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Peri-Implant Supracrestal Tissue Characteristics Related to Abutment Materials: A Comparative Histomorphometry Study

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ABSTRACT

Background: The peri-implant soft tissue integration is critical to maintain peri-implant health and the long-term success of dental implant rehabilitations.

Aim: This comparative histomorphometry study aims to characterize the peri-implant soft tissues (PIST) around experimental abutments made of titanium (Ti), dental resin (Re), and polyetheretherketone (PEEK).

Methods: Thirty bone-level implants were placed, each receiving an experimental transmucosal healing abutment made of one of the three materials. After an 8-week healing period, the abutments and surrounding tissues were harvested and prepared for histological and histomorphometric analyses. Dimensions of sulcus depth, epithelial and connective tissue adhesion were measured. In addition, the abutment surface characteristics, levels of inflammation, plaque accumulation, and peri-implant bone level changes were evaluated.

Results: The dimensions of the different components of the PIST were comparable across the three experimental groups. The mean overall dimensions of the PIST were 2.68 ± 0.51 mm for Ti, 2.66 ± 0.47 mm for Re, and 2.32 ± 0.55 mm for PEEK. Mean sulcus depth was 0.71 ± 0.69 mm for Ti, 0.74 ± 0.50 mm for Re, and 0.68 ± 0.63 mm for PEEK. Mean junctional epithelium was 1.82 ± 0.67 mm for Ti, 1.56 ± 0.47 mm for Re, and 1.53 ± 0.40 mm for PEEK. Mean harvested connective tissue (until abutment platform) was 0.30 ± 0.29 mm for Ti, 0.36 ± 0.38 mm for Re, and 0.09 ± 0.10 mm for PEEK. However, the resin group exhibited significantly more supramucosal biofilm adhesion ($p = 0.026$).

Conclusion: The PIST around abutments made of PEEK, resin, or titanium tend to develop in a similar pattern. However, longer observation periods are required to evaluate the long-term effects.

1 | Introduction

Mucosal integration, the interface between soft tissues and the transmucosal implant components, plays a critical role in maintaining long-term implant health by providing a seal against bacterial colonization [1–3]. The peri-implant supracrestal soft tissues consist of sulcus, junctional epithelium, and connective tissue, with a typical total height of approximately 4 mm [4–6].

Complete soft tissue healing around the transmucosal component is typically achieved within – 8 weeks following implant placement [7–10]. In contrast to natural teeth, where collagen Sharpey's fibers are anchored tightly and perpendicularly to the root surface, the collagen fibers within the implant supracrestal complex are oriented parallel or circumferentially to the implant surface and display a more fragile adhesion. This anatomical arrangement renders the peri-implant soft tissue interface more susceptible to bacterial infiltration and mechanical disruption [3, 11].

Several factors influence the peri-implant soft tissue integration, including the amount of keratinized mucosa, mucosal phenotype, and environmental factors such as smoking [12]. Other features of the transmucosal components, such as design [13–15], surface characteristics [16], and manipulations [17], as well as material [18] were also suggested to influence the peri-implant soft tissue interface [19]. In the era of immediacy, an increasing number of temporary materials for provisional crowns and/or anatomical abutments are used in the transmucosal portion [20–22]. Particularly, resin is often used for provisional crowns, and PEEK (polyetheretherketone) can be used as provisional socket sealing abutments (SSA) [23, 24] or definitive restorations [25]. The use of resin remains controversial due to potential long-term cytotoxicity [26]. However, some authors demonstrated that, in vitro, resin exhibits similar fibroblast attachment and proliferation patterns to titanium [27] as well as comparable fibroblast proliferation rates reported on different types of resin BisGMA-TEGDMA/bioactive glass (BAG) composites [28].

PEEK has been shown to promote higher fibroblast viability, adhesion, and proliferation compared to titanium [29]. Preclinical studies have also observed similar histological peri-implant soft tissue integration between resin-based and titanium abutments [30, 31]. However, in these minipig models, a high proportion (> 95%) of epithelial adhesion and less connective tissue (CT) interface (less than 5%) was found when compared to data reported in other preclinical studies in which 30%–50% of the soft tissue interface was a CT interface [32, 33]. In a preclinical study comparing PEEK to Ti, Rea and coworkers observed comparable PIST dimensions, ranging from 3.2 to 4.2 mm, with a mean CT interface of 1.2 mm and 1.7 mm, respectively, for Ti and PEEK [18]. Nevertheless, the authors suggest that preclinical models might be more biotolerant than humans [31]. On the other side, Caballé-Serrano identified multinucleated giant cells more frequently associated with PEEK abutments than on titanium [34].

