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Comparative Cytological and Histopathological Study of Peri-Implantitis and Periodontitis

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Abstract

Peri-implant diseases, such as peri-implant mucositis and peri-implantitis, pose significant challenges to the long-term prognosis of dental implants. This study aimed to comprehensively compare peri-implantitis with periodontitis from cytological and histopathological perspectives, shedding light on the morphological characteristics associated with peri-implantitis. Thirteen patients, including six with peri-implantitis and seven with periodontitis, were included in the study. Cytological examination of affected gingival mucosa revealed distinct differences between the two conditions. Peri-implantitis exhibited an inflammatory background predominantly composed of neutrophils with lobulated nuclei, accompanied by stratified squamous epithelial cells showing signs of keratinization. In contrast, periodontitis showed a similar neutrophilic inflammatory background but with non-keratinized epithelial cells. Histopathological examination further confirmed these differences, with peri-implantitis showing keratinized epithelium in the inner epithelial layer. This histological finding aligns with the notion that peri-implantitis has a distinct mucosal profile compared to periodontitis. Additionally, cytological analysis revealed that peri-implantitis had a lower occurrence rate of Light green-positive cells, indicating a tendency toward keratinization. This finding suggests that the presence of keratinized mucosa might be associated with peri-implant health, although further research is needed to clarify this relationship. Overall, this study demonstrates the potential of cytological examination and Papanicolaou staining for assessing mucosal inflammatory conditions and distinguishing between keratinized and non-keratinized cells. These findings underscore the utility of oral mucosal smears as a valuable tool for diagnosing peri-implantitis and enhancing our understanding of its pathogenesis.

Keywords

Peri-Implant Disease, Peri-Implantitis, Oral Cytology

1. Introduction

Peri-implant diseases are among the prognostic factors for dental implants [1] [2], of which peri-implant mucositis and peri-implantitis are representative [1]. Peri-implant mucositis is a localized lesion of the mucosa surrounding the implant [1]. On the other hand, peri-implantitis encompasses pathological changes not only within the mucosa, similar to peri-implant mucositis, but also involving the bone surrounding the implant [1]. These conditions bear resemblance to gingivitis and periodontitis.

One of the causes of peri-implant diseases is believed to be bacterial plaque; however, consensus on the composition of the bacterial biofilm remains unsettled [3] [4]. In addition, the progression from peri-implant mucositis to peri-implantitis exhibits similarities to the pathogenesis of periodontal disease. Therefore, investigating the pathogenesis of peri-implantitis is significant in comparison with periodontitis.

Periodontal disease stands as a primary cause of tooth loss. In Japan, according to the 2022 Survey of Dental Diseases' prevalence, 47.9% of individuals aged 15 years and older were reported to have periodontal pockets measuring 4 mm or more, indicating a high prevalence of periodontal disease [5]. On the other hand, the patient-level prevalence of peri-implantitis in Japan is reported to be 9.7%, a lower incidence than periodontal disease [6]. However, internationally, the prevalence of peri-implant diseases is reported to be 80% for peri-implant mucositis and 28% - 56% for peri-implantitis [1]. Thus, considering the long-term stability of implants, peri-implant diseases emerge as significant conditions that cannot be ignored [1].

Titanium, the primary component of implant fixtures, is known for its biocompatibility. However, it has been reported to be susceptible to attachment by periodontal pathogens, with characteristics that do not hinder bacterial proliferation [7] [8]. Consequently, in cases where a proper oral environment cannot be maintained, peri-implantitis can ensue. For achieving prolonged implant stability, early detection of peri-implant diseases is paramount.

Peri-implant diseases have histological characteristics similar to advanced gingival lesions, with a higher presence of inflammatory cells compared to healthy tissue, as reported [9]. On the other hand, periodontal diseases are detectable through cell examination and their stages can be determined [10]. Therefore, oral exfoliative cytology has the potential to screen for peri-implant diseases.

Oral cytology, a cost-effective, minimally invasive, and convenient test, is commonly used in the dental field for screening oral mucosal diseases [11]. As

for the application of cytology in relation to implants, reports suggest its potential use in detecting metal particles released from metal implants [12] [13] [14]. However, limited attention has been given to the feasibility of cytology for screening peri-implantitis [15]. Thus, if the morphological features required for peri-implantitis screening through oral cytology can be elucidated, clinical benefits through early detection can be anticipated.

