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CLINICAL RESEARCH:

Influence of Abutments Surface (machined *vs.* laser-microgrooved) in Soft-tissue Response During One Year of Function: Clinical and Biochemical Outcomes of a RCT with Split-Mouth Design

Influencia de la superficie de los pilares (mecanizada *versus* microranurada con láser) en la respuesta del tejido blando durante un año de función: resultados clínicos y bioquímicos de un ECA con diseño de boca dividida

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ABSTRACT: The purpose of this study was to evaluate peri-implant soft tissue response by assessing IL-6, IL-1b and MMP-8 levels in peri-implant crevicular fluid (PICF) around machined vs. laser-microgrooved implants/healing/prosthetic abutments during 1 year of function. Twenty-four patients each received 2 one-stage implants in a split mouth design on the same jaw. In each patient, one implant, one immediate healing, and one prosthetic abutment with a machined surface (M group), and one implant, one immediate healing abutment and one prosthetic abutment with a laser-microgrooved surface (LMS group) were used. PICF sampling, pocket probing depths (PPD) and bleeding on probing (BOP) were assessed at 1, 3, and 12 months. IL-6, IL-1b and MMP-8 levels were determined by specific enzymelinked immunosorbent assay systems (ELISA). Repeated measure ANOVA was used to run comparisons with groups and between groups months at 1, 3, and 12 months. At 3 and 12 months, the LMS group showed significantly lower PD, BOP and IL-6, IL-1 β and MMP-8 levels than the M group (P<.05). This study suggests the presence of more remodeling and/or inflammatory phenomena around implants/ abutments with a machined surface than around implants/abutments with a laser-microgrooved surface.



KEYWORDS: Abutments surface; Machined; Laser-microgrooved; Soft-tissue response; IL-1_β; MMP-8.

RESUMEN: El propósito de este estudio fue evaluar la respuesta del tejido blando periimplantario mediante la evaluación de los niveles de IL-6, IL-1b y MMP-8 en el líquido crevicular periimplantario (PICF) alrededor de implantes mecanizados versus microranurados con láser/curación/ pilares protésicos durante 1 año de función. Veinticuatro pacientes recibieron cada uno 2 implantes de una etapa con diseño de boca dividida en la misma mandíbula. En cada paciente se utilizó un implante, un pilar de cicatrización inmediata y un pilar protésico con superficie maquinada (grupo M), y un implante, un pilar de cicatrización inmediata y un pilar protésico con superficie microranurada con láser (grupo LMS). Se evaluaron el muestreo PICF, la profundidad de sondaje de las bolsas (PPD) y el sangrado al sondaje (BOP) a los 1, 3 y 12 meses. Los niveles de IL-6, IL-1b y MMP-8 se determinaron mediante sistemas de ensayo inmunoabsorbente ligado a enzimas específicos (ELI-SA). Se utilizó ANOVA de medidas repetidas para realizar comparaciones con grupos y entre grupos meses a 1, 3 y 12 meses. A los 3 y 12 meses, el grupo LMS mostró niveles de PD, BOP e IL-6, IL-1β y MMP-8 significativamente más bajos que el grupo M (P<0,05). Este estudio sugiere la presencia de más fenómenos de remodelación y/o inflamaciones alrededor de implantes/pilares con superficie mecanizada que alrededor de implantes/pilares con superficie microranurada con láser.

PALABRAS CLAVE: Superficie de los pilares; Mecanizado; Microranurado por láser; Respuesta de los tejidos blandos; IL-1β; MMP-8.

INTRODUCTION

Soft tissues around implants differ from that around teeth regarding the amount of blood supply, the direction of connective tissue fibers, the number of fibroblasts and collagen fibers, and the extension of junctional epithelium (1-3). Compared to teeth, differences at the transmucosal portion of dental implants have been related to the interference of wound healing events with biomaterial and the adaptation of the soft tissue to this biomaterial (4, 5). It has been documented in vitro that a smooth surface leads to a flat arrangement of fibroblasts attachments and a consequent extensive spreading and de-differentiation (6, 7). Conversely, microgeometric surface modifications induce fibroblast stabilisation and differentiation, according to the so-called "contact guidance" concept, which promotes connective tissue adaptation to the transmucosal part of the implant (8). Microgeometric properties of the surface can also influence bacterial colonization around implants and abutments (9), which in turn can induce an inflammatory reaction (10) with possible progressive bone loss around implants (11). Intimate contact between the marginal mucosa and implant abutment that protects the implant body from pathological microbial communities of the mouth is believed to be important for the maintenance of healthy conditions and for the long-term success of the implant (1). Strategies aimed at promoting connective tissue adaptation to the transmucosal part of the implant and at reducing bacterial adhesion and biofilm formation on implant abutment surfaces are of pertinent clinical interest and can be used for the maintenance of soft tissue health or possibly in the prevention of peri-implant inflammatory diseases.

