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Original Article

Effects of restraint stress and surface treatments on the stability of titanium dental implant osseointegration in dogs: An in vivo comparative study

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الملخص

اهداف البحث:هدفت الدراسة الى تقييم تاثير الاجهاد المقيد, المعالجة الرملية, المعالجة الرملية مصاحبة للمعالجة الحامضية, استخدام جهاز الليزرنوع الإربيوم والإيتربيوم ,الكروم والسكانديوم والجاليوم, وكذلك الطلاء باستخدام مادة العكبرلمعالجة سطوح الغرسات السنية ومدى تاثيرهم على ثبات الزرعات السنية نوع الرابع لمادة التيتانيوم ومدى تكون العظم حولها في الكلاب.

طرق البحث: شملت الدراسة من مامجموعه ثمانية وأربعين غرسة أسنان , CPTi حيث تم تقسيمهم ألى أربع مجموعات وفقًا للمعالجات السطحية على النحو التالي: المجموعة أ: المعالجة الرملية المصاحب بالمعالجة الحامضية، المجموعة ب: المعالجة الرملية باستخدام اوكسيد الالمنيوم، المجموعة ج: ،باستخدام جهاز الليزرنوع Er,Cr:YSGG ، المجموعة د: طلاء سطح الغرسة بمستخلص العكبر. انجزت الدراسة على ستة عشر كلباً من السلالة المحلية تتراوح أعمارهم بين 1-1.5 سنة ووزن 22 ± 3 كجم. تم تقسيم الكلاب إلى مجموعتين رئيسيتين (عدد = 8 كلاب): المجموعة الأولى: غير متوترة من دون اجهاد، المجموعة الثانية: متوترة مع الاجهاد المقيد. تم تقسم كل مجموعة الى اربع مجموعات بحسب المعالجة السطحية للزرعات السنية(كلبان لكل مجموعة وفيه ثلاث غرسات), تم اختبار قياس ثبات الزرعة عند الاوقات التالية وهي اليوم الاول, بعد اربعة عشر يوما, بعد تسعون يوما باستخدام جهاز ®EasyCheck. لتحليل الكورتيزول في مصل الكلاب لكل من المجموعات الحيوانية المجهدة وغير المجهدة عند اوقات التحليل التالية ليوم 0, 15,30,45,60, و90 يومًا باستخدام ELISA لفحص الكورتيزول في الكلاب. تم تحليل البيانات إحصائيا باستخدام تحليل التباين أحادي الاتجاه (ANOVA) واختبار *Post Hoc* Tukey's عند 0.05 من مستوى الاختلاف .

النتائج: اختبار قياس ثبات الزرعة اثبت أن المعالجة الرملية للمجموعة غير المجهدة (المجموعة الأولى) كان لأعلى قيمة متوسط (88.0) في تسعون يومًا بينما أظهرت المعالجة الرملية مع المعالجة الحامضية عند اربعة عشر يومًا أقل قيمة متوسط (82.6). كان للمعالجة باستخدام الرمل في (المجموعة الثانية) أعلى قيمة متوسط (88.3) في تسعون يومًا بينما أظهرت المعالجة السطحية الليزرية في اربعة عشر يومًا أدنى قيمة متوسط (72.00). كان مصل الكورتيزول في تسعون يومًا مرتفع بشكل معنوي مقارنة بالاوقات الاخرى المعتمدة في كل من المجموعات غير المجهدة والمجهدة ايضا ($P < 0.05$)

الاستنتاجات: إن اختبار قياس ثبات الزرعة يعتمد على المعالجة السطحية. كانت قيمة ثبات الزرعة أعلى في المعالجة الرملية للمجموعات المجهدة وغير المجهدة عند تسعون يومًا. كان لدى مجموعة الحيوانات المجهدة مستويات أعلى من الكورتيزول في الدم لجميع الغرسات المعالجة سطحيًا مقارنة بالمجموعة غير المجهدة.

الكلمات المفتاحية: الاجهاد المقيد؛ الثباتية؛ معالجة السطح؛ غرسات التيتانيوم السنية

Abstract

Objectives: This study was aimed at assessing the effects of restraint stress and sandblasting; sandblasting with acid etching; Er–Cr: YSGG laser treatment; and propolis coating of implant surfaces on the implant stability quotient (ISQ) of grade 4 titanium dental implant osseointegration in model dogs.

Methods: A total of forty-eight CPTi dental implants were divided into four groups according to surface treatment: group A: sandblasting with acid etching; group B: sandblasting with Al_2O_3; group C: Er–Cr: YSGG laser; and group D: propolis coating. Sixteen male dogs of local breed,1–1.5 years of age, weighing 22 ± 3 kg, were divided into two main groups (n–8 dogs

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each): a non-stressed group (group I) and stressed group (group II). Each of these groups is further divided into four implant groups per surface treatment, A, B, C, and D (two dogs per implant group), each dog has three implants. The ISQ was tested at 0 (baseline), 14, and 90 days with a noninvasive EasyCheck® device. Serum cortisol in the stressed and non-stressed groups was analyzed at 0, 15, 30, 45, 60, 75, and 90 days with a canine cortisol ELISA kit. The data were statistically analyzed with one-way analysis of variance and Tukey's *post hoc* test at a 0.05 level of significance.

Results: For implant stability quotient (ISQ), sandblasting in the non-stressed (group I) had the highest mean value (88.0) at 90−days, whereas sandblasting with acid etching at 14−days had the lowest mean value (82.6). Sandblasting in the stressed (group II) had the highest mean value (88.3) at 90−days, whereas the laser surface treatment had the lowest mean value (72.00) at 14−days. Serum cortisol (ng/μl) at 90 days (143.10 and 195.33 for non-stressed and stressed groups respectively), was significantly higher than other time intervals ($P < 0.05$).