The aim of this study was to comprehensively compare peri-implantitis and periodontitis from both cytological and histopathological perspectives, thereby elucidating some of the morphological characteristics associated with peri-implantitis.

2. Materials & Methods

2.1. Participants

The participants were 13 patients who visited the Irie Dental Clinic between July 2021 and November 2022, and who were adequately informed about the study and provided their consent. Cases in which a biopsy of the inflamed region was conducted after cyto-logical examination were included. There were 6 cases of peri-implantitis (mean age ± standard deviation: 78.3 ± 5.0 years, male:female = 1:5) and 7 cases of periodontitis (mean age ± standard deviation: 74.7 ± 7.3 years, male:female = 3:4).

In this study, the diagnostic criteria were established as follows.

2.2. Peri-Implantitis

The diagnostic criteria for peri-implant disease were based on the sixth European Workshop on Periodontology (EWP) [1], the American Academy of Periodontology, and the eighth EWP (**Table 1**) [16] [17].

2.3. Periodontitis

Adhering to the criteria established by Maurizio *et al.* [18], individuals were classified based on the following: 1) Presence of probing depths involving two or more non-adjacent teeth in the interdental area; 2) Presence of clinical attachment levels (CALs) of 3 mm or greater on the buccal or oral aspect with pocket depths greater than 3 mm involving two or more teeth. Moreover, CALs must not be attributed to any of the following factors other than periodontal disease: 1) Gingival recession due to trauma; 2) Extensive caries spreading to the cervical region; 3) CAL present on the distal surface of second molars and associated with the positional anomaly or extraction of third molars; 4) Marginal periodontal tissues serving as discharge routes for periapical lesions; 5) Presence of vertical root fractures (**Table 1**).

In addition, in this study, individuals meeting any of the following conditions were excluded from the study: ongoing bisphosphonate therapy; prior history of radiation therapy after implant placement; or less than one year since attachment of the implant-supported prosthesis.

Table 1. Diagnostic criteria in this study.

	Periodontitis	Peri-implantitis
Inclusion Criteria	1. Interdental CAL is detectable at ≥2 non-adjacent teeth. 2. Buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth.	1. BOP with or without pus. 2. Radiographic changes in the bone level compared to baseline.
Exclusion Criteria (If any of the criteria are satisfied)	1. Gingival recession of traumatic origin. 2. Dental caries extending in the cervical area of the tooth. 3. The presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar. 4. An endodontic lesion draining through the marginal periodontium. 5. The occurrence of a vertical root fracture.	1. Taking bisphosphonate medication. 2. History of radiation therapy following implant placement. 3. Less than one year since placement of implant-supported prosthetic device.
Reference	Tonetti, M.S. *et al.* [18] https://doi.org/10.1002/JPER.18-0006	Lindhe, J. *et al.* [1] https://doi.org/10.1111/j.1600-051X.2008.01283.x Sanz, M. *et al.* [16] https://doi.org/10.1111/j.1600-051X.2011.01837.x Rosen, P. *et al.* [17] https://doi.org/10.1902/jop.2013.134001

2.4. Cytological Examination

In both the peri-implantitis and periodontitis groups, oral exfoliative cytology was performed to collect cytological samples from the affected gingival mucosa. The collected samples were processed following standard Papanicolaou protocols. All cytological observations were per-formed by two cytologists and three oral pathologists. For semi-quantitative evaluation, cellular images were captured using using a 20× objective lens.

For Orange G-positive cells (Orange G-positive non-nucleated cells, Orange G-positive nucleated cells), Eosin Y-positive cells, and Light green-positive cells, the appearance rates per field were calculated for each stain (appearance rate per field = number of specific cells/total number of cells), and the average value across 10 fields was considered the value for that particular case.

2.5. Histopathological Examination

For histopathological examination, formalin-fixed paraffin-embedded blocks were used, which were prepared during tissue processing for histopathological analysis from samples obtained during implant removal or periodontal surgical procedures. Ten cases of fibrous epulis diagnosed at the Department of Diagnostic Pathology of Nihon University Hospital at Matsudo, were selected as controls. The formalin-fixed paraffin-embedded blocks were sectioned to a thickness of 4 μm using a microtome. Deparaffinization was per-formed using a xylene-alcohol series, followed by Papanicolaou staining. Dehydration and clearing were carried out using an alcohol-xylene series, followed by embedding in Marilon. Papanicolaou-stained slides were prepared as permanent samples and observed under an optical microscope for histopathological examination. All histopathological examinations were performed by three oral pathologists.