One of these strategies is represented by a controlled laser ablation, which allows the creation of microgrooves with resolution within a micrometric range. In vitro experimental studies (12, 13) provided the hypothesis that laser-produced microgrooves in the range of 8µm could be used to create a predetermined site on which a physical connective tissue attachment can be achieved. Subsequent histologic studies on humans demonstrated that unlike fibers aligned in a direction parallel and circumferential to the traditional transmucosal part of dental implants as a fibrous capsule, fibers around an 8µm laser-microgrooved surface (LMS) have a perpendicular, functional physical attachment (14-16). Recent in vitro (17) and in vivo (18) studies showed that the microgooved topography produced with laser ablation can influence bacterial adherence too. Since bacterial cells are more rigid than mammalian cells and are not able to deform to accommodate surface constraints, a micro-grooved topographic pattern that promotes adhesion of mammalian cells can usually reduce bacterial adherence (19, 20).

In most clinical studies related to the evaluation of dental implants and soft tissue health, the state of the peri-implant mucosa is monitored by probing pocket depth (PPD) and bleeding on probing (BOP). However, the use of such "periodontal" parameters has been criticized because they lack sensitivity to assess the biological response of the peri-implant mucosa. Analysis of peri-implant crevicular fluid (PICF) levels of proinflammatory cytokines, such as interleukin IL-1B and IL-6, and enzymes, such as metalloproteinasis-8 (MMP-8) allow the detection of an inflammatory state in periimplant tissues (21). IL-1B and IL-6 have similar and synergistic effects promoting the synthesis of endothelium-binding molecules and inflammatory cells, such as neutrophils, monocytes, and fibroblasts (21, 22). Overproduction of IL-1B and IL-6 is consistent with the presence and progression of inflammation (21, 22). MMP-8 is an enzyme involved in the degradation of extracellular matrix proteins such as laminin, collagens, proteoglycans and fibronectin, leading to increased migration of inflammatory cells and destruction of tissue structure (23). Nowadays, it is believed that the measurement of IL-1B, IL-6 and MMP-8 concentration in PICF can be very helpful to assess the degree of early inflammation in peri-implant tissues. Its advantage over traditional diagnostic methods (e.g., PPD, BOP and radiographic examination) is the increased diagnostic sensitivity and specificity, including the ability to predictively detect health status or/and inflammatory status before clinical and radiographic measurements indicate pathological changes. The purpose of this study was to assess the soft tissue response of healing and prosthetic abutments with a machined surface (M) and a laser-microgrooved surface (LMS) by comparing associated biochemical and clinical parameters, to assess peri-implant inflammatory conditions. The null hypothesis was that perimplant soft tissues adjacent to M and LMS healing and prosthetic abutments would exhibit similar characteristics of health and biochemical features during the first 12 months of function.

MATERIALS AND METHODS

Since 2016, we have treated a series of 24 non-smoking partially edentulous patients (11 males and 13 females, mean age 47.3 years), requiring implant therapy for a prosthetic rehabilitation in at least two contralateral sites of the mandible or maxilla. With a split-mouth design, each patient received two different implants (TRX and TLX BioHorizons, AL, USA). TRX and TLX were randomly allocated (block randomization) based on sequentially numbered opaque sealed envelopes. TRX and TLX implants have the same tapered macro design and the same body grit-blasted surface; TRX implants have the most coronal 0.3mm of collar smooth/machined surface (M), while TLX implants have the most coronal 1.8mm of the collar laser-microgrooved surface (LMS). After placement, TRX implants received an immediate healing abutment with a machined surface (Standard Healing Abutment, BioHorizons, AL, USA) while the contralateral TLX implant received an immediate healing abutment with a laser-microgrooved surface (Laser-Lok© Healing Abutments, BioHorizons, AL, USA) (Figure 1).

