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JENIFFER PERUSSOLO

Influence of the keratinized mucosa on the stability of peri-implant tissues and brushing discomfort: a 4-year follow-up study

Influência da mucosa queratinizada na estabilidade dos tecidos peri-implantares e no desconforto à escovação: 4 anos de acompanhamento

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Dissertation presented to the Postgraduate Program in Integrated Dentistry of the Departament of Dentistry of the State University of Maringá as partial requirement to obtain the Master's Degree.

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Influência da mucosa queratinizada na estabilidade dos tecidos peri-implantares e no desconforto à escovação: 4 anos de acompanhamento

RESUMO

Objetivo: O objetivo do presente estudo foi avaliar por 4 anos a influência da mucosa queratinizada (MQ) peri-implantar no nível ósseo marginal (NOM), na saúde dos tecidos peri-implantares e no desconforto à escovação.
Material e Métodos: Oitenta pacientes foram recrutados durante sua visita de manutenção de janeiro a outubro de 2013 e alocados em dois grupos de acordo com a largura da MQ em torno dos implantes: MQ ≥ 2 mm; e MQ <2 mm. O nível ósseo marginal (NOM), índice de placa modificado (IPm), profundidade de sondagem (PS), nível de inserção clínica (NIC), sangramento à sondagem (SS) e desconforto à escovação (DE) foram obtidos na avaliação inicial (T0) e após 4 anos (T4). O teste Mann-Whitney, o teste Wilcoxon e um modelo multinível foram utilizados para análise estatística ($p<0,05$).
Resultados: Cinquenta e quatro pacientes com 202 implantes retornaram para T4. Os pacientes do grupo com MQ <2 mm apresentaram maior perda óssea marginal ($p=0,015$), IP ($p=0,002$), SS ($p=0,026$) e DE ($p=0,029$) do que aqueles no grupo com MQ ≥2 mm. A análise multinível sugeriu que a largura da MQ e o tempo em função tiveram efeito sobre o NOM ($p = 0,035$).
Conclusões: Os achados indicam que a largura de MQ tem efeito sobre as mudanças do NOM, acúmulo de placa, inflamação dos tecidos peri-implantares e desconforto à escovação. Portanto, a perda óssea marginal foi mais evidente em torno de implantes com MQ <2 mm do que em torno de implantes com MQ ≥2 mm

Palavras-chave: Implante dental, mucosa queratinizada, nível ósseo marginal, inflamação, desconforto à escovação

Influence of the keratinized mucosa on the stability of peri-implant tissues and brushing discomfort: a 4-year follow-up study

ABSTRACT

Objective: The purpose of the present 4-year follow-up study was to evaluate the influence of the peri-implant keratinized mucosa (KM) on marginal bone level (MBL), peri-implant tissues health and brushing discomfort.

Material and Methods: Eighty patients were initially recruited during their maintenance visit from January to October 2013 and allocated in two groups according to KM width around implants: Wide group (KM ≥2 mm) and Narrow group (KM <2 mm). At the 4-year follow-up visit (T4), marginal bone level (MBL), modified plaque index (mPI), probing depth (PD), clinical attachment level (CAL), bleeding on probing (BoP) and brushing discomfort (BD) were assessed and compared to results obtained in the initial assessment (T0). Paired t-test, Wilcoxon signed-rank test and a multilevel model were used for statistical analysis ($p<0.05$).

Results: Fifty-four patients with 202 implants returned for T4. Patients in the Narrow group presented more marginal bone loss ($p=0.015$), mPI ($p=0.002$), BoP ($p=0.026$) and BD ($p=0.029$) than those in the Wide group. Multilevel analysis suggested that KM width and time in function had an effect on MBL ($p=0.035$).

Conclusions: Findings indicate that KM width has an effect on MBL changes, plaque accumulation, tissue inflammation and brushing discomfort. As a result, marginal bone loss was more evident around implants with KM <2 mm than around implants with KM ≥2 mm.

Key-words: Dental implants, keratinized mucosa, marginal bone level, inflammation, brushing discomfort

SUMMARY

LITERATURE REVIEW

1.1 Introduction

Dental implant therapy is consider an effective and predictable alternative for rehabilitation of edentulous regions. Implant-supported restorations have demonstrated a high long-term survival rate (Fiorellini et al. 1998). Ekelund et al. (2003) observed in a 20-year follow-up study a 98.9% survival rate for implants supporting mandibular fixed prostheses. However, despite the reported high success rate of dental implants, failures may occur. In a longitudinal study, Karoussis et al. (2003) observed that after 10 years, the incidence of biological complications around implants was 5.8%. Thus, in addition to survival rate, maintenance of peri-implant tissues health and stability, as well as the reestablishment of aesthetics, are essential to achieve clinical success of implant-supported restorations (Karoussis et al. 2004; Papaspyridakos et al. 2012).

Peri-implant diseases are the most frequent complications that may affect dental implants as a result of the imbalance between the bacterial challenge and the host defenses (Heitz-Mayfield 2008; Zitzmann & Berglundh 2008; Tomasi & Derks 2012). A literature review demonstrated that peri-implant mucositis (inflammation restricted to the peri-implant mucosa) occurred in approximately 80% of individuals and 50% of implants, while peri-implantitis (inflammation with additional bone loss) was found in 28-56% of patients (12%-43% of implants) evaluated (Zitzmann & Berglundh 2008). One of the major challenges in implant therapy is the ability to identify factors that may be associated with the onset of these biological complications. Several local and systemic factors, such as untreated periodontal disease, poor oral hygiene, diabetes, and smoking have been considered risk factors for the development of peri-implant diseases (Heitz-Mayfield 2008; Tomasi & Derks 2012).

Additionally, the lack of an “adequate” band of keratinized mucosa (KM) around implants has also been suggested as a risk factor to mucositis and peri-implantitis (Roos-Jansåker et al. 2006; Costa et al. 2012; Canullo et al. 2016). However, the actual need for this “adequate” band of KM around dental implants for the maintenance of peri-implant tissues health has been a matter of controversy. Thus, the present literature review aimed to assess the previous and current literature on the influence of the KM on the health and stability of peri-implant tissues.

1.2 Influence of the keratinized mucosa on the health and stability of peri-implant tissues

A search in MEDLINE-PubMed was conducted to identify evidence supporting the present literature review. The following keywords were used for literature search: dental implant (Mesh) OR implants AND keratinized mucosa OR masticatory mucosa OR attached mucosa OR attached gingiva AND inflammation OR bleeding OR bone level OR bone loss. A total of 19 articles that analyzed or related the amount of KM with the health and stability of peri-implant tissues were included in this literature review. The exclusion criteria were not to be published in English, (ii) pre-clinical studies and (iii) lack of information on clinical variables of the peri-implant tissues (Tables 1-3).

The main variables analysed by the selected studies were: plaque index (PI) (Löe 1967; Silness & Loe 1964), and modified plaque index (mPI) (Mombelli et al. 1987); bleeding on probing (BoP), bleeding index (BI) (Silness & Loe 1964), and modified bleeding index (mBI) (Mombelli et al. 1987); gingival index (GI) (Löe 1967), and modified gingival index (mGI) (Mombelli et al. 1987); and probing depth (PD). Some studies also evaluated the variables mucosal recession (MR); clinical attachment level (CAL); bone loss (BL); marginal bone level (MBL); and brushing discomfort (BD) (Souza et al. 2015).

CROSS-SECTIONAL STUDIES

Nine cross-sectional studies were selected and included in this literature review. Table 1 displays the information collected and analyzed in each study, such as the number of patients/implants, type of prosthesis/implants, variables collected, KM analysis, statistical analysis, results, conclusions, and comments.

Oral Hygiene

Oral hygiene was assessed in 7 studies. Of these, five showed that implants with KM <2 mm presented more plaque accumulation than those with KM ≥ 2 mm (Chung et al. 2006; Bouri et al. 2008; Adibrad et al. 2009; Ladwein et al. 2015; Souza et al. 2015). Chung et al. (2006) found that, regardless of the type/surface of the implant, poor plaque control was observed around implants with an "inadequate" band of KM. Nonetheless, no significant differences in plaque index were found in two of the studies (Wennström et al. 1994; Kim et al. 2009).

Inflammation

Peri-implant inflammation was identified by various indexes and evaluated in 7 studies included in this review. According to the results from 5 studies, implants with a narrow band of KM presented more signs of inflammation than those with KM ≥ 2 mm (Chung et al. 2006; Bouri et al. 2008; Adibrad et al. 2009; Ladwein et al. 2015; Souza et al. 2015). Bouri et al. (2008) reported that implants with KM <2 mm were more prone to bleeding on probing, even after factors such as time in function, smoking, gingival thickness, and PI were taken into account. The authors suggested that a broad band of KM may offer greater protection against the masticatory forces and frictional contact during brushing. Thus, the lack of an "adequate" band of KM would create an environment more susceptible to discomfort and irritation during brushing.

In the studies by Wennstrom et al. (1994) and Kim et al. (2009), the width of the KM was not significantly related to tissue inflammation. However, Wennstrom et al. (1994) found a higher proportion of bleeding sites in the group with KM <2 mm than in the group with KM ≥ 2 mm (69% vs. 54%, respectively).

Probing depth and clinical attachment level

Most studies did not observe statistically significant differences between groups regarding the PD (Chung et al. 2006; Bouri et al. 2008; Adibrad et al. 2009; Kim et al. 2009; Ladwein et al. 2015; Souza et al. 2015). Wennstrom et al. (1994) observed that sites with KM ≥2 mm showed a lower frequency of shallow sites (29% vs. 49%), and a higher proportion of deep sites (8% vs. 1%) than areas with a narrow band of KM.

Out of the 2 studies evaluating CAL (Adibrad et al. 2009; Souza et al. 2015), only 1 showed a statistically significant difference between groups. Adibrad et al. (2009) found higher CAL values in the group with KM <2 mm ($p=0.04$). A negative correlation between KM and CAL was also observed ($p<0.05$), demonstrating that the wider the KM the lower the attachment loss.

Soft tissue/ mucosal recession

Among the studies included in the present review, 2 verified the effect of peri-implant KM on soft tissue recession (Adibrad et al. 2009; Kim et al. 2009). The results from both surveys

showed that areas lacking an “adequate” band of KM presented more MR than regions with KM ≥2 mm ($p<0.05$).

Marginal bone level and/or bone loss

Five studies considered the variable marginal bone level or bone loss (Chung 2006; Bouri et al. 2008; Adibrad et al. 2009; Kim et al. 2009; Ladwein et al. 2015). The studies by Bouri et al. (2008) and Kim et al. (2009) demonstrated statistically significant differences in mean marginal bone loss between the groups, which was higher around implants with an “inadequate” band of KM. The remaining studies failed to demonstrate any influence of KM on MBL.

Soreness/Discomfort during oral hygiene

Souza et al. (2015) evaluated brushing discomfort in 80 patients with the aid of a visual analog scale (VAS). The investigation revealed that patients with implants lacking an “adequate” band of KM presented higher levels of brushing discomfort. The authors suggested that this discomfort was related to the anatomical characteristics of the tissue, since the masticatory mucosa would allow better sensorial isolation during brushing when compared to the lining mucosa (Souza et al. 2015).

Peri-implant diseases

Some studies evaluated the lack of an “adequate” band of KM as a risk indicator for peri-implant diseases (Roos-Jansåker et al. 2006; Canullo et al. 2016). Roos-Jansaker et al. (2006) showed that KM width was one of the variables that explained the presence of mucositis and peri-implant bone loss. Canullo et al. (2016) observed a higher prevalence of peri-implantitis at implants bordered by a narrow band of KM and demonstrated that an "adequate" band of KM significantly reduced the probability of the implant developing peri-implantitis (OR=0.36).

