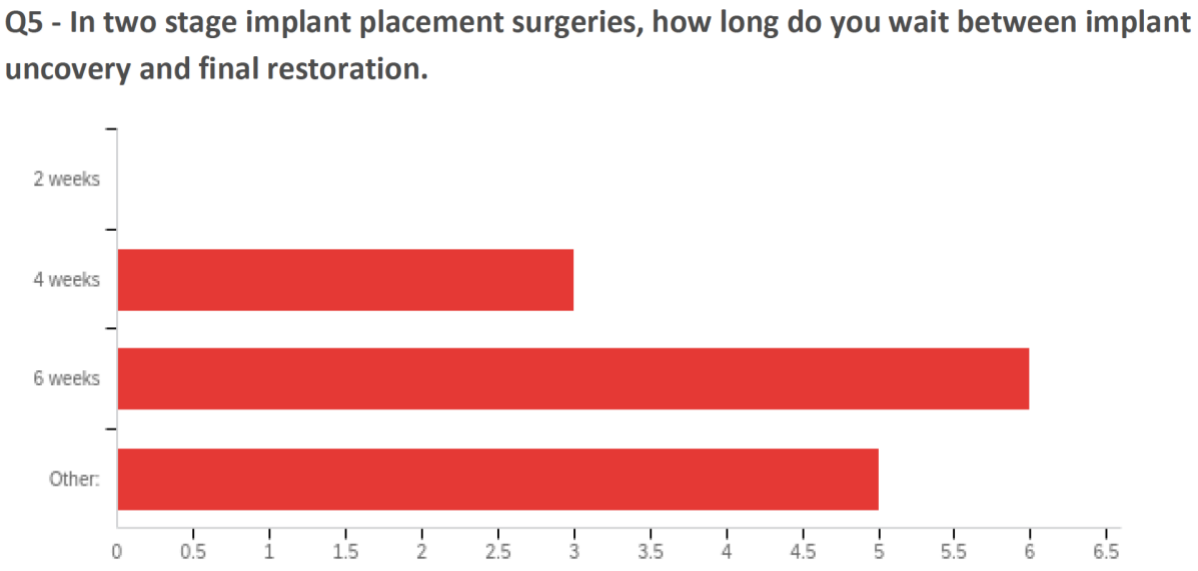

Figure 21. Survey response based on duration of time from day of implant uncovery to final restoration in two stage implant placement surgeries. The mean was 3.14 months in response to the survey question.

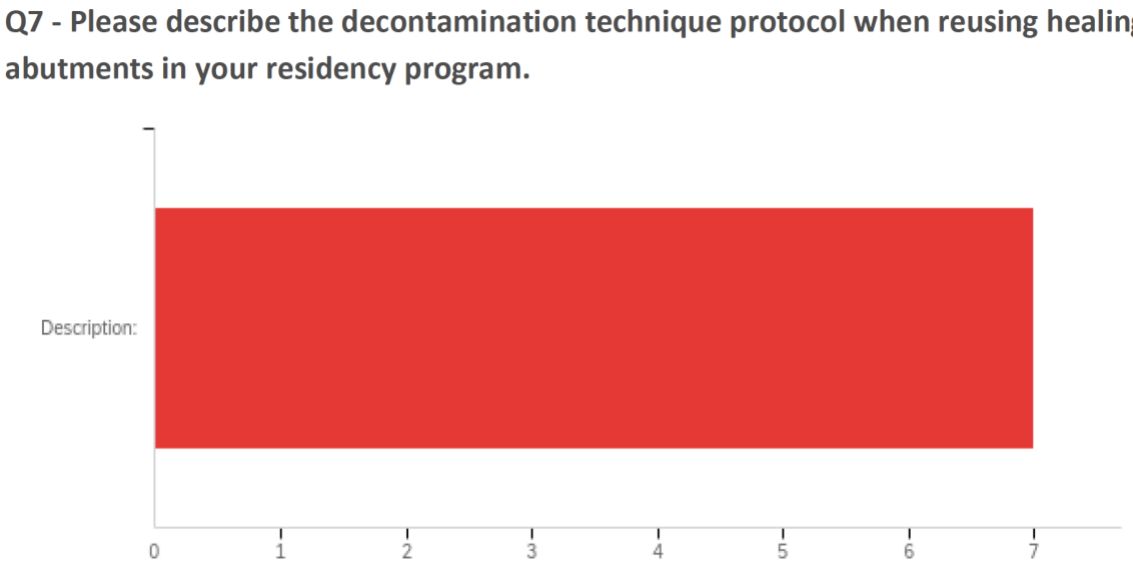

Figure 22. Survey response based on whether healing abutments are reuse between patients in their residency program.