The machined abutments were entirely machined, while the laser-microgrooved abutments exhibited a partially (0.7mm) microgrooved surface. For further details regarding study population, exclusion/inclusion criteria and materials and methods, refer the study by Guarnieri *et al.* (24), that previously reported comparative treatment outcomes including sulcus fluid volume, IL-6, and II-1 β concentrations during 12 weeks of healing.

The present study was designed as a continuation of the previous RCT, to compare periimplant soft tissue response after 1 year of functional loading between machined implants/healing/ prosthetic abutments (M group) and laser-microgrooved implants/healing/prosthetic abutments (LMS group). Treatments were performed according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects. The study was approved by the Research Ethics Committee of the Sapienza University of Rome (#4790). ClinicalTrials.gov NCT04415801, registered 03/06/2020, <u>https://register.clinicaltrials.</u> gov/prs/app/action/SelectProtocol?sid=S0009 V52&selectaction=Edit&uid=U0003LQX&ts=24 &cx=-mviyyi

During the prosthesis session, machined healing abutments were replaced with machined definitive prosthetic abutments (Custom Castable UCLA Abutments, BioHorizons, AL, USA) and laser-microgrooved healing abutments were replaced with laser-microgrooved definitive prosthetic abutments (Laser-Lok© Easy Ti Abutments, BioHorizons, AL, USA) (Figure 2).



Figure 1. Schematic view of implant and healing abutment with a LMS surface (on the right) and implant and healing abutment with M surface (on the left).



Figure 2. Schematic view of implant and prosthetic abutment with a LMS surface (on the right) and implant and prosthetic abutment with M surface (on the left).

The machined prosthetic abutments were entirely machined, while the laser-microgrooved abutments exhibited a partially (0.7mm) microgrooved surface. All restorations were single screw retained.

None of the recruited patients had received antibiotics in the last 3 months prior to examination.

Each patient was enrolled in a periodontal/ peri-implant maintenance program with an interval of 4/6 months that included staining of plaque, followed by re-instruction and re-motivation, professional tooth cleaning, followed by polishing using rubber cups and polishing paste with exhaustive application of a fluoride gel.

The clinical examination during the 1-year follow-up period (at 1, 3, and 12 months) included an assessment of the full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS). Moreover, plaque index (PI), gingival index (GI), pocket probing depth (PPD), and bleeding on probing (BOP) at six sites around each implant were recorded. Mucosal recession (REC) was also assessed.

PICF Sampling and Biochemical analysis: all procedures were conducted as in our previous report as follows: the PICF was collected with standardized paper strips (Periopaper[™], Proflow, Amityville, NY) at 1, 3, and 12 months by one clinician (RG). Following the isolation of the sampling area with sterile cotton gauzes, accurate suction was performed, and experimental sites were gently air-dried to reduce any possible contamination with saliva. Supragingival plague was removed with teflon or plastic curettes for implant maintenance. Extreme care was taken to minimize mechanical irritation during PICF sampling because this is known to affect the actual fluid volume. Two paper strips were placed at each sampling site at the same time (mesially and distally) and were left in situ for 30 seconds. Paper strips contaminated by blood were excluded. Before volume measurement, a blank paper strip was placed in the device and the reading dial was set to zero. For the biochemical analysis, the paper strips were placed in a single Eppendorf vial containing 100 µl phosphate-buffered saline and stored at -80°C. Interleukins IL-1B and IL-6 were quantified by enzyme-linked immunosorbent assay (ELISA) kits following the procedures recommended by the manufacturer (Duoset kit; R&D,Minneapolis, MN, USA). The standard solution and samples were added to wells, which had been precoated with specific monoclonal capture antibodies. After 3 hours, polyclonal antibodies conjugated with horseradish peroxidase were added to each well and incubated for 1 hour. A substrate solution containing hydrogen peroxidase and chromogen was added and allowed to react for 20min. The biomarker levels were assessed by a micro-ELISA reader (Ultramark, Bio-Rad, CA, USA) at 450nm and normalized to the abundance of standard solution. The MMP- 8 level in PICF was determined by using MMP- 8-specific sandwich enzymelinked immunosorbent assay (ELISA: Boster Biological Technology Co Ltd). The standard curve was plotted as described in the ELISA kit manual, and the levels of MMP-8 were determined in PICF samples accordingly. All biochemical analyses were performed by a blinded researcher.