In order to identify if this in vitro and preclinical data can be translated into clinical practice, our group recently explored the host-related response to titanium, resin, and PEEK. Compared to the Ti and PEEK groups, Re abutments revealed a higher infiltration of macrophages in the connective tissue ($p = 0.04$) and neutrophils in the adjacent epithelium ($p = 0.03$). In the Re abutments, peri-implant bone remodeling was higher compared to the other groups ($p = 0.01$) [35]. Milinkovic et al. observed greater inflammation with PEEK abutments compared to titanium abutments [36]. In contrast, in the study of Enkling, neutrophil cell counts on PEEK abutments were slightly lower compared to titanium abutments [37]. While the PIST dimension on humans has been explored on titanium abutments [6, 38, 39], to our knowledge, it has never been explored on resin or PEEK.

A recent EAO consensus report emphasized the need for further research into the specific biological interaction between abutment material and the soft tissues [19]. Particularly, a limited number of studies have focused on the biological impact of temporary materials such as resin and PEEK on their surrounding soft tissues.

Thus, the primary aim of this study was to investigate, in humans, the peri-implant supra-crestal soft tissue characteristics and their interface with different transmucosal materials (Ti, Re, and PEEK) with histology. Additionally, peri-implant soft tissue inflammation, plaque accumulation, and radiographic peri-implant bone levels were assessed.

2 | Material and Methods

2.1 | Study Design

The study was approved by the Ethical Committee of the University of Liege, Belgium under the number N°B707201628072; was registered on clinicaltrials.gov under number: NCT05843526; and was performed according to Consolidated Standards of Reporting Trials (CONSORT) guidelines and European Medicines Guidelines for Good Clinical Practice.

This study was designed as an experimental human trial aimed at studying the histological interface between peri-implant soft tissue and three implant abutment materials: grade 5 titanium (Ti), resin (Re) (Optibond FL, Kerr Dental), and polyetheretherketone (PEEK). The primary endpoint was the histometric measurements of the different components of the peri-implant supra crestal soft tissue (sulcus, junctional epithelium and connective interface). The secondary objective was to investigate the presence of biofilm on the transmucosal component and the level of inflammation in the supra crestal peri-implant soft tissue.

Thirty bone-level implants (Institut Straumann AG, Basel, Switzerland) were placed with abutments being randomly allocated to the three materials (Ti, Re, or PEEK). Each patient could receive a maximum of two experimental abutments of different materials. Experimental abutments, together with a biopsy of the surrounding tissues, were removed 8 weeks after implant placement, allowing for PMMA histological analyses. X-rays were performed at baseline and just before