Conclusions: The ISQ was dependent on surface treatment, and was higher with sandblasting than the other treatments in the stressed and non-stressed groups at 90 days. For all surface-treated implants, the stressed group had significantly higher serum cortisol levels than the non-stressed group.

Keywords: Restraint stress; Stability; Surface modification; Titanium dental implants

Introduction

Dental implants are usually made of materials such as metal. These materials should have autogenous tissue-equivalent biomechanical properties. Qualities, such as biocompatibility, biofunctionality, bioadhesion, and corrosion resistance indicate a material's suitability for biomedical implant applications.[1] Stainless steel, cobalt alloys, titanium, and titanium alloys are the primary metallic biomaterials. Materials such as commercially pure titanium (CPTi) range in grade from 98% to 99.6% pure titanium (grades 1, 2, 3, 4, and grade 5 Ti-6Al-4V alloys), which differ in ductility, strength, and corrosion resistance. For the fabrication of dental implants, a biocompatible material with adequate strength, hardness, corrosion resistance, and fracture resistance would be ideal; these properties are typically associated with the oxygen residues. Grade 4 CPTi is the most commonly used type of titanium in dental implants because it has the highest oxygen content (0.4%) and mechanical strength.[2–4]

The success of dental implants depends primarily on osseointegration. Direct contact and interaction between peri-implant tissues and implant surfaces are necessary for optimal osseointegration, to avoid interference in the connective tissue layer.[4,5]

Numerous surface modification techniques, such as laser surface modification, anodization, hydroxyapatite coating, sandblasting, acid etching, combinations thereof, and Ti plasma spray coating, have been developed to enhance the compatibility of titanium and the osseointegration of surrounding bone structures.[5] Bone-to-implant contact can be improved by the presence of a rough surface; variations in implant surface roughness also significantly influence the healing of the bone surrounding the implant.[6–8] Thus, roughness is a key factor influencing dental implant osseointegration.

Implant stability, the ability of an implant to resist vertical, horizontal, and rotational forces is among the most important factors for successful dental implant treatment and serves as an indirect indicator of osseointegration and successful healing. Depending on the biomaterial used, various bone morphological features may benefit implant stability. According to the local tissue qualities, the choice of suitable biomaterial is now mandatory.[9] However, the primary stability of the implant should not be excessive, to prevent bone necrosis due to overloading of bone tissue.[10]

Chronic emotional stress has been found to be a major risk factor contributing to the onset of various diseases and negatively affecting the bone-to-implant connection.[11] The hypothalamic paraventricular nucleus enhances secretion of corticotrophin-releasing hormone when the hypothalamic-pituitary-adrenal system is activated by emotional stress. By encouraging adrenocorticotropin secretion from the anterior pituitary gland, this mechanism induces the release of adrenal corticosteroids.[12]

Despite mounting evidence indicating that chronic stress impairs wound healing, the effects of stress on the bone−implant connection have not been well explored in the literature and remain under debate.

This study was aimed at assessing the effects of restraint stress and sandblasting; sandblasting with acid etching; Er−Cr:YSGG laser treatment; and propolis implant surface coating treatments on the Implant Stability Quotient (ISQ) of grade 4 titanium dental implant osseointegration in model dogs. The null hypothesis was that restraint stress and the various implant surface treatments would not affect the ISQ or canine serum cortisol levels.

Materials and Methods

The Ethical Committee at the College of Dentistry, University of Mosul, Mosul, Iraq, approved the current study involving animal research (UoM. Dent/A.67/22). This experimental animal study was conducted at the experimental surgical center of the Veterinary Teaching Hospital, College of Veterinary Medicine/University of Mosul, Mosul, Iraq, between January 2023 and September 2023.

Study setting

Sixteen healthy mature adult local breed male dogs 1−1.5 years of age, weighing 22 ± 3 kg, were included in the current study. The animals received care in accordance with the institutional guidelines, including appropriate veterinary

care and standard laboratory nutritional support throughout the study period. While the dogs were caged, their behavior was observed as a basis for divided them into non-stressed and stressed groups before stressors were applied.

Throughout the experimental study, healthy dogs were housed individually in 1.5 m^2 cages under a 12-hour light/dark cycle, and natural food and water were freely available. The dogs underwent oral hygiene and plaque control by mechanical cleaning of both teeth and implants with a mechanical toothbrush and 0.2% chlorhexidine mouth irrigation once per week.[13] From the first day of implant installation, the body weight of each dog was measured every 15 days.

Study design

The oral cavity in adult dogs has been used as an experimental site for implant installation. The dogs were divided into two main groups, each consisting of eight dogs:

Group I: non-stressed animal group.
Group II: stressed animal group.

As described in a previous study,[14] the titanium dental implants investigated in this study were Dentium standard screw-type dental implant systems (Dentium Co., Ltd. Seoul, Korea), with a diameter of 4 mm and length of 10 mm.

A total of 48 commercially pure titanium dental implants were randomly divided into the following four groups according to surface modification:

Group A: n = 12 titanium dental implants subjected to sandblasting and acid etching (SLActive) surface treatment (etched with a warm hydrochloric acid concentration of HCl 37% at 60 °C for 5 min, rinsed and cleaned by ultrasonication in ultra-pure water, and dried).
Group B: n = 12 titanium dental implants subjected to sandblasting surface treatment (air-abraded with aluminum oxide (Al_2O_3) particles of 50 μm particle size for 15s at 0.6 MPa, 6 bars of pressure).
Group C: n = 12 titanium dental implants subjected to Er–Cr: YSGG laser surface treatment (at a wavelength of 2780 nm, set at 100 mJ/pulse, with a power of 2.5 W, frequency of 30 Hz, and pulse duration of 60 s, accompanied by 40% water and 50% air spray).
Group D: n = 12 titanium dental implants subjected to propolis surface coating treatment (ethanolic extracted of Iraqi propolis was applied in drops and then brushed on the implant surface).

