Longitudinal study on the effect of keratinized mucosal augmentation surrounding dental implants in preventing peri-implant bone loss Takeshi Kikuchi¹, Masahrio Wada², Tomoaki Mameno², Daisuke Hasegawa², Giovanni Serino³, Kazunori Ikebe² ¹ Department of Periodontology, School of Dentistry, Aichi Gakuin University, Nagoya city, Aichi, Japan ² Osaka University Graduate School of Dentistry, Department of Prosthodontics, Gerodontology and Oral Rehabilitation, Suita, Osaka, Japan ³ Department of Periodontology, Södra Älvsborg Hospital, Brämhultsvägen, Borås, Sweden. Corresponding Author: Masahrio Wada² Department of Prosthodontics, Gerodontology and Oral Rehabilitation, Osaka University Graduate School of Dentistry 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan Email address: masahiro@dent.osaka-u.ac.jp

39 40 **Background** Dental implant therapy is a well-established method of prosthetic rehabilitation of missing 41 42 teeth. To maintain the health of the surrounding tissue, management of risk factors/indicators and daily maintenance are important. It still remains controversial whether a certain amount of 43 44 keratinized mucosal width is essential for maintaining the health of peri-implant tissue. 45 The purpose of this multicenter retrospective study was to assess the correlation between bone loss around dental implant and the amount of keratinized tissue width. 46 47 Methods A total of 1644 implants were evaluated. Data was collected about participants' general and 48 49 dental history, as well as implant details. Bone resorption around implant was calculated from 50 intra-oral radiographs taken after 1 year and more than 3 years of function. Implants were 51 classified into three groups; received free gingival graft or apically repositioned flap surgery for 52 increasing the keratinized mucosa ≥ 2 mm width (group A), keratinized mucosa width ≥ 2 mm 53 (group B), and keratinized mucosa width < 2 mm (group C). These data were analyzed by propensity score analysis and a generalized linear regression analysis was performed to compare 54 55 the bone resorption among groups. 56 Results 57 Mean functional time was 55.8 months (SD = 20.5) in group A, 67.6 months (SD = 28.1) in 58 group B, and 74.5 months (SD = 32.9) in group C. Mean bone resorption of group A, B, and C were 0.08 mm (SD = 0.40), 0.18 mm (SD = 0.66), and 0.44 mm (SD = 0.40). Groups A and B 59 60 had significantly lower bone resorption than group C. 61 Conclusion 62 The results in this study show the importance of keratinized mucosa in maintaining the periimplant bone. Our findings also suggest that mucosal transplantation is useful, as opposed to 63 64 narrowing of the keratinized mucosa. 65 66 67

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68 69 70 Abstract

71 Introduction 72 Dental implant therapy is a well-established method to rehabilitate missing teeth and is highly 73 beneficial for the recovery of masticatory function (Esposito et al., 2015). However, peri-implant 74 diseases, a complication, occur at a constant rate. Peri-implant diseases are divided to two 75 pathological situations. Peri-implant mucositis is defined as the presence of a plaque-related 76 inflammatory soft tissue infiltrate with-out concurrent loss of peri-implant bone tissue, while 77 peri-implantitis demonstrate inflammation in combination with bone loss. Peri-implant mucositis 78 is reversible, meanwhile peri-implantitis is irreversible. Therefore, importance of prevention of 79 peri-implantitis was high-lighted, as mucositis was found to be potentially progressing into peri-80 implantitis. To maintain the health of the surrounding tissue, management of risk 81 factors/indicators and daily maintenance are important. In a retrospective longitudinal study, 82 poor oral hygiene, loss of occlusal support, maxillary implant site, cement-supported 83 superstructure, and width of keratinized mucosa were risk indicators for peri-implantitis 84 (Mameno et al., 2020). Many studies have suggested a prominent association between 85 periodontitis-related factors such as a history of periodontitis and poor oral hygiene and peri-86 implantitis (Renvert & Quirynen., 2015; Schwarz et al., 2018). However, just as peri-implant tissue and the periodontium are deceptively similar, peri-implantitis and periodontitis have 87 88 different basic properties while having common characteristics (Kotsakis & Olmedo., 2021). 89 It is commonly believed that width of keratinized and attached gingival width is not important 90 in the progression of periodontal disease if good oral hygiene is maintained (Kennedy et al., 91 1985). Many studies have focused on the significance of keratinized mucosa surrounding dental 92 implants for peri-implant health (Wennstrom & Derks., 2012; Lin, Chan & Wang., 2013; Thoma, 93 Muhlemann & Jung., 2014). The authors also had previously reported a link between peri-94 implantitis and width of keratinized mucosa (Wada et al., 2019; Mameno et al., 2020). A 95 systematic review found that an appropriate amount of keratinized mucosa is required to maintain the health of the tissue surrounding the implant (Pranskunas et al., 2016). 96 97 It has been reported that an insufficient amount of keratinized mucosa, up to 2-mm, tends to 98 cause discomfort during brushing, plaque accumulation, and inflammation of the tissue 99 surrounding the implant (Souza et al., 2016). The width of the keratinized mucosa on the buccal 100 side of the implant is generally about 1 mm shorter than the width on the palatal/lingual side of