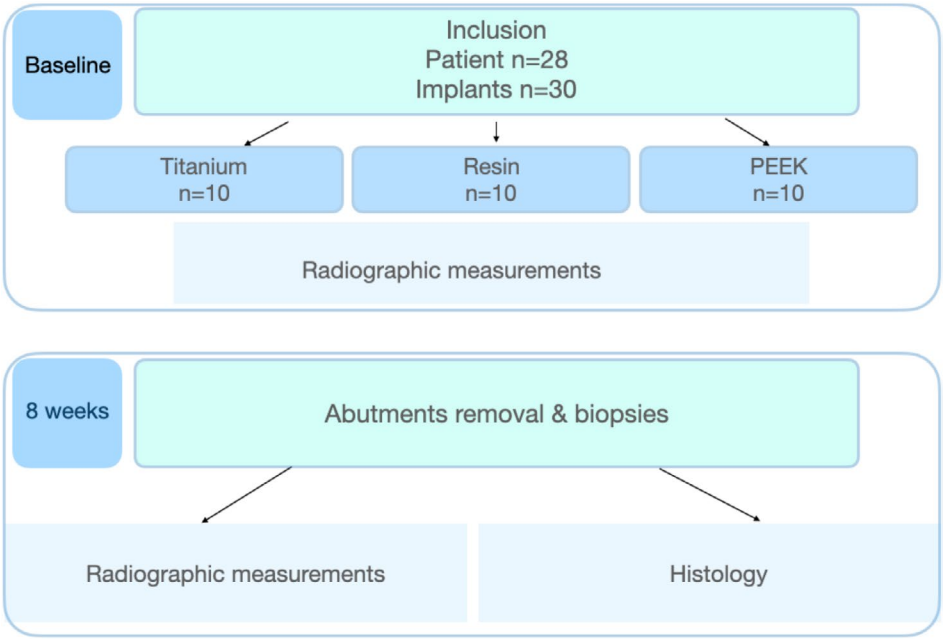

FIGURE 1 | Study design flowchart.

abutment removal. The study design flowchart is represented in Figure 1.

2.2 | Abutment Design and Manufacturing

Experimental CAD-CAM abutments were manufactured by the Proscan company (Zonhoven, Belgium) according to a design validated in a previous study [40]. Abutments were milled in Titanium (grade 5 titanium) and PEEK (polyetheretherketone) blocks (Figure 2). The resin experimental abutments were obtained by sandblasting the Ti abutment and covering it with resin (Optibond FL, Kerr Dental). The anatomical design of the experimental abutments was specifically developed to allow gingival tissue harvesting using a 4.5 mm diameter biopsy punch, resulting in a tissue ring approximately 1 mm in thickness. The horizontal platform diameter of the abutments was 3.8 mm, and the screw channel diameter was 2.7 mm.

2.3 | Abutment Surface Characterization

Surface morphology of the experimental abutments was evaluated on three components of each kind at the Oral Research Laboratory, Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, Norway. Samples were mounted on aluminum stubs with conductive carbon tape and for PEEK and resin coated with gold (Cressington 108 Auto, Cressington Scientific Instruments, Watford, UK) and analyzed with an analytical benchtop scanning electron microscope (TM3030, Hitachi High-Technologies Europe GmbH, Krefeld, Germany). Images were taken with an accelerating voltage of 15 kV. An S Neox optical profiler (Sensofar, Spain) controlled with the SensoSCAN 6.3 software (Sensofar, Spain) was used to perform the profilometry. Samples were imaged with an EPI 50× objective using the confocal mode at six random non-overlapping positions with an area of $350.88 \times 264.19\,\mu m^2$ (1360×1024 px). Surface parameters were obtained from image analysis and processing was done using SensoMap Standard 7.3 (Sensofar, Digital Surf's Mountains Technology, Spain). Topographical assessment of the abutments was performed by optical profilometry. The scanned images were flattened by subtraction, and the cylindrical form of the abutments was corrected before the images were numerically processed and the topographical parameters were obtained. The average surface roughness (S_a), root mean square roughness (S_q), the kurtosis (S_{ku}) equivalent to the sharpness of the roughness profile, the skewness (S_{sk}) representing the asperity, and the developed interfacial area ratio (S_{dr}) associated with the surface texture were measured. In addition, scanning electron microscopy (SEM) (TM3030, Hitachi, Germany) examined the surface morphology. The acceleration voltage was set at 15 kV, and images were taken at 5000 magnification.

2.4 | Inclusion and Exclusion Criteria

All patients scheduled for the placement of one or two implants in the posterior area of the maxilla or the mandible from May 2019 to May 2021 were invited to participate in this study. Patients signed an informed consent and were enrolled if they met the following criteria: patients aged 18 or over, one or more missing teeth in the maxillary or mandible area, seeking implant therapy, in good systemic health (ASA I/II), absence of contraindications for oral surgical interventions, and a full mouth plaque score (FMPI) lower than or equal to 25%.