Clinical evaluation

Pre-operatively, all dogs were clinically examined and evaluated intraorally to ensure the presence of jaws with fully erupted permanent intact dentition, absence of occlusal trauma, and presence of healthy periodontium. All dogs were free from viral or fungal infections and had good systemic health. All dogs were radiographically examined with an X-ray scanner (POX-300 BT, Toshiba, Rotanode™, Japan) and evaluated pre-operatively for jaw bone width, length, density, and dimensions at the site of implant rooming.

Blood sampling

For the analysis of biochemical parameters, the dogs were fasted for 12 h before the collection of blood samples in both the stressed and non-stressed groups at 0(baselines), 15, 30, 45, 60, 75, and 90 days (Figure 1). Almost all samples were collected between 10.00 and 11.00 AM, to minimize errors that might potentially have been introduced by changes in collection times. Five-milliliter blood samples from each dog were drawn via jugular vein puncture, placed into plain filter tubes, allowed to rest protected from light, stored temporarily at 4 °C, and centrifuged for 10 min at 3000 rpm. The separated serum was removed with a micropipette and transferred to sterile Eppendorf tubes, which were stored vertically at 20 °C, and kept for a maximum of 3 months to preserve the stability of cortisol until analysis (at least 1 ml per sample). The frequency of sample collection depended on the sampling date, but the maximum storage period was never reached.[15]

Serum cortisol as a stress biomarker

The generation of animal models of restraint stress is based primarily on alternation of multiple stressors. The cortisol units were 1 μg/dL–27.59 nmol/L. The normal baseline cortisol level in dogs is 20–250 nmol/L. In the present study, the dogs in the stressed group were exposed to dark for 12 h, noise, and hunger during the day.[15,16] The canine cortisol concentration was analyzed with an ELISA kit (Elabscience® Quickey Pro canine cortisol, competitive ELISA, USA).

Surgical procedure

Before each surgical procedure, the dogs fasted for 12 h, the mouth was irrigated with 0.2% chlorhexidine mouthwash, and systemic coverage with prophylactic antibiotics comprised a combination of procaine penicillin and streptomycin administered I.M. at a dose of 10,000 international units, 10 mg/kg weight, and Metalgen analgesic administered at a dose of 3 ml once daily and continuing for 4 days after the operation. On the day of the operation, the selected dog's general health was verified by a veterinarian. The dog subsequently underwent general anesthesia with intramuscular injection of ketamine hydrochloride 10% (10 mg/kg body weight) with xylazine 20% at a dose of 2 mg/kg intramuscularly, which maintained sedation for the required time with minimal pain. The dogs were pre-anesthetized and received conventional dental infiltration local anesthesia with 2% lidocaine HCL with epinephrine 1:80,000 injected into the buccal and lingual gingiva at surgical sites for hemostasis.

Extraction phase

The treatments were performed under the direction of a veterinarian (Figure 2, a–d). The mandibular left premolar teeth (P1, P2, and P3) were extracted, followed by 12 weeks of healing. A supra-crestal incision was made from the mandibular canine to the first molar M1; a mucoperiosteal full-thickness flap was peeled back and elevated both

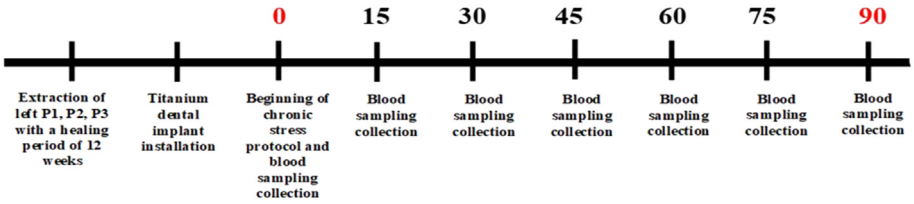

Figure 1: Experimental design with follow-up periods (**days**).

buccally and lingually, and the teeth were sectioned in the buccolingual direction at the bifurcation with a tungsten carbide bur. Roots were subsequently extracted individually with elevators, to remove any separated root remnants and lower surgical forceps without damaging the remaining socked bony walls. The dimensions of the sockets were measured with digital calipers, and mean alveolar ridge measurements were determined. The flaps were repositioned with multiple sutures for a 12-week healing period after tooth extraction. The dogs were fed a soft diet, and the sutures were removed after 2 weeks.

Implant placement

After 12 weeks of healing and adequate bone remodeling (adequate bone formation) in the socket of the extracted left mandibular premolars (P1, P2, and P3) (Figure 3:a,b), three dental titanium implants (Dentium Co., Korea) (4 × 10 mm diameter × length) were installed in the position of the previously extracted mandibular premolars (P1, P2, and P3). First, the surgical guide was fitted on the planned dog mandible, and the first guiding drill was inserted into the central implant position. The buccal bony crest was the level at which the implant was positioned. The three dental implants were inserted in the premolar region (#1−3). The surgical implant placement protocol and the sequential osteotomy were performed according to the manufacturer's guidelines at the recipient sites through the use of a

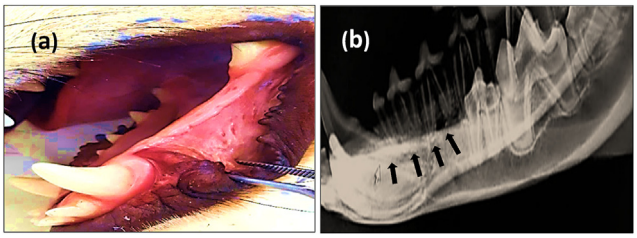

Figure 3: Twelve-week healing period after tooth extraction. (a) Clinical view. (b) Radiographical X-ray showing adequate bone formation 12 weeks after tooth extraction (black arrows).