the tooth (Chang et al., 1999; Chang & Wennstrom., 2013; Parpaiola et al., 2015). In contrast, it

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104 about 1 mm less in thickness than the gingiva (Chang et al., 1999). Considering the 105 aforementioned reports and some contradictory findings (Kim et al., 2009; Schrott et al., 2009), 106 it remains controversial whether a certain amount of keratinized mucosal width is essential for 107 maintaining the health of peri-implant tissue. 108 To clarify the risk factors of bone resorption around a dental implant, appropriate statistical 109 analysis is crucial. Propensity score (PS) analysis is a useful statistical technique to adjust for 110 confounding factors. However, few studies have investigated the necessity of keratinized mucosa using PS analysis. 111 112 The purpose of this multicenter retrospective study was to clarify the correlation between the 113 presence of keratinized mucosa and peri-implant bone resorption and the clinical efficacy of 114 augmentation techniques used to increase the keratinized mucosal width around dental implants 115 by PS analysis. 116 117 **Materials & Methods** 118 Study participants 119 The records of patients who underwent dental implant therapy between November 1996 and 120 February 2015 at two university dental hospitals (Osaka University Dental Hospital, Osaka, 121 Japan and Aichi Gakuin University Dental Hospital, Aichi, Japan) and six dental offices, which 122 also situated in Japan were analyzed. The inclusion criteria were: (1) at least one rough-surface 123 titanium implant with fixed prosthesis in function for over 4 years and availability of intraoral 124 radiographs taken at 1 year follow-up after prosthesis delivery. The exclusion criteria were: (1) 125 absence of the regular maintenance programs, (2) history of radiotherapy to the head/neck area, 126 and (3) presence of uncontrolled systemic diseases. All participants provided informed consent 127 after understanding the purpose of the study. Before implant therapy, all participants received 128 initial periodontal treatment and smoking cessation guidance, if necessary. 129 This study was approved by the Osaka University Graduate School of Dentistry Ethics 130 Committee (H28-E24). All clinical investigations were conducted according to the principles of 131 the Helsinki Declaration. This study also followed the Strengthening the Reporting of

Observational Studies in Epidemiology (STROBE) guidelines.

has been reported that the thickness of the buccal keratinized mucosa surrounding the implant is

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134 Demographic data collection 135 Data on age, sex, smoking habits (defined as smoking more than one cigarette per day), alcohol 136 consumption habits (defined by daily intake), and systemic diseases were obtained as systemic 137 factors. As intraoral factors, history and presence of periodontitis, oral hygiene status (plaque 138 control record [PCR]; O'Leary score), number of occlusal support (Eichner index; A1-3, B1-4, 139 and C1-3), existence of bruxism, and gonial-angle on the orthopantomogram (index of occlusal force) were collected (Miwa et al., 2019). The definition of periodontitis is currently followed a 140 141 new classification, which based on a multi-dimensional staging and grading categories (Papapanou et al., 2018). However, the data of this study was collected retrospectively, therefore, 142 143 definition of periodontitis in this study referred to the previous report (Derks et al., 2016). 144 According to this report, history of periodontitis was defined as recorded presence of bleeding on 145 probing or suppuration, attachment loss ≥ 2 mm, and pocket probing depth ≥ 6 mm on more than two teeth, and presence of periodontitis was defined as bleeding on probing or suppuration 146 147 attachment loss ≥ 2 mm, and pocket probing depth ≥ 6 mm on more than two teeth at the followup examination, according to a previous report of periodontitis definition. Based on these data, 148 149 the participants were divided into two groups by using a cut-off value of 20% PCR score, and 150 Eichner A1-3, B1-2, B3-4 (having at least one occlusal contact), or C1-3 (no occlusal contact). 151 Bruxism was diagnosed if the following signs were present: subjective symptoms of tooth 152 grinding or clenching, abnormal tooth wear, and transient pain or fatigue of the masseter muscle. 153 Implant data collection 154 Functional time, implant length and diameter, arch (maxillary or mandibular implant), implant 155 site (anterior or posterior, distal to the canine tooth was defined as posterior), surgical procedure 156 (one-stage or two-stage), with/without bone augmentation (guided bone regeneration [GBR], 157 sinus lift, and socket lift), with or without free gingival graft (FGG) or apically repositioned flap 158 surgery (APF), connection type (external or internal), fixation method (cement-retained or 159 screw-retained), and keratinized mucosa width (KMW). KMW was defined as the minimum 160 distance between the gingival margin and the mucogingival junction surrounding the implant. 161 APF was judged necessary when the KMW was between 1 mm and 2 mm, and FGG was judged 162 necessary when the KMW was less than 1 mm. In practice, APF and FGG were performed with