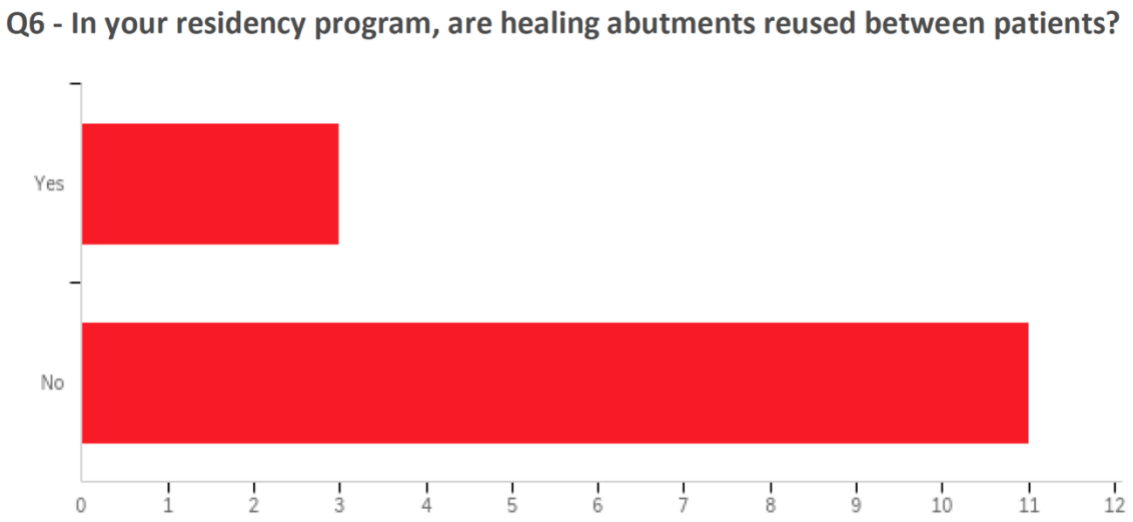

Figure 23. Survey response based on whether healing abutments are reused between patients in their residency program. Three respondents checked “yes” and eleven respondents checked “no” in response to the survey questions.

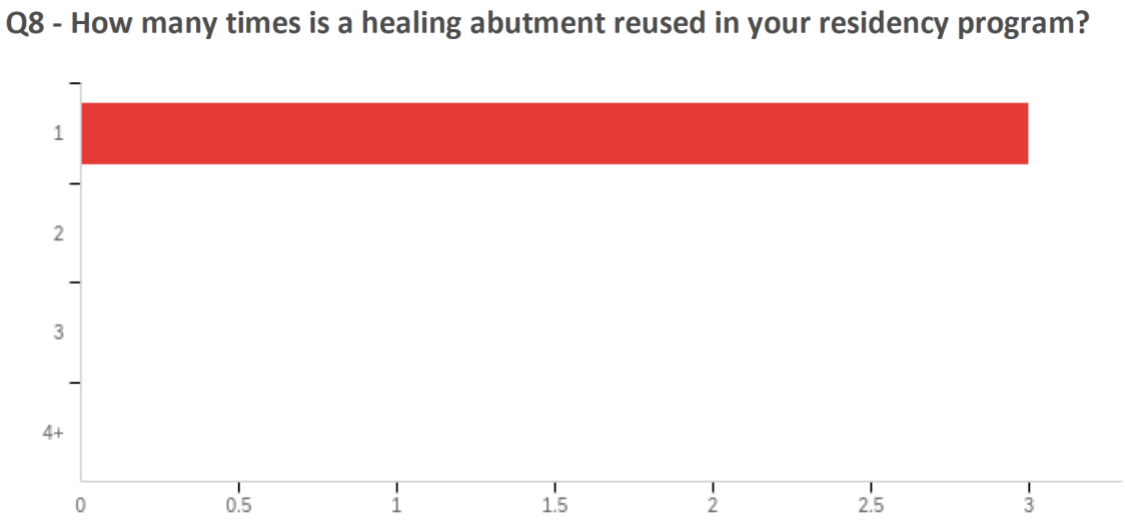

Figure 24. Survey response based on the number of times a healing abutment is reused between patients in their residency program. All respondents who said they reuse healing abutments in their residency program said they reuse it one time.