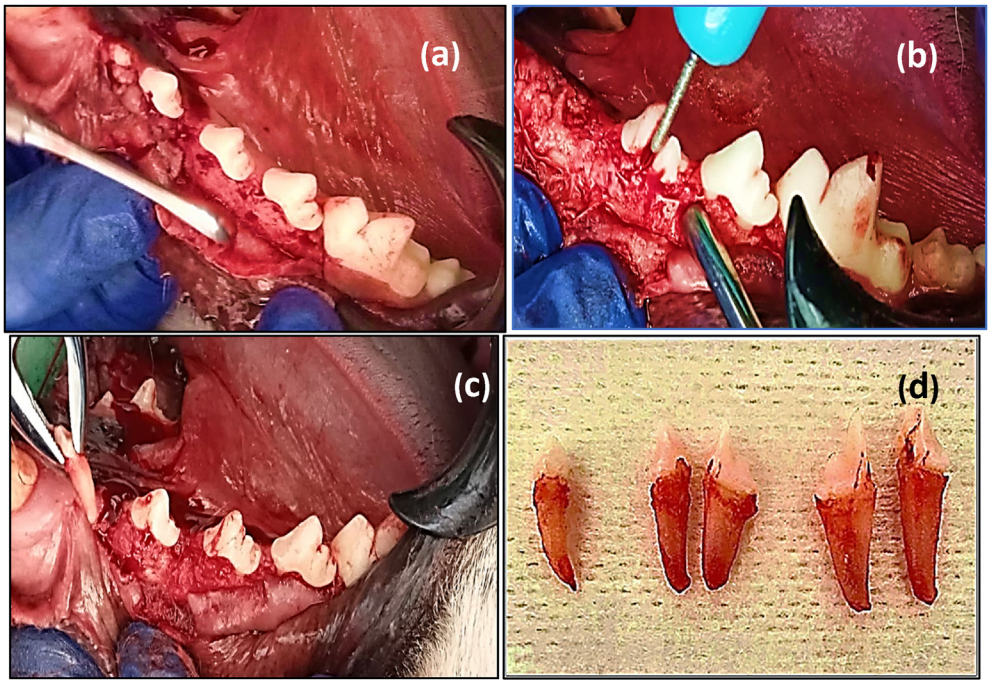

Figure 2: Surgical extraction of mandibular P1, P2, and P3. (a) A mucoperiosteal full-thickness flap both buccally and lingually was elevated. (b) The teeth were sectioned in the buccolingual direction at the bifurcation area. (c) The roots were extracted individually with elevators and lower surgical forceps to remove the separated root. (d) Extracted sectioned mandibular teeth.

surgical guide template with the sequential steps of implant placement (Figure 4: a–h). The surgical torque control (insertion torque) was ≥35 Ncm. Subsequently, cover screws (Dentium Co., Ltd. 150, Endong ro, Giheung-gu, 16985, Republic of Korea) were screwed at 10 Ncm on each implant to enable a submerged healing protocol, and the soft tissues were closed with non-resorbable sutures.

Implant stability test

To evaluate the clinical stability of the dental implants, as represented by the ISQ, we used Easy Check® (on a scale from 1 to 99) (Easy Check Genoss Co., Ltd, 821174-10, Jagok-ro, Gangnam-gu, Seoul, Republic of Korea), a new damping capacity device for noninvasive biomedical measurement of implant stability (Figure 5). The ISQ value was calibrated with a standard height of 3.5 mm and was performed at the buccal and lingual aspects according to the manufacturer's instructions, at baseline (immediately after insertion, time–0), and 14 and 90 days after implant installation. The attack pole was directly connected perpendicularly (90°) to the healing abutment as recommended by the manufacturer (Figure 6). The Dentium ISV scale indicates that values of ≤60, 65–70, and >70 are low (instability), moderate, and high (greater stability), respectively.

To evaluate the repeatability of the measurements, we conducted each measurement five times on two sides in perpendicular directions at a measurement angle of 90°, the

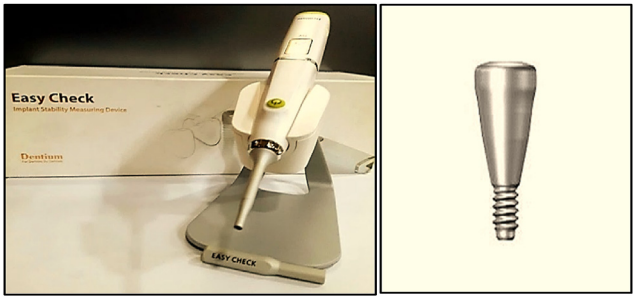

Figure 5: A: Easy Check device, B: Healing abutment of 3.5 mm height and 4 mm diameter.

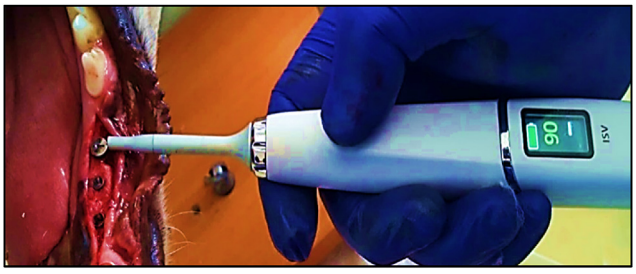

Figure 6: The attack pole was directly connected perpendicularly at an angle of (90°) with respect to 3.5 mm height healing abutment.