2.6. Compliance with Ethical Standards

This study was conducted with approval from the Institutional Review Board of our university (EC21-008A).

2.7. Statistical Analysis

The difference in appearance rates per field between periodontitis and peri-implantitis was tested using R version 4.3.1 (R Development Core Team) and the lawstat version 3.6 package. Normality of the data of each group was assessed using the Shapiro-Wilk test, and equality of variances between the two groups was assessed using the F test. If normality and equality of variances were confirmed for both groups, Student's t-test was performed. If normality could not be confirmed for either of the two groups, the Brunner-Munzel test was conducted. The significance level for all tests was set at $p < 0.05$.

3. Results

3.1. Cytological Findings

In peri-implantitis and periodontitis, an inflammatory background predominantly composed of neutrophils with lobulated nuclei was observed (Figure 1). In peri-implantitis, keratinized stratified squamous epithelial cells were observed, while in periodontitis, non-keratinized stratified squamous epithelial cells were identified (Figure 1). Both types of epithelial cells displayed signs of inflammatory changes such as nuclear enlargement and perinuclear halos (Figure 1(b) and Figure 1(d)).

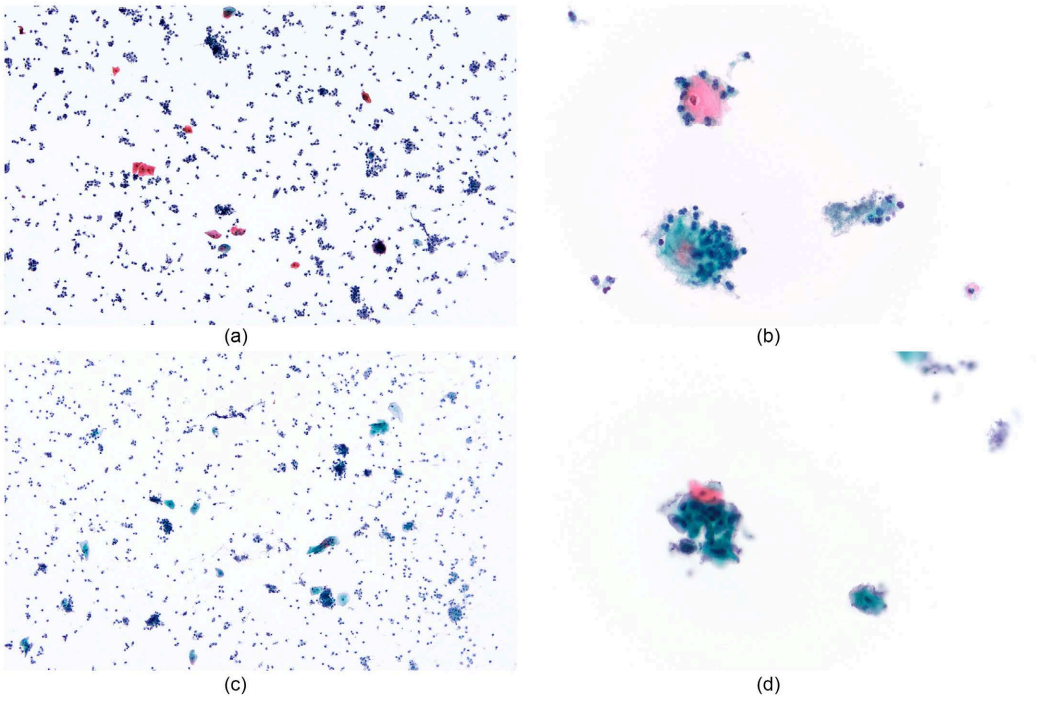


Figure 1. Cytological findings. (a) Peri-implantitis ×100; (b) Peri-implantitis ×400; (c) Periodontitis ×100; (d) Periodontitis ×400.

3.2. Cellular Occurrence Rate on Cytology

The median (interquartile range) of the occurrence rates for each analyzed cell type are presented in Table 2. For Orange G-positive cells, the occurrence rates were as follows: peri-implantitis 0.503 (0.340 - 0.620), periodontitis 0.311 (0.115 - 0.477); for non-nucleated Orange G-positive cells: peri-implantitis 0.103 (0.062 - 0.151), periodontitis 0.065 (0.033 - 0.117); for nucleated Orange G-positive cells: peri-implantitis 0.303 (0.200 - 0.521), periodontitis 0.189 (0.082 - 0.275); for Eosin Y-positive cells: peri-implantitis 0.365 (0.193 - 0.617), periodontitis 0.167 (0.073 - 0.284); for Light green-positive cells: peri-implantitis 0.075 (0.036 - 0.201), periodontitis 0.485 (0.363 - 0.539).