V. DISCUSSION

The re-use of HA by clinicians has been widely discussed. There has been debate as to whether decontamination and sterilization can effectively remove all the debris. Debris present on the HA could prevent its close adaptation to the implant, thereby causing an introduction of contaminants within the soft tissue surrounding the implant (Grecchi et al 2017b), potentially leading to peri-implant diseases namely peri-implant mucositis and peri-implantitis. Although previous studies have shown cleaning and sterilizing the abutments does not result in the complete removal of debris and ninety percent of the samples tested did show evidence of proteins and peptides remaining on the surface, this was purely qualitative, and the biologic implications of these remnants were never tested.

In this study, in addition to qualitatively assessing the HA using a modified method described by Wadhwani et al (Wadhwani, 2016e), the authors aimed to quantify the biologic response of the contaminants. Four decontamination methods, available in most clinical settings, were used prior to staining the HA to evaluate their efficacy. In addition, a survey was sent to Postgraduate Periodontics Program Directors in the United States asking questions relating to the re-use of healing abutments and the technique of decontamination sterilization prior to their re-use. Results of the qualitative assessment were in agreement with previous studies. On visual inspection, there was a significant difference in the quantity of remaining debris on the surface between groups A-D, with groups A (autoclave only) and B (ultrasonic and

autoclave) showing the highest degree of contamination. Although groups C (prophy jet and autoclave) and group D (scrub sponge and autoclave seemed to be more visually effective, there was remnants to a lesser degree remaining on the surface of the abutments in group D. The surface of group C was similar to group E and showed no evidence of any stain remaining on the surface. The most likely reason for these results was due to the lack of any manual decontamination method used to remove the debris in groups A and B. We noted similar results for group C and group E with few contaminants visible on the surface of group D, which is most likely due to the inability of the scrub sponge to reach the hard to reach screw access hole as well as between the threads of the HA. The surface of group C did however show particles of glycine powder which was attached to its surface. This finding is consistent with the protein quantification assay performed which found the highest protein content on groups A and B, 59.5μg/ml and 94.5μg/ml respectively whereas in groups C and D, a protein content of 16.4μg/ml and 19.3μg/ml was seen, respectively. The most common areas of contamination of the HA in groups A-D seemed to be in the area of the screw threads and screw access hole, both areas which have difficulty in access for cleansing for both patients and clinicians alike.

Although bacterial plaque is regarded as the most common etiology of peri-implant diseases, the intensity of the local inflammatory response to the bacterial products is increased (Fonseca et al, 2014a). Used HA contaminated from several resources may play a fundamental role in adversely shaping an immune response via the sensing of Pathogen-Associated Molecular Patterns (PAMPs) thus triggering local inflammation which may lead to bone loss. Pro-inflammatory cytokines and chemokines, such as IL-1b, TNF-a, IL6 and

IL8 have been shown to have a strong correlation with disease progression due to its ability to generate resorption of bone. (Page and Kornman, 1997). Studies have shown increased levels of these cytokines in the peri-implant crevicular fluid (PICF) from sites with peri-implantitis when compared to mucositis (Duarte et al. 2016; Javed et al. 2011, Ghassib et al. 2019).

As noted in Figure 12 and 13, a steady increase in IL-1A and IL-1B in all test groups from 4 to 48 hours with a gradual decline in levels at day 5 was observed. Locally, the progression of gingival inflammation is probably the consequence of IL-6 amd IL-1β activities, which cause damage of the tissue by the activation of osteoclasts and induction of collagen synthesis by fibroblasts (Petkovic-Curcin et al, 2011a). IL-1 receptor antagonist IL-1Ra may inhibit the pro-inflammatory effects of IL-1; (Feghali and Wright, 1997). Indeed, we noted a sharp rise in IL-1 levels from 4 to 48 hours which gradually declined at day 5 (Figure 11).

IL-6 has been shown to be upregulated in chronic inflammatory diseases and autoimmune disorders. A study by Severino et al (Severino et al, 2016) found IL-6 levels were significantly higher in the group with peri-implantitis when compared to the healthy group. According to the authors, increased IL-6 levels negatively affected the local inflammatory process contributing to clinical signs such as an increase in bleeding and probing depths found in patients with peri-implantitis. In the present study, we noted a sharp rise in IL-6 levels from 24 to 48 hours in test groups A and B which coincided with the highest concentration of debris on macroscopic evaluation and protein quantification (Figure 14).