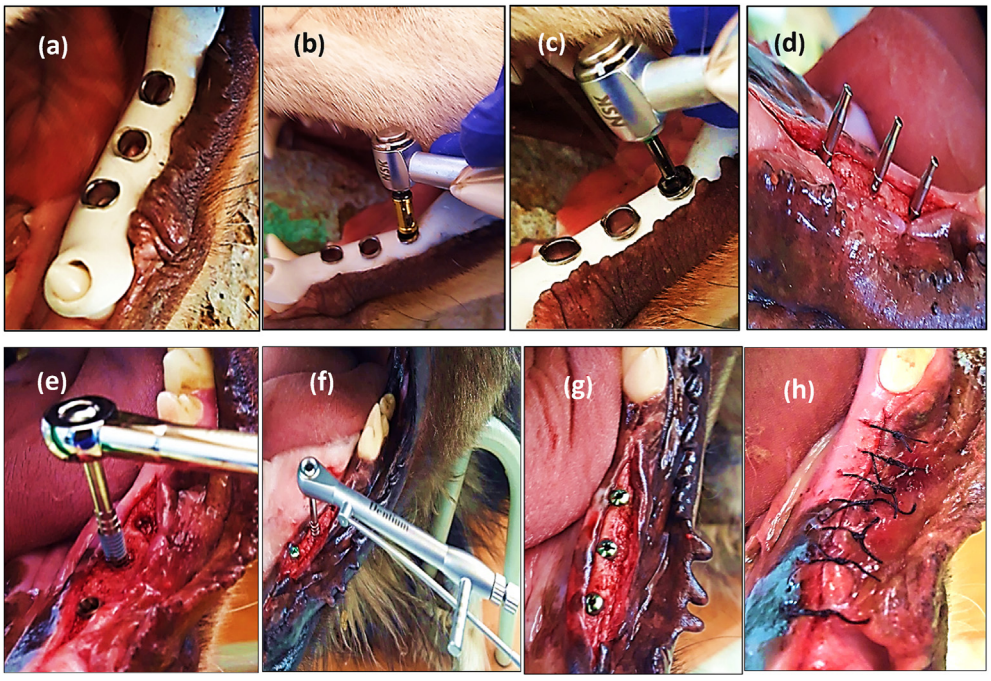

Figure 4: Sequential osteotomy and implant installation, performed according to the manufacturer's guidelines. (a) Customized surgical guide during implantation within the oral cavity. (b) Pilot drill with a stopper. (c) Final drill with a stopper at the recommended diameter and distance of entry by the guidance of the sleeve. (d) Parallel pins application for parallelism. (e) Implant installation with an insertion torque ≥35 Ncm. (f) Calibrated torque wrench. (g) Cover screws screwed at 20 Ncm torque. (h) Soft tissues closed with non-resorbable sutures.

means and standard deviations of the ten ISQ values were calculated.[17,18]

Statistical analysis

Software from SPSS Inc. (Chicago, IL, USA), version 21.0, was used to analyze the data. Both descriptive statistics and the statistical data were evaluated. All implant surface modification data were analyzed with one-way analysis of variance (ANOVA), and P values <0.05 were considered statistically significant. Groups with significant differences were compared with Tukey's *post hoc* test.

Results

Implant stability testing

A total of 48 implant sites in 16 dogs were included. The ISQ was tested at the time of implant installation (baseline), and at 14- and 90-days follow-up. Implant surface treatment groups (A, B, C, and D) were compared, and the data from all groups were analyzed. Implants with ISQ reference values $\geq$ 70 ISQs were considered stable.

The mean ISQ values and standard deviations (SDs) for the various time points after implant installation in the non-stressed and stressed animal groups are listed in Table 1. The surface treatment protocols caused significant differences in the ISQ values in the four groups ($P < 0.05$). For various implant surface modifications, one-way ANOVA indicated significant differences in mean ISQ among various surface treatments in all groups ($P < 0.05$) (Table 2). Tukey's *post hoc* test indicated that sandblasting surface treatment in the non-stressed dogs (group I) had the highest mean value (88.0) at 90 days among groups, whereas sandblasting with acid etching at14 days had the lowest mean value (82.6) (Table 1). Tukey's *post hoc* test indicated that sandblasting surface treatment in stressed dogs (group II) had the highest mean value (88.3) at 90 days among groups,

Table 1: Mean and standard deviation of ISQ in all dog groups according to surface treatment.

Animal groups	Time interval (days)	Surface treatments, mean ± SD				N
		Sandblasting with acid etching (A)	Sandblasting (B)	Laser surface treatment (C)	Propolis coating (D)	
Non-stressed	0	87.33 ± 2.51 a	87.66 ± 2.51 a	86.33 ± 1.15 b	86.66 ± 3.05 b	8
	14	82.66 ± 2.51 e	85.33 ± 2.51 c	84.00 ± 0.00 b	84.00 ± 1.00 d	8
	90	84.33 ± 1.15 c	88.00 ± 0.00 a	86.33 ± 2.08 d	86.66 ± 0.57 b	8
Stressed	0	86.33 ± 2.30 b	84.00 ± 1.00 d	87.33 ± 2.51 a	84.66 ± 5.03 c	8
	14	73.00 ± 1.73 j	78.66 ± 3.21 g	72.00 ± 2.00 l	72.33 ± 2.51 k	8
	90	80.00 ± 3.46 f	88.33 ± 1.52 a	77.00 ± 2.00 h	75.00 ± 2.64 i	8

Abbreviations: SD, standard deviation, number of dogs = 8, different letters indicated statistically significant differences according to Tukey's test, $P < 0.05$.

Table 2: One-way ANOVA of ISQ in stressed and non-stressed groups according to implant surface treatment.

		Sum of squares	df	Mean square	*F*-value	*P*-value
Non-stressed	Between groups	94.222	11	8.566	2.390	0.036[a]
	Within groups	86.000	24	3.583		
	Total	180.222	35			
Stressed	Between groups	1225.556	11	111.414	15.367	0.000[a]
	Within groups	174.000	24	7.250		
	Total	1399.556	35			

[a] Significant differences, $P < 0.05$. df = degree of freedom.