the consent of the participants. Based on this, implants were categorized as follows: received 163 164 FGG or APF (group A), KMW ≥ 2 mm (group B), and KMW < 2 mm (group C). 165 166 Radiographic evaluation 167 In this study, bone resorption around each implant was evaluated by intraoral radiography. A 168 single blinded examiner (MW) analyzed the bone level using image analysis software (ImageJ 1.49v; Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The measurement 169 170 method was as follows: the vertical distance from the implant apex to the bone crest in contact 171 with the implant was measured on the mesial and distal to the implant. The actual implant length 172 was then used to calibrate the vertical distance. This measurement was performed at baseline (1 173 year after prosthesis delivery) and at follow-up (over 3 years from baseline). The marginal bone 174 level change (MBLC) was calculated by the difference in the distance between baseline and 175 follow-up. If there was a difference between mesial and distal MBLC, a larger MBLC was used 176 for analysis. As bone resorption within the first year of implant function is often relatively high 177 because of bone remodeling, the baseline was set to 1 year after delivery of the prosthesis, which 178 is considered to be the period of completion of physiological bone resorption. It should be noted 179 that the intra-class correlation coefficient for the MBLC measurement was 0.97, which indicated 180 almost perfect concordance. 181 182 Statistical Analysis 183 The mean and standard deviation (SD) for continuous variables and percentage for categorical 184 variables are presented as descriptive statistics. Initially, the comparison analyses for each 185 variable were performed by Chi square test and Kruskal-Wallis test. Inverse probability of 186 treatment weighting (IPTW) was used to address the large differences in sample size and 187 baseline characteristics of the study population. In the IPTW method, weights are assigned to 188 implant sites based on the inverse of their probability of receiving a keratinized mucosal 189 augmentation procedure, as estimated by the propensity score (PS) analysis. The score was 190 calculated based on a multinomial logistic regression model that estimated treatment allocation 191 to the three groups, along with background factors associated with bone resorption (age, sex, 192 follow-up period, history of periodontitis, diabetes mellitus, smoking, PCR) as adjusting 193 variables. IPTW results in a pseudo-population in which implant sites with a high probability of

194 receiving the procedure have a smaller weight and implant sites with a low probability of 195 receiving the procedure have a larger weight. Therefore, the distribution of the measured 196 characteristics of implant sites that are used to calculate the propensity score becomes 197 independent of the treatment assignment. Finally, a generalized linear regression analysis was 198 performed to compare the MBLC among groups A, B, and C in the re-weighted pseudo-199 population. All statistical analyses were performed using R version 3.31 (The R Foundation for 200 Statistical Computing, Vienna, Austria). The level of statistical significance for all analyses was 201 set at 0.05. 202 203 Results A total of 1626 implants (66 in group A, 987 in group B, and 573 in group C) were placed in 204 205 545 patients with a mean age of 57.5 years (Figure 1). In group A, 18 implants (21.4%) with less 206 than 2 mm KMW at follow-up were determined to be unsuccessful and excluded from the 207 analysis. The description of all variables according to each group is presented in Table 1. Mean 208 functional time was 55.8 months (SD = 20.5) in group A, 67.6 months (SD = 28.1) in group B, 209 and 74.5 months (SD = 32.9) in group C. Mean MBLC of group A, B, and C were 0.08 mm (SD = 0.40), 0.18 mm (SD = 0.66), and 0.44 mm (SD = 0.40), respectively. Descriptive analysis 210 211 showed large variations in baseline characteristics among groups A, B, and C. Demographic characteristics known to be risk factors for the development of peri-implantitis 212 213 (age, sex, follow-up period, history of periodontitis, diabetes, smoking, plaque control record, 214 and clinician-associated factors) were used for PS analysis, because large differences were 215 adjusted for in each group. In the final analysis, unstandardized regression coefficients (B), 95% 216 confidence intervals (CI), and P-values were estimated with reference to group C. PS analysis 217 showed that both groups A (B = -0.37, 95% CI: -0.49 to -0.24, P < 0.01) and B (B = -0.21, 95% 218 CI: -0.30, -0.12, P < 0.01) had significantly lower MBLC than group C (Table 2). 219 220 Discussion 221 Prevention and control of peri-implant disease is crucial in implant therapy. Many factors, such 222 as oral hygiene, periodontal disease, smoking, systemic diseases, implant surface texture, implant 223 site, and prosthesis design, have been proposed as risk factors for peri-implant disease 224 (Pjetursson et al., 2012; Marrone et al., 2013; Aguirre-Zorzano et al., 2015; Daubert et al., 2015;