TNF-α and IL-1β have a significant effect in the immune response to microbial antigens, whereas different levels of concentration of IL-8 and MIP-1α, can affect leukocyte migration and together with TNF-α and IL1-β affect the onset, course and outcome of inflammation (Petkovic-Curcin et al, 2011b). IL-1 and TNF are synergistic in their capacity to stimulate resorption of bone and can induce the release of other mediators that amplify or maintain the inflammatory response such as prostaglandins, stimulating the production of collagenase, and enhancing the killing of bacteria and phagocytic activity (Delima et al, 2001). Figure 17 shows high levels of TNFα in Groups A and B at 4 hours which decreased significantly at further time intervals.

The link between neutrophil dysfunction and the presence of periodontal disease has been reported (Fenoti et al, 2017, Marawar et al, 2013, Tsai et al, 1995). In response to Interleukin-8 neutrophils display the migration of cells and several other changes intra- and extracellularly. Connective tissue constituents are efficiently degraded by neutrophil enzymes, released upon activation. Figure 15 shows a high level of IL-8 which was released by macrophages in all the test groups, the highest levels of which were seen in Group A and B at 4 hours followed by a sharp decline in levels thereafter.

Studies have indicated that CXCL10 plays an key role in leukocyte homing to inflamed tissues and in the sustenance of inflammation and thus may significantly contribute to tissue damage via RANKL, which has been shown to be responsible for the resorption of periodontal bone (Aldahlawi et al, 2018). Figure 16 shows levels of CXCL10 which were

highest at 4 hours in Groups A and B. However, levels decreased in all test groups at later time points.

A study investigated titanium-induced expression of chemokines and cytokines that play a key role in osteoclastogenesis. The results from this study suggested that titanium ions play possibly recruit osteoclast precursors to the bone-implant junction by increasing CCL22 expression and by upregulating the CCR4 receptor eventually leading to aseptic loosening of the implant (Cadosch et al, 2009). CCL22 is produced by dendritic cells, macrophages and endothelial cells functions as an adaptive immune chemokine affecting the T-helper 2 (Th2) cells (Hallab and Jacobs, 2017). Figure 10 shows increasing levels of CCL22 from 4 hours to 48 hours in test groups A and B as well in control group E. This is most likely due to its regulatory effect on adaptive immunity with impact on inflammation (Hallab and Jacobs, 2017).

IL-10 is an anti-inflammatory cytokine known to inhibit pro-inflammatory cytokines IL1, IL2, TNF, IL6, IL8, and IFNλ (Casado et al 2013, Fonseca et al, 2014b). Figure 8 shows an increase in IL 10 levels which occurred across all the test groups with the highest levels noted in Groups A and B at 24 hours.

Although IL-12p40 has been shown to be an antagonist, competitively binding to the IL-12 receptor, Abdi (Abdi, 2002) showed IL-12p40 may also have an agonistic role in the development of an immune response (Cooper et al, 2006).

The possible role of IL-12 in the pathogenesis of chronic periodontitis has been shown by Tsai et al (Tsai et al, 2005) where they found the levels of IL-12 were significantly higher in patients diagnosed with chronic periodontitis than in patients with gingivitis or periodontal health. Figure 9 however shows a non-significant rise in production levels amongst all test groups A-D.

We sent surveys to Postgraduate Program Directors in the Unites States to determine the extent of the reuse of HA and methods used for decontamination. Responses from the survey showed (3/14, 21.43%) indicated hundreds of dental implants are placed every year and healing abutments are re-used between patients. Responses as to the description of the decontamination protocol varied from manual debridement, ultrasonic cleaning and rinsing prior to heat sterilization in an autoclave. These results indicate HA which are designed for single use by manufacturers, are re-used on a large scale and no standardized method of decontamination exist prior to their re-use in an educational setting.

Limitations of this study include a smaller sample size with only 10 HA/ group examined. Two brands of healing abutments were used in this study so the effect of different titanium alloys from different brands is unknown. The effect of HA with different diameters and lengths and their correlation to an increase or decrease in residual protein concentration and cytokine release was not measured in this study. The HA used in this study were used only one time. The effects of multiple use and sterilization of the HA prior to use may affect the implant abutment seal thereby giving a different outcome of quantity of residual protein.

Lastly, only four common methods of decontamination of HA were used. Alternate methods of decontamination may be used by clinicians however their efficacy has not been studied.

Our goal to show the efficacy of decontamination methods available in most clinical settings determined that used HA are never truly “decontaminated” since they trigger an immune response by macrophages despite the lack of visible protein. Cumulatively, we conclude that group A and group B seemed to be the least effective method of decontamination while group C and group D seemed to be more effective. Nevertheless, caution should be exercised when considering reusing HA due to the potential to initiate an inflammatory response, even after common “decontamination” procedures are utilized. However, the cause and effect relationship linking used HA to peri-implant inflammation awaits further study. Further research is also required to identify suitable methods of decontamination prior to reusing HA.