Table 3: Analysis of variance (ANOVA) in the two animal groups according to surface treatment and time interval.

	Surface treatment	Time (days)	Sum of squares	df	Mean square	*F*- value	*P*- value
Non-stressed × stressed	Sandblasting with acid etching	0	1.500	1	1.500	0.257	0.639
		14	66.667	1	66.667	8.000	0.047*
		90	28.167	3	7.333	4.2250	0.019*
	Sandblasting	0	22.000	1	0.667	0.7460	0.554
		14	66.667	1	66.667	8.0000	0.047*
		90	24.000	1	24.000	48.000	0.002*
	Laser treatment	0	1.500	1	1.500	0.3910	0.566
		14	308.167	1	308.167	73.960	0.001*
		90	73.500	1	73.500	36.750	0.004*
	Propolis coating	0	6.000	1	6.000	0.3460	0.588
		14	204.167	1	204.167	55.682	0.002*
		90	204.167	1	204.167	55.682	0.002*

* Significant differences, $P < 0.05$.

Table 4: Mean and standard deviation of body weight in animal groups according to time interval.

Animal groups	Time intervals (days)	Weight (kg) ± SD	*P*-value
	0	26.96 ± 2.66 f	0.000*
	15	24.76 ± 0.25 g	
	30	27.03 ± 1.00 e	
Non-stressed	45	28.16 ± 1.04 d	
	60	32.73 ± 0.25 a	
	75	32.30 ± 0.26 b	
	90	31.73 ± 0.25 c	
	0	27.20 ± 0.72 a	0.000*
	15	24.13 ± 3.59 b	
	30	20.23 ± 0.25 c	
Stressed	45	19.23 ± 1.93 d	
	60	17.80 ± 1.66 e	
	75	15.66 ± 0.28 f	
	90	15.13 ± 0.23 g	

Abbreviations: SD, standard deviation.
* Significant differences: different letters indicated statistically significant differences according to Tukey's test, $P < 0.05$.

Table 5: Mean and standard deviation of serum cortisol (ng/μl) in the stressed and non-stressed groups according to time interval.

Animal groups	N	Time intervals (days)	Cortisol (ng/μl) ± SD	*P*-value
		0	53.66 ± 0.57 f	0.000*
		15	104.53 ± 0.05 e	
		30	111.86 ± 0.05 d	
Non-stressed		45	127.16 ± 0.15 c	
		60	142.06 ± 0.05 b	
		75	143.10 ± 0.10 a	
		90	143.10 ± 0.10 a	
		0	129.10 ± 1.01 g	0.000*
		15	142.36 ± 0.77 f	
		30	148.13 ± 5.34 e	
Stressed		45	164.13 ± 0.32 d	
		60	194.16 ± 0.76 c	
		75	194.80 ± 0.72 b	
		90	195.33 ± 0.76 a	

Abbreviations: SD, standard deviation.
* Significant differences: different letters indicated statistically significant differences according to Tukey's test, $P < 0.05$.

whereas laser surface treatment at 14 days had the lowest mean value (72.00) (Table 1).

In a comparison among animal groups at the same time interval for each implant surface treatment, one-way ANOVA (Table 3) indicated significant differences in the mean ISQs for sandblasting, sandblasting and acid etching, laser, and propolis surface treatments ($P < 0.05$) at 14 days and 90 days.

Body weight

The mean body weight of all dogs was ~25 kg at the beginning of the study. One-way ANOVA (Table 4) indicated a significant difference in mean body weight between treatment groups ($P < 0.05$). During the period in which restraint stress was applied, dogs in the non-stressed group exhibited an increase in body weight, whereas those in the stressed group exhibited significant weight loss ($P < 0.05$) (Table 4).

Stress biomarker analysis

All samples were collected between 10.00 and 11.00 AM, to minimize potential errors due to changes in collection time. The analyzed cortisol levels were significantly higher in the stressed group than in the non-stressed group ($P < 0.05$). The serum cortisol levels (Table 5) in the 90−day experimental period were significantly higher than in other time intervals ($P < 0.05$). The serum cortisol levels in both stressed and non-stressed animal groups significantly increased from the first stress cycle to the 60-day time point (in which animals were adapted to the stressor) when compared with other baseline time intervals (Table 5).

Discussion

The current research was aimed at assessing the effects of chronic restraint stress in a dog model as well as the effects of sandblasting with acid-etching, sandblasting, laser ablation, and propolis implant surface treatments on the grade 4 titanium implant osseointegration stability. The null hypothesis was rejected.

The sandblasting surface treatment in the non-stressed (group I) had the highest mean value (88.0) at 90 days among groups, whereas sandblasting with acid etching after a 14−day time interval resulted in the lowest mean value (82.6) (Table 1). The sandblasting surface treatment in the stressed group (group II) had the highest mean value (88.3) at 90 days among groups, whereas the laser surface after a 14−day time interval treatment showed the lowest mean value (72.00) (Table 1). These findings were attributed to various factors affecting the ISQ, owing to surface roughness produced in Ti dental implants based on their surface treatment protocol.

At the time of implant placement (day−0) (Table 3), no significant differences in primer stabilization measurements were observed among groups. The identical design, implant geometry, and macroscopic groove properties might have contributed to the similarity of the ISQ readings. The stability implant ISQ in all animal groups with various surface treatments (Table 1), initially decreased at 14 days and then increased at 90 days after implant placement. The weakest correlation was observed after 2 weeks, possibly because of individual variations in the loss of primary stability, thereby resulting in broad variations in ISQ values. The decreased implant stability after 14 days of healing should be considered a common event that should not require changes in routine follow-up.[17]

According to our results (Table 1), the stability of implants was weakest in the 2−4 weeks after implant installation, and any activities producing micromotion of the implant, such as tightening of the healing abutment during measuring should be avoided. The implant stability was considered low (ISQ <60); therefore, periods longer

than 3 months are required before the attachment of prosthesis components.