Table 1. Cross-sectional studies

Authors (year), Country	N/n	Type of prosthesis/implant (Loading period)	Variables collected	KM analysis	Statistical analysis	Statistical Unit	Results	Conclusions	Comments
Wennstrom et al. (1994), Sweden	39/171	Full-arch (≥10 years) and partial fixed prostheses (≥5 years) restoration/Branemark system implants	PI,GI PD, BoP (3 sites), KM width and soft tissue mobility	Dichotomous: KM <2 mm / KM ≥2 mm	Multiple regression analysis	Implant	KM width was not found to significantly influence PI, GI and BoP, in any of the models.	The lack of an "adequate" KM width, and mobility of the marginal soft tissue showed no significant effect on peri-implant soft tissue health.	
Chung et al. (2006), United States of America	69/339	Fixed or removable prostheses / smooth and rough surface implants (≥3 years)	mPI, mGI, GI, PD (4 sites), KM, annual BL, type of implant	Dichotomous: KM <2 mm / KM ≥2 mm or AM <1 mm / AM ≥1 mm	Chi-square test, Student's t-test and ANOVA	Implant	mPI and GI were significantly greater in implants with KM <2 mm (0.94 and 1.51) than in sites with KM ≥2 mm (0.76 and 1.26). No significant differences in PD and annual BL were observed between groups.	The absence of an "adequate" KM or AM was associated with higher plaque accumulation and gingival inflammation but not with more annual BL, regardless the implant surface.	Implants were subdivided by type and surface .

Roos-Jansaker et al. (2006), Sweden	218/999	Fixed or removable restorations/ Branemark system implants (9-14 years)	PI, BoP, PD, KM, suppuration, % of teeth with BL before implant placement, no. of threads without bone contact	Dichotomous: KM <2 mm / KM ≥2 mm Mucositis: PD 4 mm + BoP - Peri-implantitis: BL ≥ 3 mm + BoP and/or suppuration.	Uni and multivariate logistic regression	Implant	KM and IP were explanatory variables for mucositis and bone level ($p<0.05$).	The absence of KM was associated with peri-implant mucositis and bone level.	
Bouri et al. (2008), United States of America	76/200	NR/NR (1 year)	mPI, mGI, PD (3 sites), KM, BL, gingival thickness, mobility, loading period, no. and position of implants and smoking	Dichotomous: Group A (KM ≥2 mm) and group B (KM <2 mm)	t test and Wilcoxon test, Linear and logistic multivariate regression	Implant	Group B presented significantly greater mPI, GI and BL values. Furthermore, sites with < 2 mm were more prone to bleeding (89% vs 71%; $p<0.01$) and BL. No statistical difference was observed for PD.	The study showed the relationship between KM width and peri-implant tissue health. BoP, PI and BL were greater in implants with KM <2 mm.	Tissue thickness was higher in group A. "OR" adjusted for implant installation, smoking, gingival thickness and PI.
Adibrad et al. (2009), Iran	27/66	Overdenture (mean 25.40±10.28 months)	mPI, mGI, BoP, PD (6 sites) MR, CAL, KM, MBL	Dichotomous: Group A (KM ≥2 mm) and group B (KM <2 mm)	Pearson correlation coeficient and Mann-Whitney test	Implant	Group A presented significantly greater mPI, mGI, BoP, MR and CAL. Furthermore, a negative correlation between KM width and MR	A significant influence of KM was observed on peri-implant tissue health.	

							and CAL. ($p<0.05$).		
Kim et al.(2009), South Korea	100/276	NR/Osstem, Dentium and Nobel Biocare (>6 months)	PI, GI, buccal PD, MR, crestal BL	Dichotomous: Sufficient KM (≥2 mm); and deficient KM (<2 mm)	t test	Implant	In the Deficient KM group, MR and BL were greater than in the Sufficient KM group ($p<0.05$)	The absence of KM was a risk factor for increased MR and BL. Thus, in cases requiring long-term maintenance management and esthetic, the presence of an appropriate amount of KM would be required.	
Ladwein et al. (2015), Germany	211/967	N/R/ Straumman®-Tissue Level SP/S (≥10 years)	mPI, mGI, PD (4 sites), BoP, KM, MBL, no., mobility and position of implants.	Dichotomous: Absence (KM =0 mm); and presence (KM >0 mm).	Mann-Whitney and Chi-square test.	Implant	mPI, mGI and BoP were significantly higher in implants lacking KM ($p<0.05$). No statistical difference was observed for PD and MBL.	Results indicated that the presence of KM had a positive effect on peri-implant tissue health, but did not seem to influence peri-implant bone level.	MBL measurements were carried out with panoramic radiographs.
Souza et al. (2015), Brazil	80/269	NR/NR (≥1 years)	mPI, PD, CAL, BoP (3 sites), KM and brushing discomfort	Dichtomous: Wide group (≥2 mm) and Narrow group (<2 mm)	Mixed linear model and chi-squared test	Implant and Subject	Implants with KM <2 mm had greater discomfort levels ($p<0.001$), mPI ($p=0.0021$) and BoP ($p=0.017$). No statistically significant	The study demonstrated that patients with KM <2 mm exhibited higher levels of peri-implant discomfort during brushing, plaque, and peri-implant	

							differences were observed in PD and CAL values among groups.	inflammation.	
Canullo et al. (2016), Spain	534/1507	NR/ NR	mPI, BoP, PD (6 sites), KM, suppuration, gingival biotype, bacterial count Peri-implantitis: BL >3 mm + PD ≥4 mm + BoP and/ or suppuration	Dichtomous: Presence (KM ≥2 mm); and Absence (KM <2 mm)	Chi-squared test and multivariate analysis	Subject and implant	Implants diagnosed with peri-implantitis showed higher PI, BoP and number of implants with KM <2 mm. Presence of plaque and BoP > 30% of sites and a narrow band of KM were associated with greater probability of patients developing peri-implantitis.	The results seemed to indicate that inadequate oral hygiene and the presence of BoP in patients with dental implants were associated with higher prevalence of peri-implantitis.	

N/n – Number of patients/implants; NR – Not reported; KM – Keratinized mucosa; AG – Attached gingiva; PI – Plaque index; mPI – Modified plaque index; BoP – Bleeding on probing; BI – Bleeding index; mBI – Modified bleeding index; GI – Gingival index; mGI – Modified gingival index; PD – Probing depth; MR – Mucosal recession; CAL – Clinical attachment level; BL – Bone loss; MBL – Marginal bone level

LONGITUDINAL STUDIES

Seven longitudinal studies were selected and included in this review. Table 2 presents the collected information such as follow-up period, the number of patients/implants, type of prosthesis/implant, collected variables, KM analysis, data analysis, results, and conclusions.

Oral hygiene

The effect of peri-implant KM on oral hygiene condition was reported in 5 studies (Mericske-Stern et al. 1994; Schrott et al. 2009; Crespi et al. 2010; Boynueğri et al. 2013; Roccuzzo et al. 2016). Of these, 3 studies showed significantly more plaque around implants bordered by KM <2 mm than implants with KM ≥2 mm (Schrott et al. 2009; Crespi et al. 2010; Boynueğri et al. 2013). Scrott et al. (2009) found a negative correlation between plaque accumulation and KM width only at lingual sites. A 10-year follow-up study assessed the significance of peri-implant KM on tissue health and stability. At the end of the observation period, plaque accumulation was found to be higher around implants placed in areas with KM than with alveolar mucosa (AM) (Roccuzzo et al. 2016). In contrast, a 5-year follow-up study evaluating implants supporting overdentures in elderly subjects did not reveal significant differences among groups (Mericske-Stern et al. 1994).

Inflammation

Five studies reported data on peri-implant tissue inflammation (Mericske-Stern et al. 1994; Schrott et al. 2009; Crespi et al. 2010; Boynueğri et al. 2013; Roccuzzo et al. 2016). Three studies observed increased tissue inflammation around dental implants with an "inadequate" KM width (Schrott et al. 2009; Crespi et al. 2010; Boynueğri et al. 2013). Schortt et al. (2009) observed that at lingual sites, the presence of KM ≥2 mm reduced the probability of bleeding by 40% (OR=0.60, 95% CI=0.48-0.74). However, at buccal sites no association was found. According to the authors, the higher indexes at lingual sites may be influenced by the fact that lingual regions may be associated with the shallower floor of the mouth, making oral hygiene access difficult. In two studies, no statistically significant differences between groups were observed (Mericske-Stern et al. 1994; Roccuzzo et al. 2016).

Probing depth and clinical attachment level

Three studies revealed no significant differences in PD among groups (Mericske-Stern et al. 1994; Crespi et al. 2010; Boynueğri et al. 2013). Attachment loss was assessed only in one study (Mericske-Stern et al. 1994), which demonstrated that implants with KM <2 mm

presented significantly more CAL at lingual sites in the final assessment.

Soft tissue/mucosal recession

Four studies presented data on MR. The studies by Crespi et al. (2010), Schrott et al. (2009) and Rocuzzo et al. (2017) demonstrated that implants with the absence or an "inadequate" band of KM showed more MR after 1, 5 and 10 years, respectively. Bengazi et al. (1996) observed that sites bordered by a lining mucosa showed greater mean MR than sites with KM at the 6-month follow-up. However, no further increase in the mean amount of MR had occurred at the 2-year follow-up. Thus, the authors concluded that the lack of KM was not found to affect the amount of MR.

Marginal bone level and/or bone loss

The role of KM on MBL stability was assessed in two longitudinal surveys (Crespi et al. 2010; Roccuzzo et al. 2016). Both investigations found that the presence of KM was not a critical factor in MBL stability after 4 and 10 years, respectively. In the study by Costa et al. (2012) the variable "bone loss" was used to characterize the groups.

Soreness/Discomfort during oral hygiene

One study asked patients to indicate whether soreness/discomfort was present (YES/NO) during oral hygiene (Roccuzzo et al. 2016). The findings revealed that in the KM group no pain or discomfort in oral hygiene procedures were reported by patients, while 42.9% of the patients in alveolar mucosa group reported discomfort in performing oral hygiene ($P<0.001$). Additionally, patients showing inadequate plaque control due to soreness/discomfort were offered the option to receive an additional surgical procedure (free gingival graft).

Proinflammatory cytokines

One survey (Boynueğri et al. 2013) verified the effect of KM on peri-implant clinical and biochemical parameters and showed that sites lacking KM have higher levels of TNF-α when compared to sites with the presence of KM ($p<0.05$). Besides, an increase in TNF-α levels was observed after 12 months.

Peri-implant diseases

In a study by Costa et al. (2012), the authors evaluated risk indicators for peri-implant diseases over a period of 5 years. According to their findings, the occurrence of peri-

implantitis was related to factors such as the presence of periodontal disease, plaque accumulation, the percentage of sites with bleeding on probing and a reduced band of KM (Costa et al. 2012).