The tooth at the implant site(s) must have been extracted at least 12 weeks prior to implantation. The bucco-lingual dimension of the keratinized mucosa must be at least 3 mm. The available bone volume must permit the placement of an implant with a diameter of 4.1 mm. Patients were not enrolled in case of any of the following exclusion criteria: autoimmune disease requiring medical treatment, medical conditions requiring prolonged use of steroids, use of bisphosphonates and

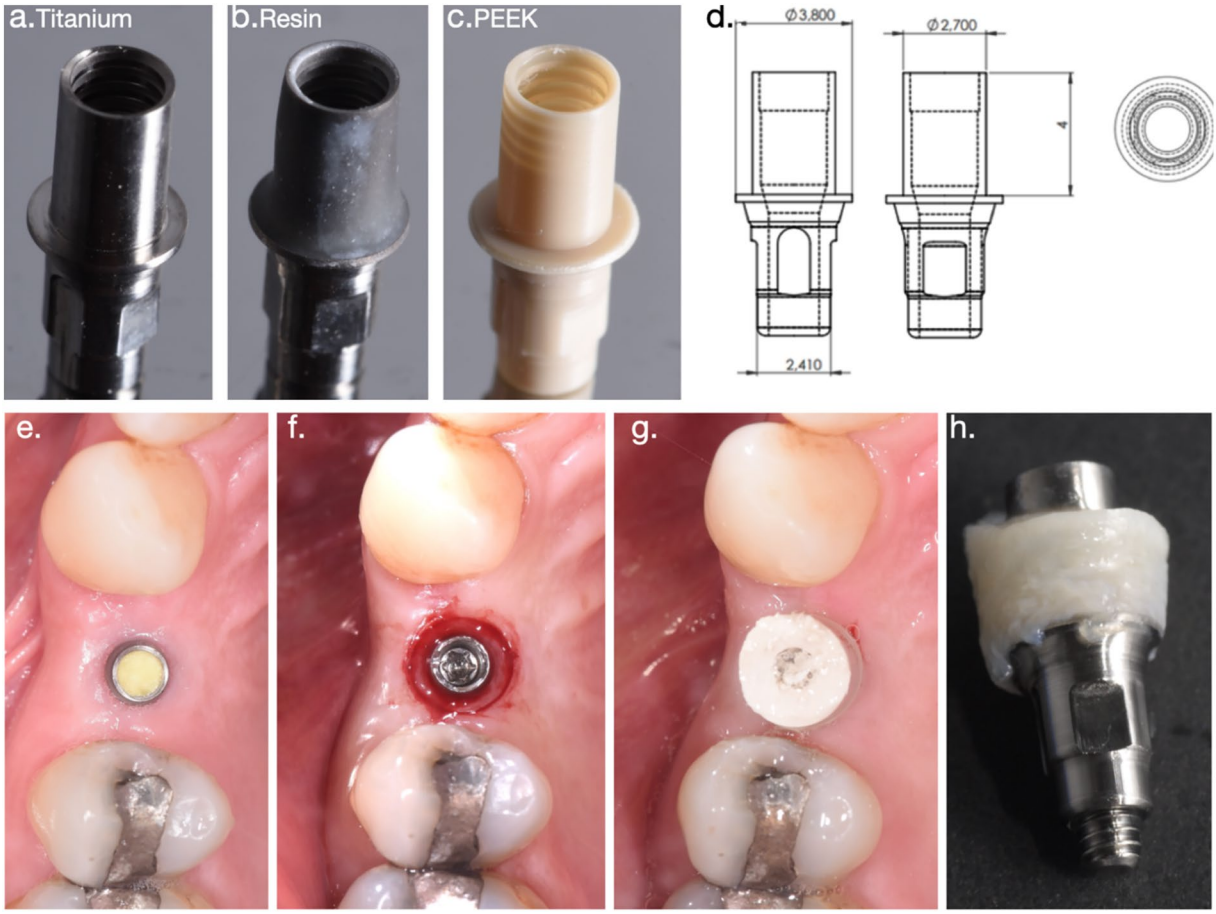

FIGURE 2 | Experimental abutments. titanium (a), resin (b), and PEEK (c), anatomy of the experimental abutment (d), occlusal view after 8 weeks of healing (e), peri-implant mucosal biopsy after the use of punch device (f), screw-retained abutment in place (g), harvested biopsy on experimental abutment (h).

denosumab intravenously or in oral use, current pregnancy or breastfeeding women, alcoholism or chronic drug abuse, immunocompromised patients, uncontrolled diabetes, smokers, implant diameter under 4 mm (narrow implant), or local or systemic infection. The site was excluded if it was previously treated with socket preservation techniques, presented untreated local inflammation, mucosal diseases, or oral lesions, had a history of local irradiation therapy, or had a persistent intraoral infection.