In a comparison of the 14– and 90–day time intervals, the final ISQ average value was different among groups. This finding may be explained by improvements in dental implant biomedical applications as a result of the surface modifications. Changes in surface composition, incorporation of various components, or changes in surface energy have been proposed to alter the surface physicochemical characteristics or topography, thereby resulting in more favorable load transmission, bone healing, implant biocompatibility, bioactivity, and osseointegration.[19–21]

Because surface roughness affects surface free energy, surface charge, hydrophilicity, and chemical composition, titanium surface modifications demonstrate an improvement in the osseointegration of dental implants.[22]

Furthermore, variations in surface energy alter the implant surface's hydrophilicity, wettability, and potential for early interaction with biological fluids (changes in surface chemistry).[23] The increased surface area of dental implants with somewhat rough surface characteristics enhances bone ingrowth. Implant stability is improved by surface changes that increase titanium roughness (which is beneficial for osteoblast proliferation and bone formation).[24] The surface topography of dental implants is crucial for osteoblast adhesion and differentiation during early phases of osseointegration as well as long-term bone remodeling.[25]

Alumina is the most widely used particle in the sandblasting process because of its many benefits, including low cost, hardness, and superior needle shape. Furthermore, Al_2O_3 particles of varying sizes and consistent roughness levels can be obtained and can modify osteoblast activity and promote cell adhesion to bone.[26,27] Some alumina particles may be retained after blasting and may contaminate the implant surface; therefore, careful cleaning with acid etching is necessary. Alumina and other retained blasting elements may impair bone growth. In the current study, acid etching was used to produce activated and homogeneous surface roughness and remove the outermost layers of the implant surface; moreover, to minimize surface tension and eliminate any remaining alumina particles.[28]

The surface grain boundaries disappeared after sandblasting, which probably because stress-related diffusion along grain boundaries resulted in a negative surface charge.[29] According to Guo et al.,[30] a negative surface charge enhances cell adhesion and osteoblastic development, thus facilitating protein adsorption necessary for cellular growth and development, as well as bone production.[31] Hsu et al.,[32] have reported that irregularities in these surfaces enable osteogenic cells to join and deposit bone, thus forming a bone-to-implant interface and improving mechanical interlocking between the roughened surface and the bone. This increased bone-to-implant contact increases the implant's resistance to compression, tension, and shear stress. A review of results obtained by other authors confirmed that surface modifications that increase titanium roughness improve implant stability beyond that of implants with turned surfaces.[33]

Therefore, we used an Er–Cr:YSGG laser to modify the titanium implant surface. The titanium implant's mechanical properties, such as excellent surface roughness, outstanding biocompatibility, high hardness value, wear resistance, low friction coefficients, excellent corrosion, implant surface bioactivity, non-toxicity, and porosity, were enhanced and the surfaces remained undamaged under a 2.5 W power protocol for laser surface modification. Laser treatment modifies the titanium oxide layer on the implant surface, which is essential to act as a diffusion barrier in achieving osseointegration. In natural bone metabolism, the oxide film formed after laser ablation restricts the release of ionic or molecular from the titanium surface, and protects the biological environment from the highly reactive Ti metal.[34–36]

This laser treatment produces complex surface geometries and biomedical implant surfaces and can be used to quickly manufacture high-resolution complex microstructures free from contamination at nano- and micrometer scales.[37–39] Thus, laser irradiation considerably alters the rough surface of the implant.

Propolis coating has also been used in dental implants to accelerate osseointegration. Plant resins, which are responsible for a variety of biological activities, are a source of flavonoids, which are believed to be an essential biochemical component in propolis.

However, the roles of several flavonoids in bone are controversial. For example, quercetin has been found to induce both osteoblastogenic and osteoclastogenic effects.[40,41] The effects of flavonoids in cell culture and animal model systems have been extensively studied, and the findings strongly support the roles of flavonoids in bone formation *in vitro* and *in vivo*.

Most flavonoids exert effects on bone by promoting osteoblastogenesis, which ultimately leads to bone formation.[42] No prior studies have described propolis-coated dental implants in canine models and some uncertainty remains regarding the long-term stability of these coatings, which are currently used on a small percentage of clinical implants. Any improvement from propolis coatings would need to be further investigated, because of the strong bioactivity of titanium alloy surfaces and their consistent capability to undergo osseointegration. Current coatings have not yet demonstrated the necessary levels of improvement.

Primary stability, a critical factor for achieving osseointegration, is the stability of the implant at the time of implant insertion. The resistance of the bone during implant insertion reflects the clinical significance of primary stability.[43] After surgery, dental implants' primary stability must be sufficiently high to prevent micromotion at the bone–implant interface. If micromotion exceeds 50–100 μm, osseointegration may be impaired, and fibrous tissue, rather than the desired bone, may form around the implant.[44] The primary ISQ values of an implant are similar at baseline time–0 and after implant installation, as shown in Table 1; this similarity is a function of the implant's stiffness in the surrounding bone and the level of the marginal bone. The stiffness of an implant placed in the recipient's bone is influenced by the stiffness of the implant itself, the implant/tissue interface, and the surrounding bone.[45] After the implant has been integrated, total implant stability is based entirely on biological or secondary stability. The proportional relationship between the influences of primary and secondary stability changes over the course of the healing process runs its course.[46]

Secondary stability is a biological form of stability achieved at the implant−tissue interface through bone remodeling and regeneration. According to Baldi et al.,[47] the period of wound healing compromises secondary stability.