Table 2. Longitudinal studies

Authors (year), country	Follow -up	N/n	Type of prosthesis / implant (Loading period)	Variables collected	KM analysis	Data analysis	Statistical analysis	Results	Conclusions	Comments
Mericske-Stern et al. (1994), Switzerland	5 years	33/ 64	Overdenture/ ITI implants® (5 years)	mPI, mBI, PD, CAL, (4 sites) KM, BoP (final assessement)	Dichotomous: KM ≥2 mm (presence); and KM <2 mm (absence)	T test and Wilcoxon test	Implant	The results did not reveal significant differences in the clinical parameters. Implants with KM <2 mm presented significantly more CAL at lingual sites.	Implants supporting overdentures in elderly subjects could be mantained with healthy peri-implant tissues after 5 years irrespective of the presence of KM.	
Bengazi et al. (1996), Sweden and Italy	2 years	40/ 158	Full-arch and particial fixed restoration/ NR (2 years)	PI, GI, PD, KM, soft tissue recession and mobility	Dichotomous: Lining mucosa; and masticatory mucosa	Linear regression model	Implant	The lack of KM and peri-implant soft tissue mobility did not affect the amount of recession.	Soft tissue condition and recession during the 2-year follow-up were not significantly influenced by marginal tissue type or mobility.	
Schrott et al. (2009), United States of America and England	5 years	58/ 307	Mandibular full-arch fixed prosthesis / Straumman® / NR	mPI, mBI (sites), KM, MR	Dichotomous: Presence (KM ≥2 mm); and Absence (KM <2 mm)	Multivariate logistic regression, multivariate ordinal logistic regression, generalized estimating	Implant	After 5 years implants with KM <2 mm presented greater lingual plaque and tissue inflammation, as well as buccal MR than implants with	In patients exercising adequate oral hygiene and receiving regular implant maintenance therapy, implants with a reduced KM width were more prone to lingual plaque accumulation and	Evaluations were performed at 0, 3, 6, 12, 18, 24, 36, 48, and 60 months after prothesis delivery.

						equations, and Bonferroni's correction.		KM ≥2 mm.	bleeding, as well as buccal soft-tissue recession over a period of 5 years.	
Crespi et al. (2010), Italy	4 years	29/ 164	NR/NR	mPI, GI, mBI, PD (4 sites), MR, KM, BL (baseline, 1, 2 and 4 years after implant placement) Sucess: stability, and absence of radiolucency around the implant, suppuration and pain.	Dichotomous: Group A (KM ≥2 mm); and Group B (KM <2 mm)	Student t-test	Implant	Survival rate of 100%. An "inadequate" KM width was significantly related to greater plaque accumulation, inflammation, and MR. No statistically significant differences regarding PD and BL were observed between groups.	KM was not a crutial factor for the stability of interproximal bone level. However, a narrow band of KM was associated with more signs of inflammation, plaque accumulation and mucosal recession.	Immediate implant placement with immediate loading.
Costa et al. (2012), Brazil	5 years	80/ 336	NR/ Nobel Biocare, 3i and Intra-Lock (NR)	mPI (4 sites), BoP, PD, KM, BL. Mucositis: Inflammation (visual) + BoP. Peri-implantitis: PD ≥5 mm + BoP and/or SU + BL	Group 1: Maintenance therapy (MT) (≥ 5 appointmens during the study period); Group 2: no maintenance therapy (absence of visits during the study period) KM ≤ 1 mm and	Chi-square, Mann–Whitney and Fischer test. Multivariate logistic regression.	Subject	KM ≤1 mm was associated with the occurrence of peri-implantitis both in patients with MT (p=0.001) and without MT (p=0.048).	KM was significantly associated with the occurrence of peri-implantitis.	Periodontal clinical parameters were also assessed.

					KM ≥ 2 mm					
Boynuegri et al. (2013), Turkey	1 year	15/ 36	Overdenture/ Straumman® / NR	PI, GI, BoP, PD (6 sites) and IL-1β and TNF-α levels	Dichotomous: Presence (KM ≥2 mm); and absence (KM =0 mm)	Anova, Bonferroni and Wilcoxon test	Implant	GI, PI and TNF-α were higher in implants lacking KM.	The presence of KM was associated with less plaque accumulation, inflammation and TNF-α levels (*p<0.05*).	Measurements performed: immedialtely, 6 and 12 months after prosthesis installation.
Rocuzzo et al. (2016), Italy	10 years	98/ 98	Single crown or fixed dental prosthesis/ Straumman® (10 years)	PI, BoP, PD (4 sites), MR, MBL, implant loss, smoking habit, no. of sites requiring additional treatment, presence of soreness/ brushing discomfort.	Dichotomous: Keratinized tissue (KT) Alveolar mucosa (AM)	Kruskal–Wallis and Mann–Whitney test with Bonferroni's adjustment, Chi-square or Fisher's exact test (categorical variables)	Subject	42.9% of the patients in AM group reported brushing discomfort. Of these 11 subjects were submitted to an additional procedure. Plaque and MR was significantly more frequent in AM sites (p=0.007). No significant differences were found with respect to BoP, PD and MBL.	Soft-tissue grafting seemed benefitial in posterior mandibular sites, especially concerning: i) patients complain of soreness during oral hygiene; ii) ongoing MR iii) plaque control was less than ideal but was facilitated by better topography.	Radiographic data were collected, after prosthesis installation (baseline)

N/n – Number of patients/implants; NR – Not reported; KM – Keratinized mucosa; PI – Plaque index; mPI – modified plaque index; BoP – Bleeding on probing; BI – Bleeding index; mBI – modified bleeding index; GI – Gingival index; IGm – modified gingival index; PD – Probing depth; MR – mucosal recesion; CAL – Clinical attachment level; BL – Bone loss; MBL – Marginal bone level; SU – Suppuration.

SYSTEMATIC REVIEWS AND META-ANALYSIS

Three systematic reviews, one without (Wennstrom & Derks 2012) and two with meta-analysis (Lin et al. 2013; Gobbato et al. 2013), were also included. Table 3 shows the number and type of studies included in each systematic review, as well as the variables analyzed, results, and conclusions obtained.

Wennström & Derks (2012) performed a systematic review to verify the role of KM in the maintenance of peri-implant tissue health and stability. According to the authors, there was limited evidence on the theme. The review suggested that in clinical situations, where adequate plaque control can be performed, the presence of KM did not seem to be crutial. However, they pointed out to the fact that some patients might experience pain or discomfort during brushing at implants areas with an "inadequate" band of KM, preventing proper oral hygiene.

Most of the studies included in the meta-analysis were cross-sectional studies (Lin et al. 2013; Gobbato et al. 2013). The variables analyzed in both reviews were similar except for implant survival, only present in the study by Gobbato et al. (2013), and the variable marginal bone loss only included in the study by Lin et al. (2013). Furthermore, Gobatto et al. (2013) excluded from the meta-analysis the variables CAL and MR. The authors justified the exclusion by the lack of standardization among selected studies. Both meta-analysis (Gobbato et al., 2013, Lin et al., 2013) revealed that an “inadequate” band of KM (<2 mm) was associated with more plaque accumulation and tissue inflammation. Additionally, the study by Lin et al. (2013) demonstrated that implants with KM <2 mm presented higher MR and CAL values. The authors did not observe statistically significant differences between groups when the variable bone loss was analyzed ($p>0.05$).

Table 3 – Systematic reviews and meta-analysis

Author (year), Country	No. of studies included	Study type	Variables analysed	Meta-analysis	Results	Conclusions
Wennström & Derks (2012), Switzerland	19 studies	17 cross-sectional or longitudinal studies, and 2 preclinical studies	PI, mPI, GI, mGI, BI, mBI, BoP, PD, CAL, MR, BL and survival rate	No	Evidence with regard to the need for presence, or a certain amount, of KM around implants to maintain health and tissue stability was limited. Longitudinal and cross-sectional studies in well-maintained populations showed no significant association between "inadequate" KM and inflammation. However, in studies in less well-maintained populations, a significant association was reported. MR was reported in the first 6-12 months after rehabilitation and may be more pronounced at sites lacking KM.	The results suggested that in clinical situations, where proper plaque control can be performed, the presence of peri-implant KM may not be essential.
Li et al. (2013), China an United States of America	11 studies	7 cross-sectional studies and 4 longitudinal studies (prospective and retrospective)	PI, mPI, GI, mGI, BI, mBI, BoP, PD, CAL, MR and BL	Yes	Meta-analysis demonstrated that the variables PI, mPI, mGI, CAL and MR were significantly different between groups "with" and "without" KM ($p<0.05$). No significant differences regarding BoP, mBI, GI, PD and BL were found between groups ($p>0.05$).	The review suggested that an "inadequate" KM width was associated with poor plaque control, tissue inflammation, mucosal recession and attachment loss.

Gobbato et al. (2013), United States of America	8 studies	6 cross-sectional studies and 2 prospective studies	PI, mPI, GI, mGI, BI, mBI, BoP, PD and survival rate	Yes (7 studies included)	Meta-analysis revealed that implants with KM <2 mm present greater PI, mPI and GI. The variable PD was not significant between groups. BoP and implant survival rate were not included in the analysis due to the lack of information.	A band of KM <2 mm was associated with clinical signs of inflammation. However, information showing the importance of KM was still limited.

N – Number of studies included; KM – Keratinized mucosa; PI – Plaque index; mPI – Modified plaque index; BoP – Bleeding on probing;

BI – Bleeding index; mBI – Modified bleeding index; GI – Gingival index; mGI – modified gingival index; PD – Probing depth;

MR – Mucosal recession; CAL – Clinical attachment level; BL – Bone level.

1.3 Conclusion

Despite the controversy on the matter, some studies have demonstrated that implants with KM <2 mm present more plaque accumulation and signs of inflammation than implants with KM ≥2 mm. Implants sites with the absence or an "inadequate" band of KM may show more mucosal recession than sites with KM ≥2 mm. Recent studies have also suggested that the lack of KM may be associated with higher levels of discomfort during brushing. However, most studies were cross-sectional studies, and evidence with regard to the actual need of a certain amount of KM to maintain peri-impant health and marginal bone level stability is still limited.

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INFLUENCE OF THE KERATINIZED MUCOSA ON THE STABILITY OF PERI-IMPLANT TISSUES AND BRUSHING DISCOMFORT: A 4-YEAR FOLLOW-UP STUDY

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INFLUENCE OF THE KERATINIZED MUCOSA ON THE STABILITY OF PERI-IMPLANT TISSUES AND BRUSHING DISCOMFORT: A 4-YEAR FOLLOW-UP STUDY

ABSTRACT

Objective: The purpose of the present 4-year follow-up study was to evaluate the influence of the peri-implant keratinized mucosa (KM) on marginal bone level (MBL), peri-implant tissues health and brushing discomfort**.**

Material and Methods: Eighty patients were initially recruited during their maintenance visit from January to October 2013 and allocated in two groups according to KM width around implants: Wide group (KM ≥2 mm); and Narrow group (KM <2 mm). At the 4-year follow-up visit (T4), marginal bone level (MBL), plaque index (mPI), probing depth (PD), clinical attachment level (CAL), bleeding on probing (BoP), and brushing discomfort (BD) were assessed and compared to results obtained in the initial assessment (T0). Mann-Whitney, Wilcoxon signed-rank test and a multilevel model were used for statistical analysis ($p<0.05$).

Results: Fifty-four patients with 202 implants returned for T4. Patients in the Narrow group presented more marginal bone loss ($p=0.015$), mPI ($p=0.002$), BoP ($p=0.026$) and BD ($p=0.029$) than those in the Wide group. Multilevel analysis suggested that KM width and time in function had an effect on MBL ($p=0.035$).

Conclusions: Findings indicate that KM width has an effect on MBL changes, plaque accumulation, tissue inflammation and brushing discomfort. As a result, marginal bone loss was more evident around implants with KM <2 mm than around implants with KM ≥2 mm.

INTRODUCTION

The peri-implant mucosa is formed during the wound healing process that follows implant/abutment placement. The main function of the mucosal seal is to protect the osseointegration process and the underlying bone from injuries (Berglundh et al. 1991). Soft-tissue healing may result in the establishment of a border tissue composed by a masticatory mucosa or a lining mucosa. The masticatory or keratinized mucosa (KM) consists of a dense connective tissue, rich in collagen fibers, connected firmly to the periosteum and covered by keratinized epithelium. In contrast, the lining mucosa is covered by nonkeratinized epithelium and presents a lamina propria rich in elastic fibers that allow the tissue to adapt to muscle tensions (Ten Cate 1994). The amount of keratinized mucosa surrounding the implant is determined by (i) the original amount of gengiva, (ii) the amount of post-extraction soft tissue remodeling and (iii) the position of the implant surface in relation to the muco-gingival line (Chappuis et al. 2017). According to the literature, 46-74% of implants are surrounded by an "inadequate" band of KM (Adell et al. 1986; Lekholm et al. 1986; Apse et al. 1991; Mericske-Stern et al. 1994).

The importance of KM in the maintenance of peri-implant tissues health and long-term success of implant therapy has been a matter of controversy. Several studies have shown poor plaque control and more peri-implant tissue inflammation with an "inadequate" amount of KM (Chung et al. 2006; Bouri et al. 2008; Adibrad et al. 2009; Ladwein et al. 2015; Souza et al. 2015). Other studies have also demonstrated that sites with KM <2 mm could present more soft tissue recession, clinical attachment loss and marginal bone loss than the sites with KM ≥2 mm (Bouri et al. 2008; Adibrad et al. 2009). Adibrad et al. (2009) evaluated KM influence on peri-implant clinical parameters and observed a negative correlation between KM band width, soft tissue recession and clinical attachment loss. In a cross-sectional study, Bouri et al. (2008) suggested that more bleeding on probing and more bone loss occurred in implants surrounded by KM <2 mm. Furthermore, it has been suggested that the presence of an "adequate" band of KM would be necessary to maintain tissue health, and to prevent the development of peri-implant diseases (Roos-Jansåker et al. 2006; Costa et al. 2012; Boynueğri et al. 2013; Canullo et al. 2016).