2.5 | Surgical Procedure

After local anesthesia (Articaine, Septanest 40 mg/mL adrenaline 1/100,000), incision for a miniflap and flap elevation was performed in the treatment site. The decision for the flap elevation technique was related to the surgeon's preference and site condition. The implantation procedure was performed according to the protocol provided by the manufacturer. A bone-level implant of 4.1 mm or 4.8 mm in diameter (Bone level SLA, Roxolid, Straumann Group, Basel, Switzerland) was placed one millimeter under bone level with an insertion torque of at least 15 N/cm. An experimental abutment was randomly allocated, placed in a non-submerged approach, and tightened at 10 Ncm. When needed, a simple interrupted suture with resorbable materials was performed directly after abutment placement, and the abutment screw channel was filled with Teflon and closed with composite (Telio, Ivoclar Vivadent, Ellwangen, Germany). A standard periapical x-ray was taken using the parallel technique. Regarding post-surgical care, patients were advised to use a mouthwash with 0.2% chlorhexidine for 10 days and analgesics (ibuprofen 400 mg, up to 4/d) if necessary. They were instructed to avoid brushing on the implant site for 1 week after surgery and to start standard hygiene procedures at 10 days when sutures were removed.

2.6 | Harvesting Procedure

After a healing period of 8 weeks, composite and Teflon were removed to access the screw channel, then a custom-made guide was placed on the experimental abutment. A local anesthesia was performed, and a punch biopsy device of 4.5 mm in diameter was used to harvest a mucosal ring of about 1 mm in thickness around the experimental abutment. The custom-made guide was unscrewed, and a burr was used to mark the buccal aspect of the abutment. The abutment and the surrounding soft tissues were then harvested together. The abutment and the attached peri-implant soft tissue were then harvested as a single unit. The punch device was designed

to cut precisely to the bone crest level, minimizing trauma to deeper tissues, and a microblade was used to cut the remaining tissue adherent to the bone. Samples in which the gingival ring detached during harvesting were excluded. It was directly placed in a 4% formaldehyde solution and transferred to a PBS solution after 24 h. The implant screw channel was rinsed with a saline solution, and a transmucosal abutment (SRA, Straumann Group, Basel, Switzerland) or a conventional healing abutment was placed. No sutures were performed (Figure 2).

2.7 | Sample Preparation

The samples were processed for histology and histomorphometry using the fracture technique, which allows the analyses of implant/soft interface at the Robert K. Schenk Laboratory of Oral Histology, School of Dental Medicine, University of Bern, Switzerland. The samples were dehydrated in graded series of ethanol followed by xylene. They were then embedded with polymethylmethacrylate.

The abutments were cut with a diamond-coated saw (VC-50, Leco) in the apico-coronal direction from mesial to distal direction, and a second cut was performed from vestibular to lingual direction. A total of 120 ground sections (4 per abutment) were available for analyses. The sections were ground to a final thickness of 150 μm (Pedemax-2, Struers) and stained with Toluidine Blue-Fuchsin (Figure 3). The sections were scanned at high resolution with a Zeiss microscope (Axio Imager. M2, Zeiss).

2.8 | Histomorphometric Analyses

Histometric measurements were performed to determine the dimensions of each structure of the PIST. The total height of the PIST was defined as: the sulcus, the junctional epithelium, and the connective tissue. The sulcus depth (S) was measured as the distance between the peri-implant mucosal margin (PIM) to the coronal end of junctional epithelium (cJE); the epithelial adhesion (EA) was measured as the distance between cJE and aJE (apical end of the visible epithelium) and the connective tissue attachment was measured as the distance between the APF (abutment platform) to aJE (Figure 4). Due to the harvesting method, a missing part of the connective tissue apical to the abutment shoulder and adherent to the bone was not reported. The measure of the connective tissue concerned only the portion adherent to the abutment. These measurements were carried out by using image analysis software (ZEN pro 2012, Zeiss).