The statistical analysis showed a significant difference between the two groups in the occurrence rate of Light green-positive cells ($p < 0.01$), as shown in Table 2.

3.3. Papanicolaou Staining Histopathological Findings

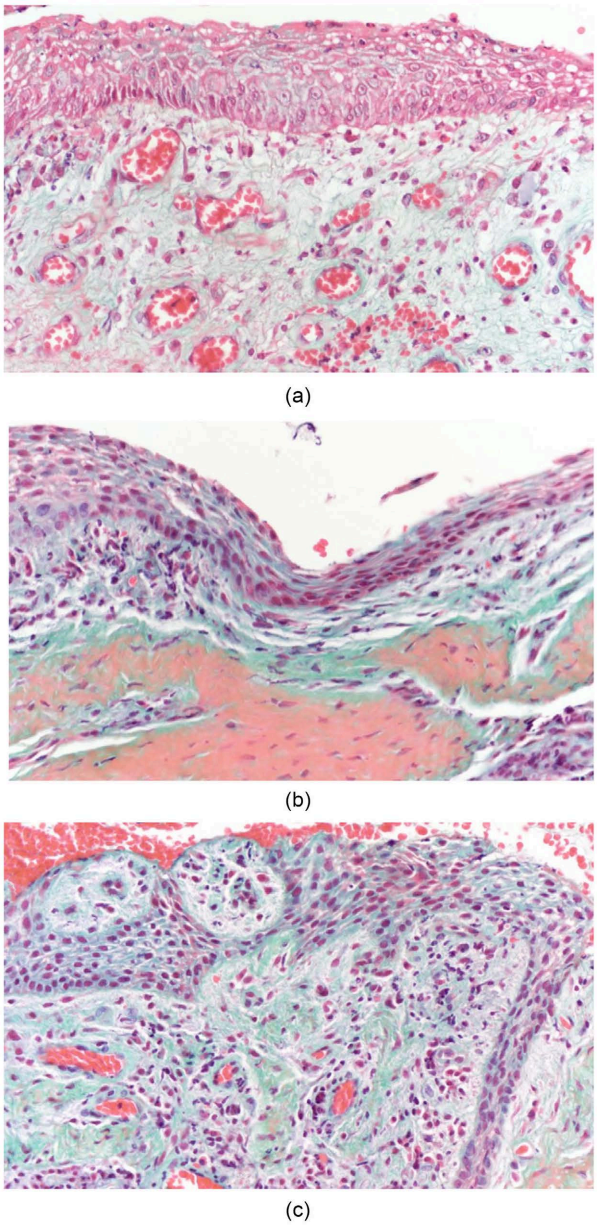
In peri-implantitis, the inner epithelium was covered by non-keratinized stratified squamous epithelium with neutrophil infiltration, showing cells with Light green positivity scattered throughout all layers of the epithelium (Figure 2(a)).

In periodontitis, the inner epithelium was covered by non-keratinized stratified squamous epithelium, with cells displaying Light green positivity observed throughout all layers of the epithelium (Figure 2(b)).

Table 2. Observed cell rate and statistical results.

	M (IQR)	Shapiro-Wilk test (*p* value)	F-test (*p* value)	Statistical method	*p* value
Orange G-positive cells					
Peri-implantitis	0.503 (0.340 - 0.620)	0.715	0.738	Student's t-test	0.168
Periodontitis	0.311 (0.115 - 0.477)	0.530			
Orange G-positive non-nuvleated cells					
Peri-implantitis	0.103 (0.062 - 0.151)	0.069	0.510	Brunner-Munzel test	0.417
Periodontitis	0.065 (0.033 - 0.117)	0.046			
Orange G-positive nucleated cells					
Peri-implantitis	0.303 (0.200 - 0.521)	0.529	0.556	Student's t-test	0.230
Periodontitis	0.189 (0.082 - 0.275)	0.645			
Eosin Y-positive cells					
Peri-implantitis	0.365 (0.193 - 0.617)	0.596	0.421	Student's t-test	0.196
Periodontitis	0.167 (0.073 - 0.284)	0.076			
Light green-positive cells					
Peri-implantitis	0.075 (0.036 - 0.201)	0.099	0.254	Student's t-test	0.002
Periodontitis	0.485 (0.363 - 0.539)	0.549			

M: median, IQR: interquartile range.