In contrast, some studies indicated that the absence of an adequate band of KM may not negatively affect peri-implant tissues health and stability (Wennström et al. 1994; Wennstrom

& Derks 2012). In a systematic review, Wennström & Derks (2012) concluded that evidence regarding the need for KM around implants was limited, and in a population with adequate maintenance, KM presence around implants did not seem to present any significance. However, the authors also stated that some patients might experience pain and discomfort during brushing at implant sites with KM < 2mm, which could hinder proper oral hygiene. In a recent cross-sectional study, Souza et al. (2015) evaluated the influence of KM on brushing discomfort in 80 patients, and showed that implants with KM <2 mm presented higher levels of brushing discomfort, poorer plaque control, and more peri-implant inflammation than sites with KM ≥2 mm. Although there is consistent evidence demonstrating more plaque accumulation and signs of inflammation at implants sites lacking an "adequate" band of KM, controlled studies evaluating longitudinally the effect of KM on clinical/radiographic variables and brushing disconfort is lacking.

Therefore, the aim of the present 4-year follow-up study was to evaluate the influence of the peri-implant keratinized mucosa on marginal bone level, peri-implant tissues health and brushing discomfort.

MATERIAL AND METHODS

Study design and sample selection

This 4-year prospective longitudinal follow-up study is an extension of a previously published study by Souza et al. (2015). The present study was performed following the criteria established by the Helsinki Declaration and has been approved by the Institutional Review Board for Research Conducted with Human Beings at the State University of Maringá, Brazil (protocol 205/2010). The study followed the STROBE statements for reporting observational studies (von Elm et al. 2007).

Patients were recruited during their routine maintenance visit to the Dental Clinic at the School of Dentistry of the State University of Maringá from January to October 2013. Subjects included were 18 years of age or older, and presented at least one implant-supported restoration in function for ≥1 year. Subjects presenting the following conditions were excluded from the study: (i) active periodontal disease; (ii) heavy smokers (>10 cigarettes/day); (iii) uncontrolled diabetes; (iv) conditions that could affect bone metabolism; (v) continuous use of anti-inflammatories or any drugs that could affect bone metabolism; (vi)

pregnancy; (vii) immunocompromised conditions; (viii) sites with implant-supported overdentures; (ix) sites with implant-supported rehabilitations presenting poor marginal adaptation (confirmed with an exploratory dental probe and radiographic examination); and (x) implant-supported rehabilitations with inadequate access to hygiene. A total of 80 subjects (25 male and 55 female) with a mean age of 52±11.7 years fulfilled the inclusion/exclusion criteria and were included in the first examination (T0) (for further details, see Souza et al. 2015).

Demographic parameters such as age, gender, smoking status, as well as data on patient's medical and dental history were obtained through a written questionnaire. All participating patients received explanations on the objectives of the study and signed a written informed consent. Clinical, radiographic and brushing discomfort assessments were performed, and each patient received complete professional dental prophylaxis and mechanical debridement, whenever necessary (T0). After a 4-year period (T4), all participants were individually reached by phone and scheduled for a new evaluation. During the period between T0 and T4, patients were enrolled in an annual maintenance program, which included oral hygiene instructions, prophylaxis, and mechanical and chemical subgingival plaque control in sites showing bleeding.

Radiographic examination and measurements

Periapical radiographs of each experimental implant site were acquired with an intraoral dental E-Speed film (Eastman Kodak®, Rochester, USA) using a plastic positioner (Maquira®, Maringá, PR, Brazil) according to the parallelism technique. Periapical radiographs were digitized with the aid of a film and slide scanner (Nikon® CoolScan IV ED, Tokyo, Japan). The resulting images were analyzed using a computer software (Image J®, National Institutes of Health, Maryland, USA), calibrated to measurements already known, such as the width of the implant platform.

Marginal bone level (MBL), defined as the distance from the implant shoulder to the first or most coronal bone-implant contact point, was measured at mesial and distal sites (Fig.1). Subsequently, the mean value of the two measurements was obtained for each implant. Bone loss in the four-year period was calculated by subtracting mean MBL found at T4 from that found at T0. Annual bone loss was estimated by dividing mean bone loss by 4. All measurements were performed by the same previously calibrated operator.

Peri-implant clinical parameters

Peri-implant clinical parameters were assessed at three sites (mesiobuccal, midbuccal, and distobuccal) at the buccal aspect of each implant with the use of a periodontal probe (Hu-Friedy® UNC 15, Chicago, USA) by two experienced examiners, previously calibrated.

The following peri-implant clinical parameters were assessed:

(i) Modified plaque index (mPI) (Mombelli et al. 1987) – Scored from 0 to 3: 0 – no plaque detection; 1 – plaque recognized by running a probe across the marginal surface of the implant; 2 – Plaque seen with the naked eye; and 3 – Abundance of soft matter.

(ii) Probing depth (PD) – measured in millimeters from the peri-implant mucosa margin to the bottom of the peri-implant sulcus.

(iii) Clinical attachment level (CAL) – measured in millimeters from the implant shoulder to the bottom of the peri-implant sulcus.

(iv) Bleeding on probing (BoP) – measured by the presence or absence of bleeding after 15 sec of gentle probing.

(v) Keratinized mucosa width, (KMw) – measured in millimeters at the mid-buccal aspect of the implant from the gingival margin to the mucogingival junction. KM and oral mucosa differences in color, texture, and mobility were considered to identify the mucogingival junction line (Fig. 2).

Implants were divided according to the KMw in two groups: Wide Group (KM ≥2 mm); and Narrow Group (KM <2 mm; Fig. 3). Furthermore, implant site location (maxilla or mandible), and type of implant-supported prosthesis (single unit, and partial and full-arch fixed restorations) were also recorded.

Brushing discomfort assessment

After the clinical and radiographic assessments, all patients received proper implant cleaning instructions. Dental brushes (Colgate® Extra Clean, Colgate-Palmolive Company, New York, USA), interdental brushes (Interdental brushes Bitufo®, Bitufo Co. Brushes LTDA, São Paulo, Brazil), and dental floss (Colgate Total® Dental Floss, Colgate-Palmolive Company) were distributed to all participants. The brushing technique adopted included vibration movements of the toothbrush with pressure at a 45° angle. Patients were invited to clean around implants using the oral hygiene devices provided for no more than 30 seconds.

Brushing discomfort experienced by patients was self-reported with the use of a Visual Analogue Scale (VAS) (Jensen et al. 1986). Immediately after the end of oral hygiene, patients were instructed to mark a point in a line ranging from zero to 100 millimeters, representing their level of discomfort during the cleaning procedure (Fig. 4). VAS scores were categorized into one of the following classes of brushing discomfort: no discomfort (VAS = 0), mild discomfort (0 < VAS < 30), moderate discomfort (30 ≤ VAS < 70), strong discomfort (70 ≤ VAS < 100) and extreme discomfort (VAS = 100).

Sample size calculation

The ideal sample size to ensure adequate statistical power was calculated using G* power 3.1 software (Heinrich Heine University Düsseldorf , Düsseldorf, Germany) considering a mean bone loss of 1.72 ± 1.18 mm in the KM < 2 mm group and 1.24±0.69 mm in the KM ≥ 2 mm group (Bouri et al. 2008). A total of 51 subjects was calculated to be necessary to provide a 95% statistical power with α=0.05.

Calibration

Each examiner was calibrated prior to clinical and radiographic measurements to ensure data collecting consistency. Intra-observer error was determined by measuring the peri-implant clinical parameters (PI, BOP and PPD) around 10 implants, five in each group, on randomly chosen patients. Each measurement was performed twice within a 2-day interval. Inter-examiner reliability was determined by Kappa correlation coefficient test, which resulted in 0.88.

Radiographic bone loss calibration was conducted according to the method described by Pennarrocha et al. 2004. To determine the intra-observer error, marginal bone loss around 30 implants was measured using the periapical radiographs. Each measurement was performed twice on consecutive days. An estimate of the intra-observer standard deviation (SD) was then determined using the following mathematical formula, $\sqrt{\frac{(\Sigma^{d})^{2}}{2n}}$, where d is the difference between the 2 measurements and n is the number of measurements made (n=30). The correlation coefficient (*Spearman's correlation*) was found to be 0.87.

Statistical analysis

Only the results from patients that returned for the 4-year follow-up examination were

considered in the analysis. MBL was the primary outcome. Descriptive statistical analysis of all data was performed to calculate the means and standard deviations (SD). Lilliefors' normality test was used to verify the normal distribution of the data. To data that followed a non-normal distribution (MBL, mPI, PD, CAL and KMw), Mann-Whitney and Wilcoxon test were used to evaluate differences between and within groups over time (T0 and T4), respectively. Otherwise, independent and paired t-test were used to verify differences in the variable BoP between and within groups. Wilcoxon test (signed rank test) was applied for categorical data. Odds ratio (OR) was used to characterize the association between groups and marginal bone loss. Confidence intervals for the odds ratio were constructed by the asymptotic normality of log OR.

Brushing discomfort was evaluated per quadrant. Thus, in each subject, the quadrants that harbored implant-supported prostheses were divided into 2 groups: quadrants with all implants with KM ≥2 mm (Wide group) or at least one implant with KM <2 mm (Narrow group). The patient was considered as experimental unit and mean value was calculated for those patients presenting more than 1 quadrant included in the same experimental group. As the variable brushing discomfort showed non-normal distribution, a nonparametric test was used.

A linear mixed model (multilevel model) for clustered longitudinal data was applied to investigate whether covariates measured at each level of the hierarchy had an impact on the dependent variable. In the present study, the dependent variable (MBL), was measured at two-time points for each implant (T0 and T4), with implants clustered within patients (Table 1). Figure 5 exemplifies the hierarchical structure of the clustered longitudinal data set using a randomly selected patient. The third patient (who represents a cluster of units) had three implants (the units of analysis), other patients could present a different number of implants.

In our model, we included fixed effects associated with all covariates under consideration (Time, Group, Gender and Age), and the two-way interactions between GROUP and each of the covariates. Also, we added the following random components to this model: random effects associated with the intercept for each patient, and random effects related to the intercept for each implant nested within a patient. Thus, marginal bone level (MBL) response on implant j nested within subject i was represented by MBL_{ij}, which is given by:

$$MBL_{ij} = \beta_0 + \beta_1 Narrowgroup_{ij} + \beta_2 Time2017_{ij} + \beta_3 Female_i + \beta_4 Age_i + \beta_5 Narrowgroup_{ij} Female_i + \beta_6 Narrowgroup_{ij} Age_i + \beta_7 Narrowgroup_{ij} Time2017_i + \{\mu_0 + \mu_{0ji} + \epsilon_{ij}\}_{Random\ Effects}$$

In this case, β_0 represents the expected value of MBL_ij for the reference levels of group, gender and time (that is, Wide Group, and Male, and 2013). The variables Narrow group, 2017 and Female were indicator variables, while Age was a continuous variable. Thus, β_1 through β_7 was the fixed effects of the covariates. Outliers were removed for better estimation and model performance.

Statistical analyses were conducted with R statistical software, version 3.3.0 Team (R Foundation for Statistical Computing, Vienna, Austria) using NLME package with the levels of significance established at 95% ($p < 0.05$).

RESULTS

Out of the 80 subjects initially assessed at T0, 54 patients (18 male and 36 females) with a mean age of 55.7±10.7 returned for the evaluation at T4. Among the 26 patients that did not return, 13 changed telephone numbers or moved to another city and could not be found, 6 preferred not to participate in the study, 5 did not come to the appointment and 2 had missing information and were excluded. An overall survival rate of 98% was observed. Out of 206 implants in the 54 patients, 4 had been lost, of which, three implants originally belonged to the Narrow Group (96.7 % survival rate).