2.9 | Descriptive and Semi-Quantitative Histological Analysis

Descriptive histological examination of each sample was performed to assess supra and/or submucosal biofilm on the mesial, distal, vestibular, and lingual surfaces. Quantification of biofilm

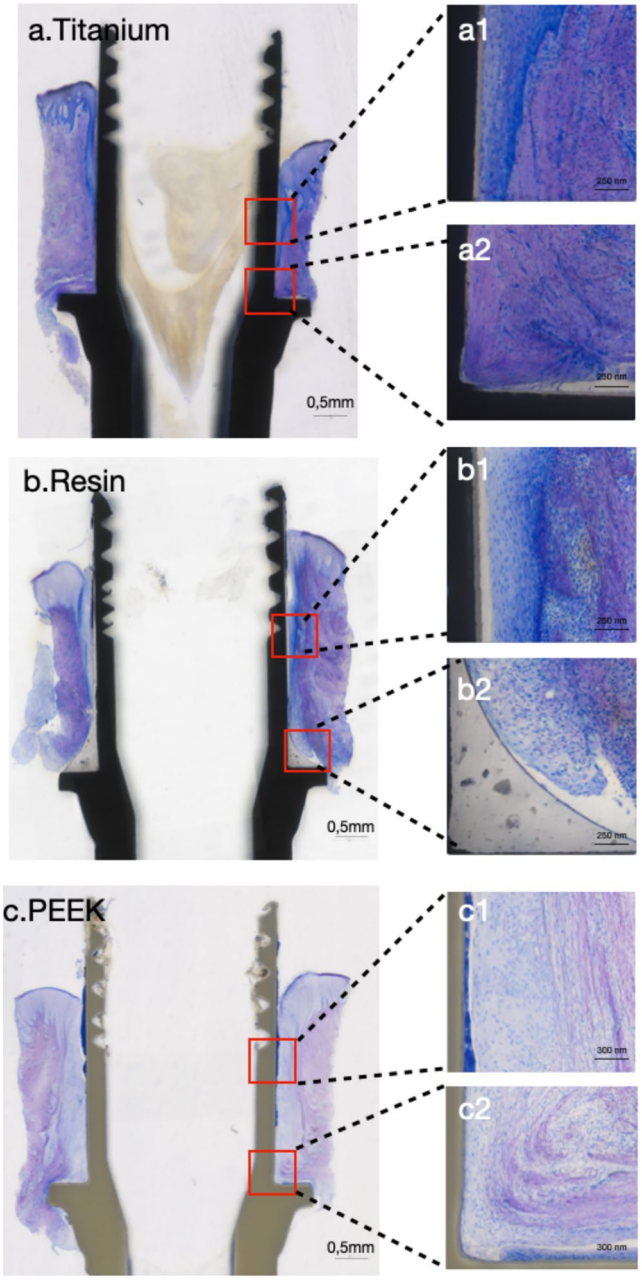

FIGURE 3 | Ground sections of the three experimental abutments. (a) Titanium, (a1) magnification of epithelial adhesion (×4120), (a2) magnification of the apical part of the abutment ×4120. (b) Resin (notice the supra layer of resin) (b1) magnification of epithelial adhesion ×3990, (b2) magnification of the apical part of the abutment ×3990. (c) PEEK, (c1) magnification of epithelial adhesion ×3990, (c2) magnification of the apical part of the abutment ×3290.

adhesion in a binary manner was conducted using image analysis software (ZEN pro 2012, Zeiss). The degree of inflammation was evaluated based on the appearance of peri-implant soft tissues observed in the sample images. The assessment was performed according to the following scale (index): 0 = normal, 1 = low local infiltration of inflammatory cells, 2 = larger infiltration area and/or more pronounced infiltration, 3 = large infiltration area, severe inflammatory signs, and advanced tissue damage and/or pus.