Primary and secondary stability together account for total implant stability. Many studies have recorded total implant stability during the implant healing period to monitor osseointegration. Most of those studies have indicated that implant stability decreases starting from the first day after implant placement. Mean stability measures are typically minimal during the first 2−4 weeks and subsequently increase and become relatively stable. This pattern has been described as a drop, or dip, in implant stability.[48]

The measurement of implant stability can be accomplished by several invasive and non-invasive clinical testing methods,[49] such as removal torque analysis, pull- and push-through tests, histomorphometry, radiographic analysis, the Periotest, and the Resonance frequency analysis (**RFA**).

Recently, the percussion-based Easy Check® device was developed to determine the stiffness between alveolar bone and implant by simply tapping at the healing abutment. In the present study, the healing abutment was not removed, and the Easy Check® device was able to test implant stability without encouraging bone resorption.[18] Because the procedure is less difficult than that with the Osstell ISQ, a superstructure connecting process such as Osstell is not necessary for measurement. Less tapping times and force were used, thus, enhancing the tapping motion and enabling safer measurement of implant stability than that with the Periotest.[50]

Cortisol, a commonly used biomarker in stress research,[51,52] is a major indicator of the stress response in most mammals, including dogs.[46] High levels of cortisol may indicate marked.

In the stressed group at baseline (Table 5), changes in how the dog models were stressed, prolonged elevated excretion of the stress hormone cortisol during the follow-up period. The regime followed to induce cortisol hormone secretion; to us, this protocol that changes in a dog's housing conditions was stressful. Therefore, many dogs in our study seemed to show significant restlessness. At the end of the observation period, the dogs in the stressed group continued to display restlessness, thus explaining the continued high secretion of cortisol. Our findings Table 5, demonstrated the significance of the individual variations in cortisol in the serum in dogs from different groups, thus indicating that cortisol is a uniform and useful biomarker of stress in dogs.[11,53]

Stress and implant osseointegration have a pathophysiological relationship that can be explained by elevated glucocorticoid levels. Dogs produce large amounts of the glucocorticoid cortisol, which is considered a valuable stress level marker. The stressed dogs in this study consistently had higher serum cortisol levels (Table 5) than the dogs in the control non-stressed group and additionally showed weight loss (Table 4), thus indicating the importance of stress management. The dog's clinical condition was indicated by a decrease in the mean implant stability (ISQ), as shown in Table 1. In the follow-up, after 60 days, the serum cortisol levels and body weight of the dogs in the stress group indicated adaptation to the stress protocol.

Peri-implantitis is an irreversible inflammatory disease affecting the soft and hard peri-implant tissue compartments. Clinically, this disease is characterized by inflammation of the soft tissue surrounding the implant, accompanied by redness, swelling, bleeding on probing, and destruction of the hard tissue surrounding the implant, thus resulting in a loss of osseointegration.[54,55]

Poorly controlled diabetes, cigarette smoking, and immunodeficiency, including AIDS/HIV infection and neutropenia are systemic risk factors for periodontitis and peri-implantitis; strong longitudinal evidence has indicated the influence of these factors on disease progression. Psychobiologic links between stress and immunologic dysregulation, microbial dysbiosis, and systemic health are beginning to emerge as more data become available.[56]

The overall association mechanisms have been hypothesized to arise from the interaction between underlying systemic inflammation and the altered dynamic balance between the host immune response and the periodontal microbiome, as well as bidirectional cross-talk between systemic and local periodontal cytokines, dysbiosis, and imbalances in the periodontal microbiome.[57,58]

Chronic stress is an environmental and genetic variable influencing the equilibrium between the periodontal immune system and microbiota. Furthermore, long-term stress has been shown to affect tissue homeostasis, which in turn may influence the onset, severity, and response to treatment of peri-implant diseases. To completely limit the risk of peri-implant disease initiation and progression and limit the development of various oral illnesses associated with inadequate immune response and poor self-care, primary and secondary preventive strategies may be considered.[56,59] Additionally, focusing on acquired health-damaging behaviors, such as alcohol consumption, smoking, poor sleep quality, and poor nutrition, is important, particularly in patients under stress.[60,61]

Chronic stress elevates exposure to glucocorticoids and thus diminishes bone mass by diverting mesenchymal stem cell differentiation from an osteoblastic lineage towards an adipogenic lineage;[62] increases osteoclast activity; suppresses osteoblastic activity; decreases bone mineral density; and accelerates bone resorption, a clinical indicator of implant failure and implant loss.[63,64] Unfortunately, few reliable studies have reported the effects of personality traits and oral health-associated quality of life on the success of dental implant treatment. Individuals who choose to receive implant therapy typically have low levels of ongoing stress and dental anxiety is advantageous in these situations because it removes a potential risk factor that might otherwise interfere with the osseointegration processes. Patient's motivation to select the most appropriate treatment is greatly influenced by their familiarity with the issue of chronic stress and the potential for finding ways to work with dental specialists to identify solutions. Further evaluation and rigorous scientific evidence are required to determine whether the psychological characteristics of such individuals can be used to predict their quality of life in terms of oral health after receiving dental implants.

Conclusions

The ISQ was dependent on surface treatment, and was higher with sandblasting than the other treatments in the stressed and non-stressed groups at 90 days. For all surface-

treated implants, the stressed group had significantly higher serum cortisol levels than the non-stressed group.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The Ethical Committee at the College of Dentistry, University of Mosul, Mosul, Iraq, approved a license to perform this animal research study (UoM. Dent/A.67/22).

Authors contributions

MAA conceived and designed the study; provided research materials; and collected and organized the data. RHH analyzed and interpreted the data. MAA and OHA wrote the initial and final drafts of the article and provided logistic support. All authors critically reviewed and approved the final draft, and are responsible for the contents and similarity index of the manuscript.

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