Of the 54 patients included in the analysis, 17 belonged to Wide group while 20 to the Narrow group. The remaining 17 subjects contributed with two quadrants, one in each experimental group. A flowchart detailing the sample is shown in Fig. 6. Two hundred and two dental implants with a mean loading time of 9.6 ± 1.2 years were examined. The number of implants in the maxilla was slightly higher than in the mandible (52.5% vs. 47.5%). In the maxilla, 26.7% and 25.7% of implants were localized at the posterior and anterior region, respectively, while the corresponding values for mandible were 41.1% and 6%. The number of single, fixed

partial restorations, and full-arch bridges supported by implants was 87, 91 and 24, respectively.

Mean MBL in the Wide and Narrow groups at T0 were 1.82 mm and 1.84 mm, respectively, while the corresponding values at T4 were 1.87 mm and 2.11 mm. The difference in mean MBL values between T0 and T4 in the Narrow group was statistically significant ($p<0.05$), while no difference was observed in the Wide group (Table 2). Mean bone loss was significantly higher for implants with a narrow band of KM ($p<0.05$; Table 3). An annual bone loss of 0.01 mm and 0.07 mm were estimated in the Wide and Narrow group, respectively. Univariate analysis demonstrated that implants with a narrow band of KM were 3.5 times more likely to have marginal bone loss ≥ 1 mm than those with a wider band of KM (adjusted OR=3.45; 95% confidence interval, 1.04 to 11.40).

Peri-implant clinical parameters mean values at T0 and T4 are shown in Table 4. Mean mPI and BoP were significantly higher in the Narrow group than in the Wide group at T0 and at T4 ($p<0.05$). No statistically significant difference between groups was observed for the PD and CAL both at T0 and T4. At T4, significantly higher values for PD and CAL were found in both groups, than at T0. A significant difference for BoP was found in the Wide and Narrow group between T0 e T4 (Table 4). The frequency distribution of mPI scores in both groups is illustrated in Table 5.

Mean VAS scores found in the Narrow group was significantly higher (mean 17.34±22.19; median 8.0 [range 0-75]) than in the Wide group (mean 5.09± 9.97; median 0.0 [range 0-41]) at T0 ($p=0.012$). Mean VAS scores were also significantly greater in the Narrow group (mean 12.28±17.59; median 2.0 [range 0-56]) than in the Wide group (mean 4.25± 8.39; median 0.0 [range 0-36]) at T4 ($p=0.029$). The frequency distribution of VAS scores according to the group at T0 e T4 are illustrated in Figures 7 and 8. At T0, no discomfort was observed in 70.6% and 46% of the patients in the Wide and Narrow group, respectively. Mild or moderate discomfort was indicated by 29.4% and 51.4% of the subjects, while the corresponding percentage for strong or extreme discomfort was 0% and 2.7% in the Wide and Narrow groups, respectively. At T4, 73.53% and 48.7% of the subjects reported no discomfort, while mild or moderate discomfort was indicated by 29.41% and 51.4% in the Wide and Narrow groups, respectively. Strong or extreme discomfort was not reported by any patient. Although

the percentage of individuals that disclosed mild or moderate discomfort in the Narrow group remained the same at the 4-year time interval, a reduction in the number of patients reporting moderate discomfort and an increase in patients with mild discomfort were observed. Mean VAS scores according to location (mandible or maxilla) at T0 and T4 are illustrated in Figures 9 and 10, respectively. At T0 VAS scores in the groups were similar in the maxilla (p=0.071), but statistically different in the mandible (mean 24.37± 28.31; median 8.75 [range 0–100] in the Narrow Group vs. mean 4.5±8.64; median 0.0 [range 0-23] in the Wide group (p=0.013).

The results from the multilevel modeling analysis regarding the effect of the covariates gender, age, group (Wide or Narrow group), and time (in function) on MBL are shown in Table 6. Significant differences were observed between the Wide and Narrow group (p=0.002). After controlling for the effects of Group, Time, Gender, Age, "Group and Gender", "Group and Age" the results suggested a positive effect of Group and Time in function on MBL. Thus, at the 4-year follow-up examination, implants sites with KM <2 mm, were predicted to have an average MBL 0.15 mm higher than implants sites with KM ≥2 mm. Although some variables were not statistically significant, they were clinically relevant and were not removed from the analysis. Figure 8 demonstrates the observed versus the fitted rates. The effect plot (Figure 12) illustrates the difference between both groups along the years considered in the analysis (4 years). The positive effect and means are closer than the empirical mean, which is an indication of a good fit.

DISCUSSION

The present 4-year prospective follow-up study evaluated the influence of the keratinized mucosa on marginal bone level, peri-implant tissues health and brushing discomfort. The study revealed significant differences on clinical and radiographic parameters for peri-implant tissues health and stability between the Wide and Narrow groups. At the 4-year follow-up assessment, implant sites with KM <2 mm exhibited significantly greater marginal bone loss, plaque accumulation, signs of inflammation and brushing discomfort than sites with KM ≥2 mm.

In the current study, MBL changes observed in the Narrow group (T0 = 1.84 mm; T4 = 2.11mm) were more significant than in the Wide group (T0= 1.82 mm; T4= 1.87 mm). Thus,

the mean MBL in the Wide group remained stable over the studied time, while a statistically significant marginal bone loss was observed in the Narrow group ($p<0.05$). In addition, the estimated annual bone loss was found to be 7 times more at the Narrow (0.07 mm/year) than the Wide (0.01 mm/year) group. A cross-sectional study carried out by Bouri et al. (2008) evaluating the role of the KM width around 200 dental implants also demonstrated more bone loss in a KM < 2 mm group (1.72 ± 1.18 mm) than in a KM ≥ 2 mm group (1.24±0.69 mm) after a follow-up of 1 year. The higher bone loss values reported by the authors in comparison with the present study may be explained by the fact that the baseline MBL was obtained at the time of implant placement. In contrast, others studies in the literature reported no association between KM width and MBL or bone loss around dental implants (Chung 2006; Crespi et al. 2010; Adibrad et al. 2009; Ladwein et al. 2015; Roccuzzo et al. 2016). In a recent study, Ladwein et al. (2015) evaluated the relationship between the presence of KM and peri-implant tissue health in 967 implants in function for at least 10 years, and observed no association between KM width and MBL. However, differently from other studies, MBL measurements were conducted with panoramic radiographs, rather than periapical ones. In addition, although no statistically significant differences in MBL were observed by Ladwein et al. (2015), their study showed more bone loss at the mesial sites of implants, as was observed in the present study. This finding may be related to patients' dexterity to perform oral hygiene, since they tend to use dental floss more effectively at distal sites than mesial sites, especially in the posterior regions.

Implants with KM <2 mm both at T0 e T4 exhibited higher mPI and BoP mean values than those with KM ≥2 mm. These results are in agreement with previous clinical studies (Chung 2006; Bouri et al. 2008; Adibrad et al. 2009; Ladwein et al. 2015; Souza et al. 2015). Meta-analysis from recent systematic reviews (Gobbato et al. 2013; Lin et al. 2013) reported significant differences in plaque and inflammation, suggesting that a narrow band of KM (<2 mm) was associated with more plaque accumulation and peri-implant tissue inflammation. Schortt et al. (2009) observed that at lingual sites, the presence of KM ≥2 mm reduced the probability of bleeding in 40% (OR=0.60, 95% CI=0.48-0.74). Sites lacking an "adequate" band of KM were also associated with higher levels of TNF-α when compared to sites with KM ($p<0.05$) (Boynueğri et al. 2013). A recent randomized clinical trial investigated the effect of free gingival graft surgery (FGGS) to increase the width of peri-implant KM based on peri-implant clinical and immunological parameters (Askin et al. 2015). After 6 months, the results revealed significant improvement of the clinical and immunological parameters in

patients treated with FGGS, unlike those with KM <2 mm that did not undergo the procedure. Substantial evidence has associated poor oral hygiene and signs of inflammation with peri-implant diseases (Roos-Jansåker et al. 2006; Costa et al. 2012; Canullo et al. 2016). A recent cross-sectional study by Canullo et al. (2015) analyzed the clinical parameters in subjects and implants affected and not affected by peri-implantitis. The investigation demonstrated that inadequate oral hygiene and the presence of BoP were associated with a greater prevalence of peri-implantitis.

VAS scores for brushing discomfort in the Narrow group were significantly higher than those in the Wide Group, both at T0 and T4. A 10-year follow-up study by Rocuzzo et al (2016) also assessed the presence of soreness or discomfort during oral hygiene and found that 42.9% of the patients in the non-KM group reported discomfort during oral hygiene ($p<0.001$) while no patient reported discomfort in the KM group. Although in the present study the percentage of individuals reporting mild or moderate discomfort in the Narrow group remained similar at T4, a migration of patients reporting lesser levels of BD was observed. A possible explanation is that some patients may have become more tolerant or even adapted to the discomfort over time. A recent study evaluated the adaptation processes to intermittent comfortable or uncomfortable stimuli and demonstrated that discomfort tended to decrease over time, with patients adapting to uncomfortable experiences (Murata & Nakamura 2017). Based on the results of the present study, the more plaque accumulation and peri-implant tissues inflammation observed around implants sites with KM <2 mm may be related to the increased BD reported by these patients. It suggests that an “adequate” band of KM may provide more satisfactory brushing, allowing patients to clean implant sites more properly and, consequently, limit bacterial infiltration.

The multilevel analysis using clustered longitudinal data set allows to investigate whether covariates measured at each level of a hierarchy have an impact on the dependent variable, which in the current study was MBL. The results of the multilevel analysis (Table 6) suggest an influence of KM width and time in function on MBL. Thus, implants sites with KM <2 mm were more prone to present marginal bone loss than implants sites with KM ≥ 2mm as time follows. Roos-Jansaker et al. (2006), who evaluated the factors related to peri-implant diseases at 999 implants 9 to 14 years after initial therapy, also observed that the amount of KM was explanatory for mucositis as well as for bone losses ≥3 mm. A recent study noted that KM ≥2 mm was found to reduce significantly the probability of an implant suffering peri-

implantitis (OR=0.36) (Canullo et al. 2016). The time interval seems to have a relevant effect on implants without an "adequate" band of KM. Roos-Jansaker et al. (2006) suggested that peri-implant lesions frequency will increase over time in function. Fransson et al. (2010) described the severity and the pattern of peri-implantitis-associated bone loss in 182 subjects, and revealed that bone loss rate presented a non-linear pattern, increasing over time.

The significance of KM on the maintenance of peri-implant tissues health and stability is probably related to the anatomical and histological features of such tissue. The keratin layer of the masticatory mucosa is responsible for providing a mechanically resistant, highly insoluble and flexible structure that protects the epithelial cells (Presland & Dale 2000). The wide stratified epitheium under the keratin layer not only provides mechanical protection to the connective tissue but also the first contact to the immune system (Presland & Dale 2000). The underlying connective tissue of the KM is dense and rich in collagen fibers which provides a great tissue adaptation to the implant abutment/implant surface, resistence to collagenasis and act as a mechanical barrier to bacterial invasion toward the bone tissue (Romanos et al. 1995). In summary, the KM around implants seems to provide a better tissue seal against bacterial challenge.

The results in the current study should be viewed within the context of some limitations. The peri-implant parameters MBL and KM width were measured in different implant aspects, interproximal and mid-buccal, respectively. Thus, to confirm the association between these two parameters more accurately, cone-beam computed tomography (CBCT) images should be considered. The proportion of patients who did not return for the 4-year follow-up may also have influenced the results, since the lost patients may have had a different prognosis than those who completed the study.

CONCLUSION

The findings from the present study indicate that implants with KM <2 mm exhibited more marginal bone level changes, plaque accumulation, tissue inflammation and brushing discomfort than sites with KM ≥2 mm. The multilevel analysis suggests KM and time in function can have an impact on MBL, with implant sites with KM <2 mm being prone to present more marginal bone loss than implants sites with KM ≥2 mm. Thus, the keratinized

mucosa around implants appears to have a protective effect on the peri-implant tissues.

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TABLES

Table 1. Clustered longitudinal data set.

Level of Data		
Cluster of Data	**Cluster (Random Factor)**	**Patient (Subject)**
(Level 3)	Covariates	Gender, Age
Unit of Analysis	**Unit of Analysis (Random Factor)**	**Implant**
(Level 2)	Covariates	Group
Time	**Time Variable**	**Time (Years)**
(Level 1)	Dependent Variable	Marginal Bone Level

Table 2. Mean and standard deviation (±SD) of the radiographic marginal bone level at distal and mesial sites at baseline (T0) and 4-year follow-up (T4) assessments in the Wide (KM ≥2 mm) and Narrow (KM <2 mm) groups.