(a)

(b)

(c)

Figure 2. Histopathological findings. (a) The inner epithelium in Peri-implantitis. (b) The inner epithelium in periodontitis. (c)The inner epithelium in health mucosa.

In healthy mucosal tissue of the control group, the inner epithelium was covered by non-keratinized stratified squamous epithelium with irregular extensions of the epithelial pegs, and Light green-positive cells were observed throughout all layers of the epithelium (**Figure 2(c)**).

4. Discussion

Though certain aspects of peri-implantitis remain unclear, its pathogenesis is considered to be similar to that of periodontitis [19]. One of the contributing factors is believed to be pathogenic bacteria [4] [19]. Epithelial tissues cover the

human body's surfaces as a defense mechanism against foreign entities such as pathogenic bacteria. The barrier function against conditions like peri-implantitis and periodontitis is provided by oral mucosal epithelium. Therefore, scrutiny of oral mucosal epithelium holds the potential to enhance our understanding of the precise nature of peri-implantitis. Cytological examination, which can be performed with low invasiveness, has been proven to be valuable in assessing oral mucosal epithelium [20]. In addition, the use of Papanicolaou staining for tissue sections allows for improved differentiation between keratinized and non-keratinized cells, thereby enhancing the contrast between cellular and tissue images [21].

Both peri-implantitis and periodontitis showed cellular images predominantly characterized by neutrophil-driven inflammatory backgrounds. This observation suggests that the act of sample collection elicited local mucosal inflammatory responses. The presence of neutrophils as the predominant inflammatory cells in periodontitis, as reported, suggests that peri-implantitis shares cytological similarities with periodontal diseases [10]. Furthermore, in peri-implantitis, the scarcity of Light green-positive cells and the prominence of Orange G or Eosin Y-positive cells indicate a tendency towards keratinization in the epithelial cells of the peri-implant mucosa. The relationship between the maintenance of peri-implant health and keratinized mucosa has been suggested, though there are still uncertainties histologically [22].

Subsequently, when comparing the occurrence rates of various cell types in cytology, it was found that Light green-positive cells were less frequent in peri-implantitis. When observing stratified squamous epithelial cells with Papanicolaou staining, keratinized cells show Orange G or Eosin Y positivity, whereas non-keratinized cells show Light green positivity [20]. According to the literature, chronic periodontitis is reported to have a similar occurrence rate of stratified squamous epithelial cells as in clinically healthy group [10]. Therefore, the statistically significant decrease in non-keratinized cells can be considered a characteristic cytological finding in peri-implantitis.

In the Papanicolaou-stained tissue images, observations were made of inner epithelium. The inner epithelium showed that, though non-keratinized stratified squamous epithelium was predominant in periodontitis and healthy tissues, peri-implantitis was characterized by keratinized stratified squamous epithelium. Regarding the histological structure around implants, Abrahamsson *et al.* reported that the outer epithelium is keratinized and continues onto the implant-facing surface as the inner epithelium equivalent [23]. However, the specifics of the inner epithelium equivalent were previously unknown. In the present study, it was shown that peri-implantitis had distinct histopathological features in the region equivalent to the inner epithelium.

5. Conclusion

The demonstrated tendency towards keratinization in the mucosa of peri-

implantitis has shed light on a portion of the histopathological differences between peri-implantitis and gingivitis. Furthermore, the cytological examination of peri-implantitis allows for an assessment of mucosal inflammatory conditions and confirmation of the state of oral mucosal epithelial cells. This shows the utility of oral mucosal smears in detecting peri-implantitis, highlighting their potential as a valuable tool for diagnosis.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary Material

Details of oral exfoliative cytological specimen collection and preparation: Specimens were collected using an interdental brush (APOTEK 0.4 mm, ELVA). The collected samples were fixed in ThinPrep solution (PreservCyt Solution 20 mL, Hologic Japan) for more than 24 hours. Monolayer slides were prepared using the ThinPrep 2000 Processor (Hologic Japan).

Details of the cytological image acquisition equipment: Cytological images were captured using an optical microscope (BX51, Olympus) and a microscope camera system (DP74, Olympus) equipped with image processing software (CellSens, Olympus).

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