The radiological examination was performed using a specialized software (DBSWIN software, Durr Dental Italy S.r.I, Italy) and digital radiographic images (parallel technique and measurements) taken at 12 months, which were compared to the radiographic image taken immediately after the implant surgery procedure (Baseline). Measurements were taken mesial and distal interproximal. The statistical analysis was made with the mean value of the two variables taken.

INTER-RATER CALIBRATION

The inter-rater calibration was performed by the two investigators (RG and LT) on 2 implants each on 6 sites (mesio-buccal, mid-buccal, distobuccal, mesio-lingual, mid-lingual, and distolingual). The statistical analysis resulted in a kappa of 0.773 and an agreement of 86.04%.

STATISTICAL ANALYSIS

The main outcome of the present study was the IL-1B, IL-6 and MMP-8 release in PICF. As indicated in our previous report, the sample size, based on the IL-6, IL-1B and MMP-8 differences between the groups, was determined according to previously published data (25). The following parameters were used for the sample size calculation: a minimum expected difference between means of 0.5, standard deviations on the difference between means of 0.7, an effect size of 0.71, a beta error of 10%, and a one-tailed alpha error of 5%, with an 80% power. This resulted in a required sample size of 12 patients. However, based on the anticipated individual variations in IL-6, IL-1B and MMP-8 responses and the specific study design accounting for potential losses and refusals, the sample size was doubled. These calculations thus estimate a minimum of 24 patients or 48 implants. Differences in each outcome were calculated, and sign tests were performed to see whether there was a difference in IL-1B, IL-6, and MMP-8 levels between M group and LMS group. Data analysis was conducted using computer software (SAS/ STAT® version 9.2; SAS Institute Inc. Cary). Data were analyzed to detect differences between M and LMS groups at 1, 3-, and 12-months postloading. A linear mixed-model regression analysis was used for repeated-measures fixed and random effects within and between groups. Initially, a random effect (intercept and slope) regression analysis was conducted to estimate the slopes of the outcome over continuous time for M and LMS groups. Repeated measure ANOVA was used to run comparisons within groups and between groups months at 1, 3, and 12 months. Sandwich estimator was used to control the correlation due to dependence of the observations among repeated measurements. The variability of the outcome within and between subjects was used to estimate standard error that tests regression coefficients. Bonferroni correction was used to conserve the overall type I error at α =.05.

RESULTS

Mean \pm SD (median) [range] values of IL-1 β , IL-6 and MMP-8 levels in M group and LMS group for different time intervals are shown in Table 1.

In the M group, mean IL-1 β , IL-6 and MMP-8 levels were significantly increased over the 1 to 3 month and 3 to 12-month interval. In the LMS, at the same time intervals, mean IL-1 β , IL-6 and MMP-8 levels did not show statistically significant increase.

Comparisons of the IL-1 β , IL-6 and MMP-8 levels between groups showed that the mean values of IL-1 β , IL-6 and MMP-8 level at 1-month, 3-month, and 12-month intervals around machi-

ned implants/abutments were statistically significant higher than around laser-microgrooved implants/abutments.

Comparisons of mean values of PPD and percentage of sites with BOP between the groups are presented in Table 2 and Table 3, respectively.

Mean PPD and percentage of sites with BOP values in group M at 3 and 12 months were statistically higher than in LMS group, whereas no differences were found in the 1-month assessment (P>.05). Table 4 shows the full-mouth plaque score and full-mouth bleeding score recorded at 1, 3 and 12 months.

Radiographic marginal bone loss at 12 months was significantly greater in M group that in LMS group (0.78 \pm 0.05mm *vs*. 0.13 \pm 0.07mm) (P<0.05).

Table 1. Comparision of IL-1 β , IL-1 and MMP-8 levels (pg/µL) at different time intervals.