	T0			**T4**		
Marginal bone level (mm)	**Wide group**	**Narrow group**	***p value***	**Wide group**	**Narrow group**	***p value***
Distal	1.85±0.81	1.89±0.89	0.407	1.91±0.80	2.15± 1.23*	0.157
Mesial	1.79±0.79	1.80±0.85	0.375	1.84±0.84	2.08±1.10*	0.148
Mean	1.82 ± 0.75	1.84 ± 0.83	0.381	1.87 ± 0.77	2.11±1.13*	0.145

*Significantly different within group, $p<0.05$.

Table 3. Mean and standard deviation (±SD) of radiographic marginal bone loss at distal and mesial sites in the Wide (KM ≥2 mm) and Narrow (KM <2 mm) groups.

Radiographic bone loss (mm)	Wide group	Narrow Group	*p value*
Distal	0.06 ± 0.55	0.26 ± 0.76	0.009
Mesial	0.05 ± 0.54	0.27 ± 0.76	0.008
Mean	0.06 ± 0.48	0.26 ± 0.71	0.015

Statistical significant, $p<0.05$

Table 4. Mean and standard deviation (±SD) of the peri-implant clinical parameters at baseline (T0) and 4-year follow-up (T4) assessments in the Wide (KM ≥2 mm) and Narrow (KM <2 mm) groups.

	T0			T4		
	Wide group	**Narrow group**	*p value*	**Wide group**	**Narrow group**	*p value*
mPI	0.45 ± 0.55	0.83 ± 0.92	*0.008*	0.54±0.48*	0.91±0.60	*0.002*
BoP	0.44 ± 0.27	0.55±0.19	*0.039*	0.56±0.26*	0.67±0.21*	*0.026*
PD (mm)	2.43 ± 0.77	2.30±0.52	*0.188*	2.76±0.75*	2.77±0.68*	*0.395*
CAL (mm)	2.56 ± 0.77	2.64±0.61	*0.325*	2.94±0.80*	3.09±0.81*	*0.319*
KMw (mm)	3.17±1.39	0.24±0.37	*<0.0001*	2.86±1.65*	0.40±0.55	*<0.0001*

mPI – Modified Plaque Index; BoP – Bleeding on Probing; PD – Probing depth; CAL – Clinical attachment level; KMw – Keratinized mucosa width.

*Significantly different within group, $p<0.05$.

Table 5. Frequency distribution (%) of plaque index scores at baseline (T0) and 4-year follow-up (T4) assessments in the Wide (KM ≥2 mm) and Narrow (KM <2 mm) groups.

Score	Baseline			Follow-up		
	Wide group	**Narrow group**	*p value*	**Wide group**	**Narrow group**	*p value*
0	66.1	48.3	*<0.0001*	51.5*	37.1*	*0.001*
1	26.1	35.6	*0.551*	38.8*	43.8	*0.543*
2	7.6	15.4	*0.116*	8.5	15.7	*0.217*
3	0.3	0.7	*0.593*	1.2	3.4	*0.328*

*Significantly different within group, $p<0.05$.

Table 6. Multilevel analysis with mean marginal bone level as the dependent variable.

Parameter	**Estimate**	**S.E.**	**95% Confidence Interval**	***p value***
β_0 (Intercept)*	1.761	0.442	(0.885,2.6341)	*0.0001*
β_1 (Narrow group)	-0.138	0.588	(-1.3001, 1.0230)	*0.8141*
β_2 (Time 2017)	0.024	0.046	(-0.0669, 0.1151)	*0.6019*
β_3 (Female)	0.257	0.175	(-0.6035, 0.0884)	*0.1435*
β_4 (Age)	0.004	0.007	(-0.0108, 0.0198)	*0.5597*
β_5 (Narrow group:Female)	0.093	0.208	(0.3186, 0.5056)	*0.6548*
β_6 (Narrow group:Age)	0.002	0.009	(-0.0162, 0.021)	*0.8299*
β_7 (Narrow group: Time 2017)*	0.145	0.068	(0.0106, 0.2798)	*0.0347*

*Statistical significant, $p<0.05$

FIGURES

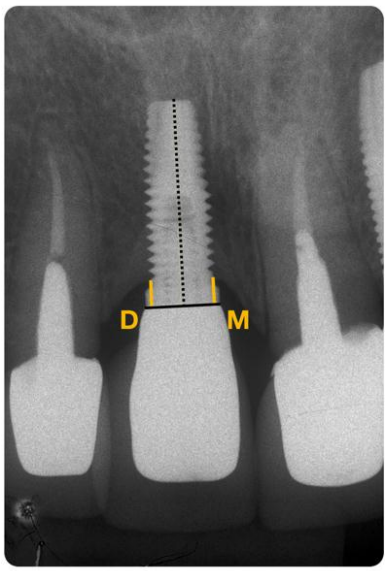

Figure 1. Marginal bone level (MBL), i.e., distance from implant shoulder to the first or most coronal bone-implant contact point, represented by the yellow line at the mesial (M) and distal (D) sites.

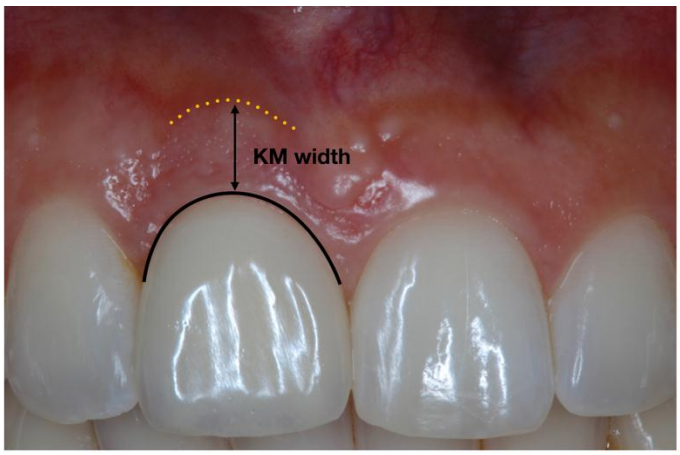

Figure 2. Keratinized mucosa (KM) width. The black line represents the gingival margin, while the yellow dotted line represents the mucogingival junction.

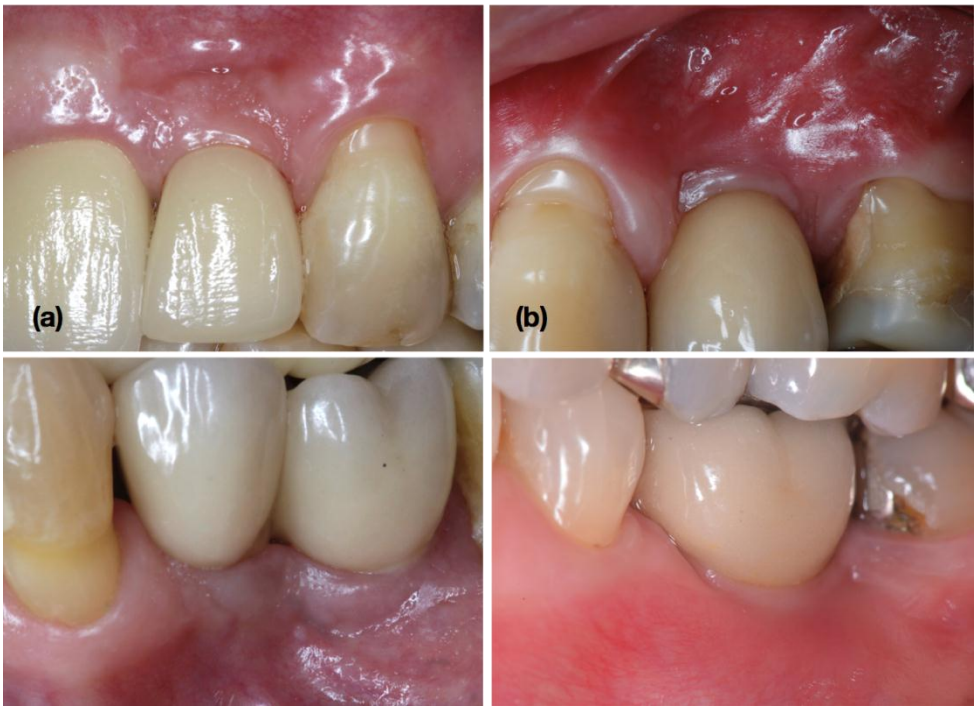

Figure 3. Photographs illustrating the two types of peri-implant tissues studied. (a) Wide Group (KM ≥2 mm) and (b) Narrow Group (KM <2 mm). Maxillary (top) and mandibular (bottom) regions.

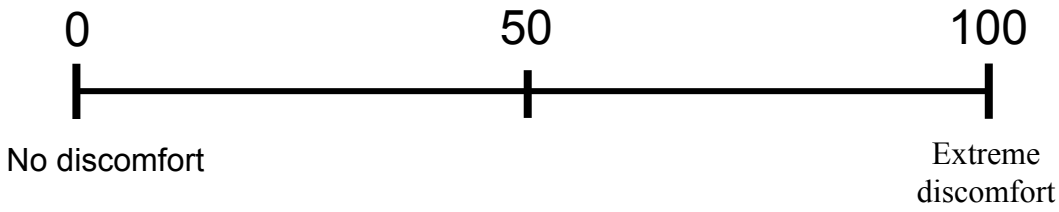

Figure 4. The Visual Analog Scale (VAS) used to measure patients' brushing discomfort.

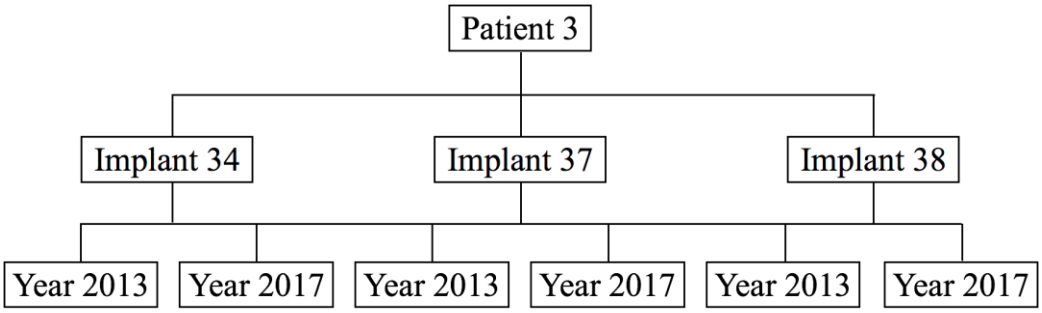

Figure 5. Clustered longitudinal data set, considering a randomly selected patient.

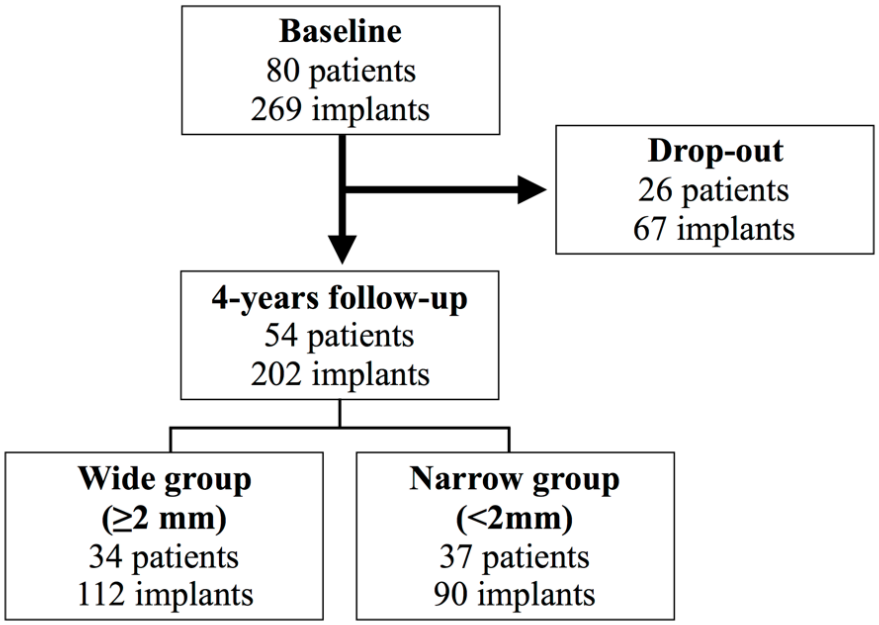

Figure 6. Sample description flowchart.