Gurgel et al., 2017; Staubli et al., 2017). In this study, we clarified the correlation between the

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229 keratinized mucosa transplantation by PS analysis, which could adjust the difference of related 230 variables. In a cross-sectional study, the lack of keratinized mucosa around the implant was 231 significantly associated with increased plaque accumulation, mucosal recession, interproximal 232 bone resorption greater than 3 mm, and peri-implantitis (Kungsadalpipob et al., 2020) while 233 another cross-sectional study suggested that a thin gingival phenotype and inadequate KMW (<2 234 mm) could be strong risk indicators of peri-implant disease and pain/discomfort during brushing 235 (Gharpure et al., 2021). In particular, a meta-analysis identified that sites with a wider range of 236 keratinized mucosa had a statistically significant advantage in terms of plaque score, modified 237 gingival index, mucosal recession, and attachment loss (Wennstrom & Derks, 2012). A 10-year 238 cross-sectional study after implant placement showed that a lack of keratinized mucosa was 239 associated with higher plaque score, even in patients with adequate oral hygiene (Roccuzzo, 240 Grasso & Dalmasso., 2016). 241 Many studies suggest that lack or narrowing of keratinized mucosa may adversely affect oral 242 hygiene; however, there is limited evidence that this factor increases the risk of peri-implantitis, 243 necessitating further research in the future. Conversely, another cross-sectional study 244 demonstrated that soft tissue transplantation with implant placement using CTG reduced 245 bleeding on probing and pocket depth and incidence of peri-implant mucositis and peri-246 implantitis and had a beneficial effect in maintaining peri-implant health (Obreja et al., 2021). A 247 systematic review showed that soft tissue transplantation provides better peri-implant 248 environment (Thoma et al., 2018). Specifically, the acquisition of keratinized mucosa using 249 autologous grafts greatly decreased the bleeding index, maintained marginal bone height, and 250 increased mucosal thickness using autologous grafts significantly decreased marginal bone loss. 251 In contrast, excessive thickness of vertical soft tissue surrounding the implant in patients with a 252 history of periodontitis was reported to adversely affect the health of implant tissue (Zhang et al., 253 2020). Not surprisingly, periodontopathic bacteria have a high risk of colonizing deep pockets 254 around implants, increasing the likelihood of peri-implant disease. Whether maintenance of soft 255 tissue transplant thickness around implants is important requires further investigation. 256 This study had some limitations. First, this was a retrospective study, and therefore, various 257 biases could not be completely eliminated. In the future the risk factors should be confirmed by 258 conducting prospective studies on diseases with a relatively high incidence, such as peri-implant

amount of keratinized mucosa and the bone loss around dental implants and the effectiveness of

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- diseases. Second, this study did not investigate local plaque deposition. As mentioned above, it is
- 261 important to investigate these factors as the existence of the keratinized mucosa is suggested to
- 262 have an effect on oral hygiene.

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264 Conclusions

- The results in this study show the importance of keratinized mucosa in maintaining the peri-
- 266 implant bone. Our findings also suggest that mucosal transplantation is useful, as opposed to
- 267 narrowing of the keratinized mucosa.

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Comparing the bone loss among 3 groups.

419	B: unstandardized regression coefficients, CI: confidence interval.	