Mea	an ± SD, (Media	p				
IL-1β	1 month	3 months	12 months	1-3 m.	3-12 m	1-12 m.
M implant/abutment	7.4±3.1 (7.1) [8-1.3]	11.8±4.1 (11.1) [14-2.7]	18.8±3.6 (18.7) [235.2]	0.024	0.015	0.032
LMS implant/abutments	1.3±0.4 (1.4) [2.4-0.4]	2.0±0.9 (2.4) [2.9-0.8]	2.9±1,3 (3.1) [3.7-0.9]	0.115	0.214	0,119
IL-1	1 month	3 months	12 months	1-3 m.	3-12 m	1-12 m.
M implant/abutment	10.4±4.3 (10.8) [13-1.1]	15.1±2.4 (15.7) [181.9]	23.81±3.1 (24.7) [29-4.2]	0.088	0.013	0.002
LMS implant/abutments	3.3±1.1. (3.4) [4.1-0.2]	4.3±1.4 (4.4) [7.8-1.6]	5.5±1.9 (5.6) [8.9-0.8]	0.324	0.317	0.222
MMP-8	1 month	3 months	12 months	1-3 m.	3-12 m	1-12 m.
M implant/abutment	7.14±3.3 (7.3) [12-1.1]	10.51±2.4 (6.7) [8.7-0.9]	15.81±4.1 (7.7) [191.2]	0.017	0.094	0.098
LMS implant/abutments	3.87±2.4 (3.3) [8.1-0.9]	4.25±1.8 (4.4) [6.4-0.7]	4.73±1.3 (4.6) [6.9-1.1]	0.115	0.214	0,119

	p					
Site	1 month	3 months	12 months	1 month	3 months	12 months
Μ	1.8±0.4	2.9±0.3	3.1±0.5	0.621	0.011	0.014
LMS	1.2±0.3	1.3±0.2	1.4±0.1			

Table 2. Intergroup comparison of mean PPD (in mm) at 1, 3 and 12 months.

Table 3. Intergroup comparison of percentage of sites with BOP at 1, 3 and 12 months.

		М			LMS			р	
	1m.	3m.	12m.	1m.	3m.	12m.	1m.	3m.	12m.
No BOP	83.7	94.4	91.8	85.4	85.4	81.5	0.665	0.021	0.018
At least 1 site with BOP	6.3	6.6	8.2	14.6	14.6	18.5			

Table 4. Patients' full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) recorded during the follow-up period.

		FMPS (%)			FMBS (%)	
	1 month	3 months	12 months	1 month	3 months	12 months
M Group	13.7	14.2	14.6	11.4	10.3	9.3
LMS Group	14.1	13.9	14.4	9.7	9.1	10.1
p	0.77	0.81	0.39	0.42	0.63	0.44

DISCUSSION

In the current study, the subjects presented at each visit with good plaque control, as evidenced by the full-mouth plaque score and Pl values, that in both groups showed no statistically significant variation at 1, 3, and 12 months. In reverse, the percentage of sites with BOP was higher in M group than in LMS group at 3 and 12 months, but not at 1 month. This higher rate suggests that the peri-implant mucosa around M implants/abutments may be mechanically more fragile than the periimplant mucosa around LMS implants/abutments. From the third month after loading, onwards, PPD mean values in LMS group were lower than in M group, indicating a stronger permucosal seal around the laser-microgrooved abutments. Moreover, M group showed a higher radiographic marginal bone loss indicating that LMS abutments may reduce marginal bone remodeling after loading. These findings are in accordance with the results of two recent literature reviews (26, 27) confirming the hypothesis of less marginal bone loss and better peri-implant soft tissue conditions around LMS implants/abutments than M implant/ abutments. At the same time intervals, the IL-1 β , IL-6, and MMP-8 levels were significantly higher around LMS