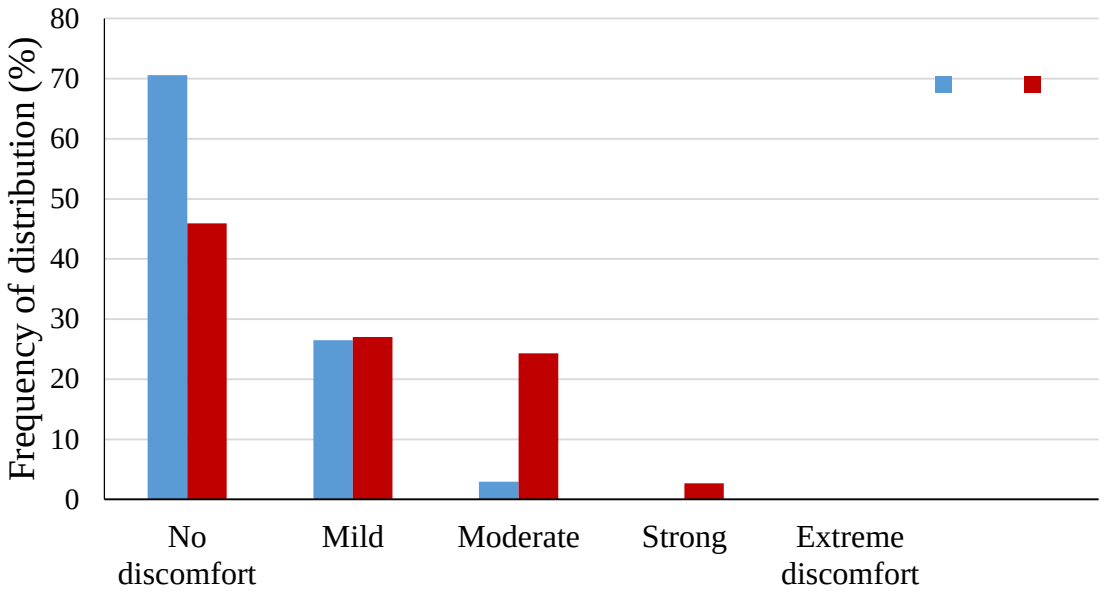

Figure 7. Graph showing the overall frequency distribution of individuals at the baseline (T0) in the Wide and Narrow groups, according to the brushing discomfort category.

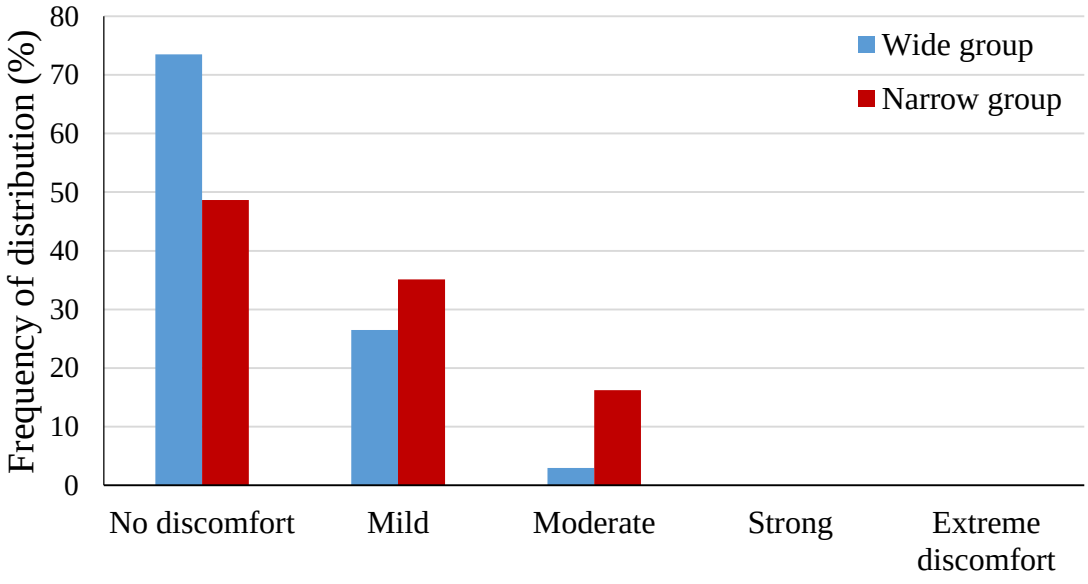

Figure 8. Graph showing the overall frequency distribution of individuals at the 4-year follow-up (T4) in the Wide and Narrow groups, according to the brushing discomfort category.

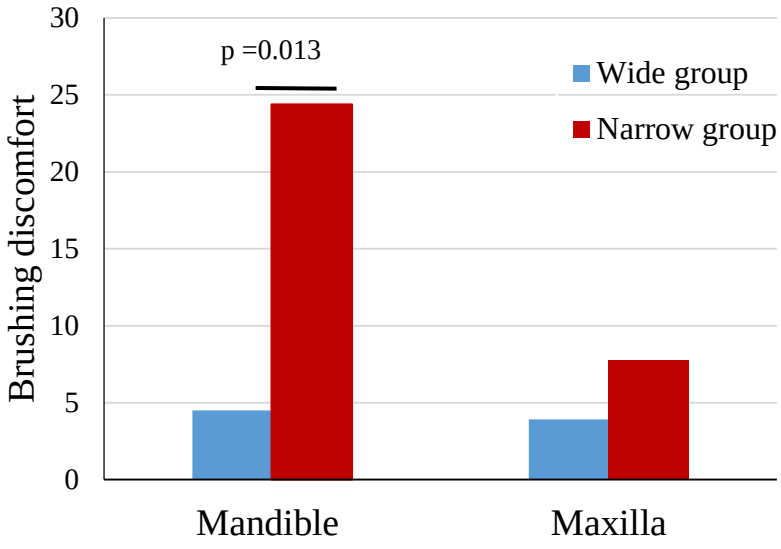

Figure 9. Graph depicting the mean values of brushing discomfort at baseline (T0) in the Wide and Narrow groups, according to location (mandible or maxilla).

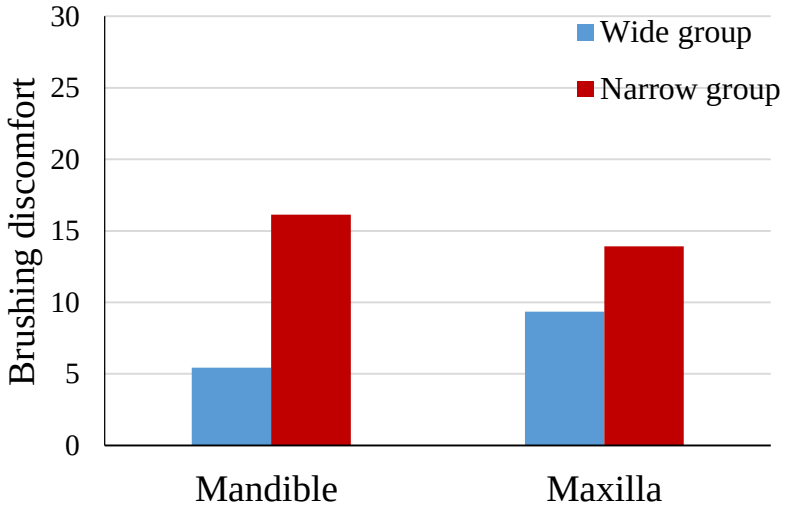

Figure 10. Graph depicting the mean values of brushing discomfort at the 4-year follow-up (T4) in the Wide and Narrow groups, according to location (mandible or maxilla).

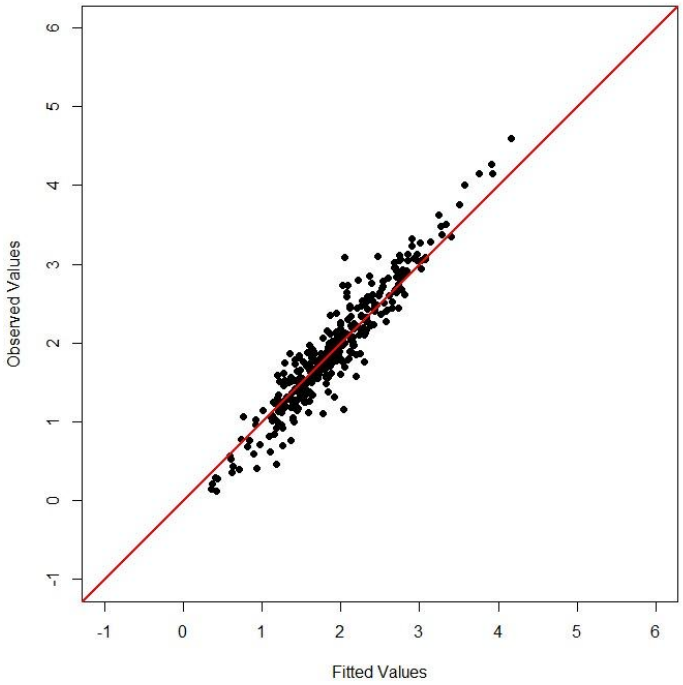

Figure 11. Observed versus the fitted rates.

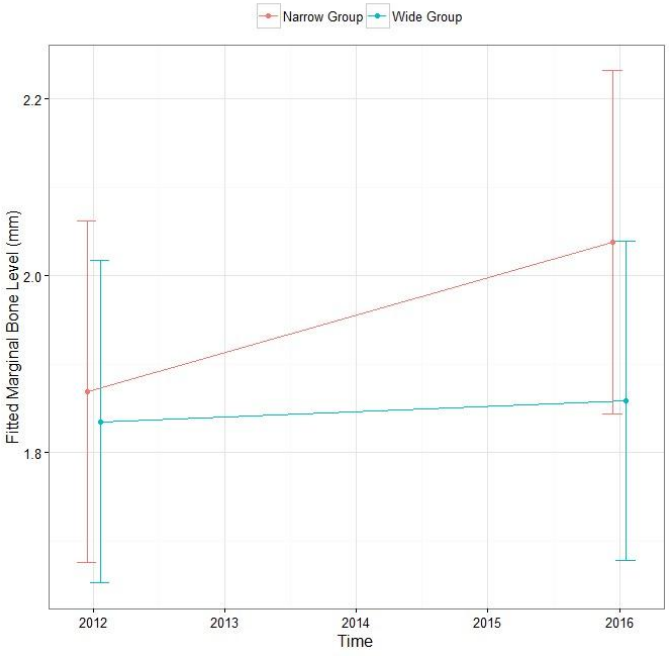

Figure 12. Effect plot.

ANNEX A – STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg

		numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

ANNEX B – *Clinical Oral Implant Research guidelines*

Author Guidelines

1. GENERAL

Clinical Oral Implants Research conveys scientific progress in the field of implant dentistry and its related areas to clinicians, teachers and researchers concerned with the application of this information for the benefit of patients in need of oral implants. The journal addresses itself to clinicians, general practitioners, periodontists, oral and maxillofacial surgeons and prosthodontists, as well as to teachers, academicians and scholars involved in the education of professionals and in the scientific promotion of the field of implant dentistry.

Clinical Oral Implants Research publishes:

Original research articles of high scientific merit in the field of material sciences, physiology of wound healing, biology of tissue integration of implants, diagnosis and treatment planning, prevention of pathologic processes jeopardizing the longevity of implants, clinical trials on implant systems, stoma-tognathic physiology related to oral implants, new developments in therapeutic concepts and prosthetic rehabilitation.