VI. CONCLUSION

Within the limitations of the study, our results demonstrate that healing abutments are contaminated with debris after use in patients and may serve as a potential risk for transmission of infectious agents. Our findings are in support of these components being viewed as single-use fixtures, unless significantly more standardized and efficient decontamination techniques can be developed and validated for use in implant dentistry. The potential risk transfer of biologic debris from one individual to another due to the re-use of these fixtures is far too great compared to the economic gain which would be encountered by the clinician and patient.

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APPENDIX

Number	Questions	
Q-1	Do you place dental implants in your residency program?	a. Yes b. No
Q-2	Approximately how many implants are placed in your residency program each year?	a. 0-50 b. 51-100 c. >100
Q-3	Are the majority of the implants placed in your residency program one stage or two stage?	a. One stage b. Two stage
Q-4	In one stage implant placement surgeries, how long do you wait before the final restoration is placed?	a. 2 months b. 3 months c. 6 months d. other
Q-5	In two stage implant placement surgeries, how long do you wait between implant uncover and final restoration?	a. 2 weeks b. 4 weeks c. 6 weeks d. other

Number	Questions	
Q-6	In your residency program, are healing abutments reused between patients?	a. Yes b. No
Q-7	Please describe the decontamination technique protocol when reusing healing abutments in your residency program.	Description
Q-8	How many times is a healing abutment reused in your residency program?	a. 1 b. 2 c. 3. d. D 4+

VITA

EDUCATION

Institution	Degree	Date Conferred	Major
Diplomate of the American Board Periodontology and Implant Dentistry		2016	
University of Illinois College of Dentistry	MS	June 2020	Oral Sciences
University of Pennsylvania School of Dental Medicine	Certificate	June 2015	Periodontics
University of Pennsylvania School of Dental Medicine	Certificate	June 2015	Periodontal Prosthesis
Dr. D.Y Patil Dental University	B.D.S.	July 2014	Dentistry

Licensure

2015 Illinois, Licensed General Dentist– Active
2015 Illinois, Licensed Specialist in Dentistry – Active
2015 Illinois, Controlled Substance License – Active
2015 Western Regional Examination Board (WREB)
2010 India Dental License – Active

PROFESSIONAL EXPERIENCE

2016 – Present	Clinical Assistant Professor, Department of Periodontics, College of Dentistry, University of Illinois at Chicago
2016 – Present	Pre- Clinical Instructor, Department of Periodontics, College of Dentistry, University of Illinois at Chicago
2016 – Present	Lecturer, Department of Periodontics, College of Dentistry, University of Illinois at Chicago
2015 – Present	Faculty Dental Practice, Department of Periodontics, College of Dentistry, University of Illinois at Chicago
2015 – 2016	Clinical Instructor, Department of Periodontics, College of Dentistry, University of Illinois at Chicago

2015 – 2016	Private practice specializing in Periodontics, Periocare Chicago, Illinois
2015	Clinical Instructor, Hoissen, Chicago, Illinois

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Journal Articles:

Ashrafi S, Narvekar A. A quick Reference guide to the evaluation of Peri-implant Tissues in Health and Disease. Access. 2019 January: 8-13

Luan K, Narvekar A, Maintaining the Oral Microbiome. CE Sponsored by Colgate in Partnership with the American Academy of Periodontology, Mar 20, 2019 (Invited)

Luan K, Narvekar A, Supporting Success in Implant Therapy. Decisions in Dentistry, October 25, 2019 (Invited)

Ashrafi, S, Narvekar, A. Evaluation of the Peri-Implant Tissues in Health and Disease. CDEWorld, American Dental Hygienist's' Association, August 2019 (CE – invited)

Narvekar, A, Luan, K, Gholami, F. Regenerative Periodontal Surgery. Clinical Dentistry Reviewed, 2019; **In Production** (Invited)

Textbook chapter:

Narvekar A, Luan KW, Gholami F. Decision Trees in Periodontal Surgery: Resective Versus Regenerative Periodontal Surgery. A Clinical Guide to Techniques and Interdisciplinary Approaches, 2019. Editor. Dr. Salvador Nares.

Society Memberships

2011 – Present	American Academy of Periodontology
2018 – Present	American Dental Education Association
2015 – 2017	American Dental Association
2015 – 2016	Illinois Society of Periodontics
2015 – 2016	Midwest Society of Periodontics
2015 – 2016	Illinois State Dental Society
2015 – 2017	Chicago Dental Society
2016	Indian Dental Association, Chicago Branch

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