implants/abutments. The null hypothesis of the current study was that peri-implant soft tissues adjacent to M and LMS healing and prosthetic abutments would exhibit similar characteristics of health and biochemical features during the first 12 months of function. The biochemical findings of our study don't support the hypothesis. Vice versa results indicated a higher inflammatory activity around M implants/abutments than around LMS implants/abutments. In the peri-implant tissue response IL-6 and IL-1 β are released in a spatial and time-controlled manner, representing two potent mitogenic and chemotactic agents for epithelial cells, fibroblasts, and neutrophils (28, 29). Moreover, they act as pro-inflammatory cytokines in CD4+ T cell differentiation (30). MMP-8 is involved in the degradation of extracellular matrix proteins such as laminin, collagens, proteoglycans, and fibronectin, which lead to increased migration of inflammatory cells and destruction of the tissue structure. MMP-8 can also process or activate host defense molecules and pro-inflammatory mediators, as well as modulate various cellular signaling pathways (31, 32). It has been documented that the concentration of IL-1B, IL-6 and MMP-8 in PICF in healthy implants is very low. whereas elevated levels have been shown to be associated with to the early active phase of periimplant soft tissue inflammation (21). Based on the results of the present study, it is possible to speculate that, as the connective tissue adhesion at the machined abutment surface has a poor mechanical resistance, a higher pro-inflammatory state could be necessary to support the anti-inflammatory response. Moreover, it is reported that the expression of IL-6 and IL-1 β is induced by the lipopolysaccharide of gram-negative bacteria (33). Accordingly, the higher levels of IL-6 and IL-1B detected in M group might indicate the presence of a higher soft tissue immunologic response that could be associated with greater protection against bacterial insult.

Several studies considered the microbiological behavior of the LMS. John et al. (34) compared the initial biofilm adhesion and development on LMS vs. hydrophobic smooth pickled titanium surfaces, hydrophilic smooth and acid etched titanium surfaces, hydrophobic sandblasted large grid and acid etched titanium surfaces. After 48 h of intraoral exposure the LMS showed significant less initial plaque area than all control groups. Cunha et al. (35) investigated a laser surface texturing as a method to reduce Staphylococcus aureus colonization and biofilm formation of Grade 2 titanium alloy surfaces. Authors found that LMS could confer antibacterial properties to the titanium surfaces when compared to the control, polished surfaces, and that the treatment caused a reduction in bacteria agglomeration, decreasing the tendency to form biofilms. Di Giulio et al. (36) compared 48h Porphyromonas gingivalis biofilm formation LMS with biofilm formation on grit-blasted and machined surfaces. The authors concluded that the LMS was significantly less colonized than grit-blasted surfaces. The same results were reported by Drago et al. (37), who compared the in vitro biofilm formation of Porphyromonas gingivalis, Staphylococcus aureus, and Pseudomonas aeruginosa on disks of the laser-treated surface and a grit-blasted surface. In a previous RCT (38), the authors of the present paper evaluated *in vivo* the differences in concentration of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, and Tannerella forsythensis around LMS vs. M implants. The total number of these periodontal pathogens in LMS implant sites was lower than that in M implant sites, indicating that the LMS on the implant collar

is less vulnerable to colonization of perio-pathogenic microflora than implants with a M collar surface. Microbial surface contamination is reported to have a negative effect on the soft tissue integration of the abutment on the implant surface (39). Moreover, it could stimulate Inflammatory responses, resulting in epithelial downgrowth and peri-implant bone loss (40, 41).

The combination of a slower biofilm formation and the advantage of being able to favor a physiological seal at the level of the implant collar could justify the results obtained in the present study around LMS *vs.* M implants/abutments.

One limitation of the present study may lie in the fact that the sites compared were not the same (for example molar *vs.* premolar areas). However, each contralateral implant site was in the same arch (mandible or maxilla) and intercalated between mesial and distal teeth with similar hard and soft tissue conditions. Other limitations include the small sample size and the short followup. Further studies with an increased number of samples and longer follow-ups are still necessary to confirm the reported findings.

CONCLUSIONS

Results of the current study suggest the presence of more remodeling and/or inflammatory phenomena around implants/abutments with a machined surface than around implants/abutments with a laser-microgrooved surface.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization: R.G. and R.R. Methodology: A.Z. Software: D.D.N. Validation: R.A. and A.Z.. Formal analysis: D.D.N. and F.P. Investigation: R.G. and F.P. Resources: L.T. Data curation: R.R. Writing-original draft preparation: R.G. and R.R. Writing-review and editing: R.G. and L.T. Visualization: A.Z. Supervision: L.T. Project administration: L.T.

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