Review articles by experts on new developments in basic sciences related to implant dentistry and clinically applied concepts.

Case reports and case series only if they provide or document new fundamental knowledge.

Novel developments if they provide a technical novelty for any implant system.

Short communications of important research findings in a concise format and for rapid publication.

Treatment rational by experts with evidence-based treatment approach.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *Clinical Oral Implants Research*. Authors are encouraged to visit Wiley-Blackwell Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

Clinical Oral Implants Research adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript have been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal. ALL named authors must have made an active contribution to the conception and design and/or analysis and interpretation of the data and/or the drafting of the paper and ALL must have critically reviewed its content and have approved the final version submitted for publication. Participation solely in the acquisition of funding or the collection of data does not justify authorship.

Clinical Oral Implants Research adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Up to 6 authors are accepted without need for justification. In the case of a specific and detailed justification of the role of every author, up to 8 authors may be mentioned. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name because readers may infer their endorsement of the data and conclusions.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical AssociationDeclaration of Helsinki (version, 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editor reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European

Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

2.3 Clinical Trials

Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist should also be included in the submission material.

Clinical Oral Implants Research encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries:www.clinicaltrials.gov, http://clinicaltrials.ifpma.org/clinicaltrials, http://isrctn.org/. The clinical trial registration number and name of the trial register will then be published with the paper.

2.4 Conflict of Interest and Source of Funding

Clinical Oral Implants Research requires that sources of institutional, private and corporate financial support for the work within the manuscript be fully acknowledged, and any potential conflicts of interest noted. Suppliers of materials should be named and their location (town, state/county, country) included. Information concerning conflict of interest and sources of funding should be included under Acknowledgements.

2.5 Appeal of Decision

The decision on a paper is final and cannot be appealed.

2.6 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

2.7 Copyright Assignment

Authors submitting a paper do so on the understanding that the work and its essential substance have not been published before and is not being considered for publication elsewhere.

If your paper is accepted, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper.

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2.8 OnlineOpen

OnlineOpen is available to authors of primary research articles who wish to make their article available to non-subscribers on publication, or whose funding agency requires grantees to archive the final version of their article. With OnlineOpen, the author, the author's funding agency, or the author's institution pays a fee to ensure that the article is made available to non-subscribers upon publication via Wiley Online Library, as well as deposited in the funding agency's preferred archive. For the full list of terms and conditions, see http://wileyonlinelibrary.com/onlineopen#OnlineOpen_Terms

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Prior to acceptance there is no requirement to inform an Editorial Office that you intend to publish your paper OnlineOpen if you do not wish to. All OnlineOpen articles are treated in the same way as any other article. They go through the journal's standard peer-review process and will be accepted or rejected based on their own merit.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be submitted electronically via the online submission sitehttp://mc.manuscriptcentral.com/coir. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete

instructions for submitting a paper is available online and below. Further assistance can be obtained from the Editorial Assistant Ms. Brigitte Baur. E-mail: coir@zmk.unibe.ch

3.1. Getting Started

Launch your web browser (supported browsers include Internet Explorer 6 or higher, Netscape 7.0, 7.1, or 7.2, Safari 1.2.4, or Firefox 1.0.4) and go to the journal's online Submission Site:http://mc.manuscriptcentral.com/coir

• Log-in or click the 'Create Account' option if you are a first-time user.
• If you are creating a new account.
- After clicking on 'Create Account', enter your name and e-mail information and click 'Next'. Your e-mail information is very important.
- Enter your institution and address information as appropriate, and then click 'Next.'
- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your area of expertise. Click 'Finish'.
• If you have an account, but have forgotten your log in details, go to Password Help on the journals online submission system http://mc.manuscriptcentral.com/coir and enter your e-mail address. The system will send you an automatic user ID and a new temporary password.
• Log-in and select Corresponding Author Center.

3.2. Submitting Your Manuscript

• After you have logged in, click the 'Submit a Manuscript' link in the menu bar.
• Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.
• Click the 'Next' button on each screen to save your work and advance to the next screen.
• You are required to upload your files.
- Click on the 'Browse' button and locate the file on your computer.
- Select the designation of each file in the drop-down menu next to the Browse button.
- When you have selected all files you wish to upload, click the 'Upload Files' button.
• Review your submission (in HTML and PDF format) before sending to the Journal. Click the 'Submit' button when you are finished reviewing.

3.3. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the entire manuscript including title page, abstract, text, references, tables, and figure legends, but no embedded figures. In the text, please reference figures as for instance 'Figure 1', 'Figure 2' etc to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below.

3.4. Blinded Review

All manuscripts submitted to *Clinical Oral Implants Research* will be reviewed by two

experts in the field. *Clinical Oral Implants Research* uses single blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper.

3.5. Suggest a Reviewer

Clinical Oral Implants Research attempts to keep the review process as short as possible to enable rapid publication of new scientific data. In order to facilitate this process, please suggest the names and current email addresses of one potential international reviewer whom you consider capable of reviewing your manuscript. In addition to your choice the journal editor will choose one or two reviewers as well.

3.6. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.7. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation email after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our email server (uranus.scholarone.com) to their whitelist.

3.8. Manuscript Status

You can access ScholarOne Manuscripts (formerly known as Manuscript Central) any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.9. Submission of Revised Manuscripts

To submit your revised manuscript, locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision' . Please remember to delete any old files uploaded when you upload your revised manuscript.

4. MANUSCRIPT TYPES ACCEPTED

Original research articles of high scientific merit in the field of material sciences, physiology of wound healing, biology of tissue integration of implants, diagnosis and treatment planning, prevention of pathologic processes jeopardizing the longevity of implants, clinical trials on implant systems, stomatognathic physiology related to oral implants, new developments in therapeutic concepts and prosthetic rehabilitation.

Review articles by experts on new developments in basic sciences related to implant dentistry and clinically applied concepts. Reviews are generally by invitation only and have to be approved by the Editor-in-Chief before submission.

Case reports and case series, but only if they provide or document new fundamental knowledge and if they use language understandable to the clinician.

Novel developments if they provide a technical novelty for any implant system.

Short communications of important research findings in a concise format and for rapid publication.

Treatment rational by experts with evidence-based treatment approach.
Proceedings of international meetings may also be considered for publication at the discretion of the Editor.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Page Charge

Articles exceeding 10 published pages are subject to a charge of USD 160 per additional page. One published page amounts approximately to 5,500 characters (excluding figures and tables).

5.2. Format

Language: The language of publication is English. Authors for whom English is a second language might choose to have their manuscript professionally edited by an English speaking person before submission to make sure the English is of high quality. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Abbreviations, Symbols and Nomenclature: The symbol % is to be used for percent, h for hour, min for minute, and s for second. In vitro, in vivo, in situ and other Latin expressions are to be italicised. Use only standard abbreviations. All units will be metric. Use no roman numerals in the text. In decimals, a decimal point and not a comma will be used. Avoid abbreviations in the title. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement. In cases of doubt, the spelling orthodoxy of Webster's third new international dictionary will be adhered to.

Scientific Names: Proper names of bacteria should be binomial and should be singly underlined on the typescript. The full proper name (e.g., Streptococcus sanguis) must be given upon first mention. The generic name may be abbreviated thereafter with the first letter of the genus (e.g., S. sanguis). If abbreviation of the generic name could cause confusion, the full name should be used. If the vernacular form of a genus name (e.g., streptococci) is used, the first letter of the vernacular name is not capitalised and the name is not underlined. Use of two letters of the genus (e.g., Ps. for Peptostreptococcus) is incorrect, even though it might avoid ambiguity. With regard to drugs, generic names should be used instead of proprietary names. If a proprietary name is used, it must be attached when the term is first used.

5.2. Structure

All manuscripts submitted to *Clinical Oral Implants Research* should include Title Page, Abstract, Main Text and Acknowledgements, Tables, Figures and Figure Legends as appropriate.

Title Page: should contain the title of the article, full name(s) of the authors (no more than 6) and institutional affiliation(s), a running title not exceeding 60 letters and spaces, and the name, telephone and fax numbers, email and complete mailing address of the author responsible for correspondence. The author must list appropriate key words for indexing purposes.
Abstract: should not to exceed 250 words. This should be structured into: objectives, material and methods, results, conclusions, and no other information.
Main Text of Original Research Article should include Introduction, Material and Methods, Results and Discussion.
Introduction: Summarise the rationale and purpose of the study, giving only strictly pertinent references. Do not review existing literature extensively. State clearly the working hypothesis.
Material and Methods: Material and methods should be presented in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made. Indicate the statistical methods used, if applicable.
Results: Present your results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all data in the tables and illustrations. The important observations should be emphasised.
Discussion: Summarise the findings without repeating in detail the data given in the Results section. Relate your observations to other relevant studies and point out the implications of the findings and their limitations. Cite other relevant studies.

Main Text of Short Communications: Short communications are limited to two printed pages including illustrations and references and need not follow the usual division into material and methods, etc., but should have an abstract.

Acknowledgements: Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name because readers may infer their endorsement of the data and conclusions. Sources of financial support should be acknowledged.

5.3. References
References should quote the last name(s) of the author(s) and the year of publication (Black & Miller 1988). Three or more authors should always be referred to as, for example, (Fox et al. 1977).
A list of references should be given at the end of the paper and should follow the recommendations in Units, symbols and abbreviations: a guide for biological and medical editors and authors (1988), p. 52, London: The Royal Society of Medicine.

a) The arrangement of the references should be alphabetical by author's surname.

b) The order of the items in each reference should be:
(i) for journal references:
name(s) of author(s), year, title of paper, title of journal, volume number, first and last page

numbers.
(ii) for book references:
name(s) of author(s), year, title of book, edition, volume, chapter and/ or page number, town of publication, publisher.

c) Author's names should be arranged thus: Daniels, J.A., Kelly, R.A. & Til, T.C.
Note the use of the ampersand and omission of comma before it. Author's names when repeated in the next reference are always spelled out in full.

d) The year of publication should be surrounded by parentheses: (1966).

c) The title of the paper should be included, without quotation marks.

f) The journal title should be written in full, italicised, and followed by volume number in bold type, and page numbers.
Examples:
Tonetti, M. S., Schmid, J., Hämmerle,C. H. & Lang, N. P. (1993) Intraepithelial antigen-presenting cells in the keratinized mucosa around teeth and osseointegrated implants. *Clinical Oral Implants Research* **4**: 177-186.
Poole, B., Ohkuma, S. & Warburton, M. (1978) Some aspects of the intracellular breakdown of erogenous and endogenous proteins. In: Segal, H.S. & Doyle, D.J., eds. Protein turnover and lysosome function, 1st edition, p. 43. New York: Academic Press.
We recommend the use of a tool such as Reference Manager for reference management and formatting. Reference Manager reference styles can be searched for here:www.refman.com/support/rmstyles.asp

5.4. Tables, Figures and Figure Legends

Tables: Tables should be numbered consecutively with Arabic numerals. Type each table on a separate sheet, with titles making them self-explanatory. Due regard should be given to the proportions of the printed page.

Figures: All figures should clarify the text and their number should be kept to a minimum. Details must be large enough to retain their clarity after reduction in size. Illustrations should preferably fill a single-column width (81 mm) after reduction, although in exceptional cases 120mm (double-column) and 168 mm (full page) widths will be accepted. Micrographs should be designed to be reproduced without reduction, and they should be dressed directly on the micrograph with a linear size scale, arrows, and other designators as needed. Each figure should have a legend

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use

pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley-Blackwell's guidelines for figures:http://authorservices.wiley.com/bauthor/illustration.asp

Check your electronic artwork before submitting it:http://authorservices.wiley.com/bauthor/eachecklist.asp

Permissions: If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

6. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

6.1 Proof Corrections

The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site:www.adobe.com/products/acrobat/readstep2.html . This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs. Proofs must be returned to the Production Editor within three days of receipt.

Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.
Articles should not normally exceed 10 printed pages, including illustrations and references. Additional pages will be charged to the author(s) at the rate of USD 160 per page.

6.2 Early View (Publication Prior to Print)

Clinical Oral Implants Research is covered by Wiley-Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated.

Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

6.3 Author Services

Online production tracking is available for your article through Wiley-Blackwell's Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The author will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.