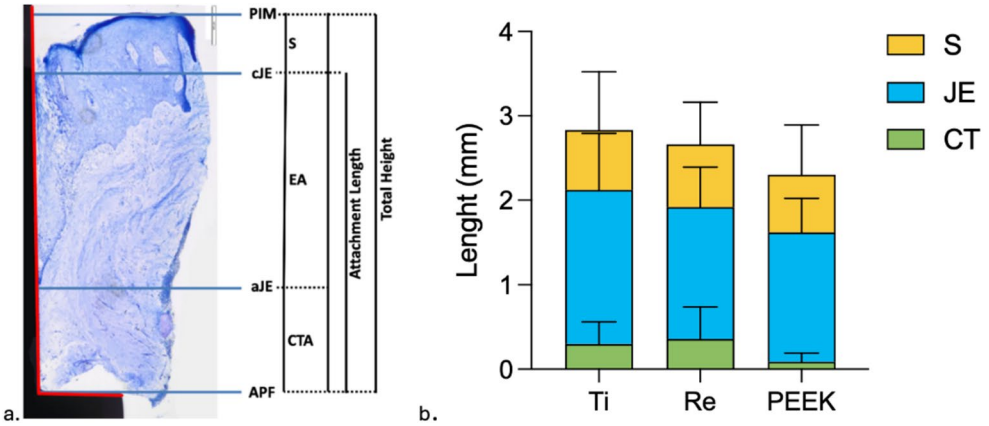

FIGURE 4 | (a) Structure of the peri-implant soft tissues. aJE = apical end of junctional epithelium; APF = abutment platform; cJE = coronal end of junctional epithelium; CTA = connective tissue adhesion; EA = epithelial adhesion; PIM = peri-implant mucosal margin; S = sulcus. (b) Repartition of Sulcus, epithelium, and connective tissue depending on the three experimental materials. CT = connective tissue; JE = junctional epithelium; S = Sulcus depth.

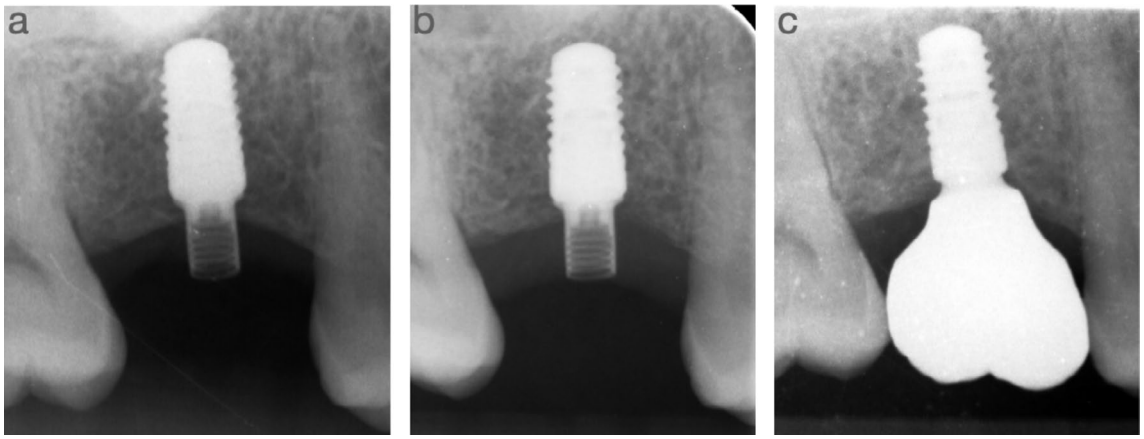

FIGURE 5 | Standardized radiography procedure: (a) after surgery and placement of the experimental abutment, (b) at the 8 weeks control, and (c) at the 3 months control after crown placement.

2.10 | Radiographic Analyses

The evaluation of peri-implant bone levels was conducted at baseline and 8 weeks later, based on periapical radiography (Figure 5). This assessment was carried out by two independent evaluators (postgraduate students in Periodontology). Prior to conducting the radiographic evaluations, these evaluators engaged in two calibration sessions spaced 1 week apart, with the resultant measurements subsequently undergoing thorough discussion. The radiographic images were acquired using the non-standardized parallel technique and a phosphor plate receptor. Subsequent calibration of these radiographs was achieved by employing the software Image FIJI [41], utilizing the known distance between implant threads for reference. Using the same software, the linear distance from the implant shoulder to the initial point of bone-to-implant contact (DIB, in millimeters) was measured on both the mesial and distal sides of bone-level implants [42]. The average of these mesial and distal measurements was then recorded as the final DIB value. Additionally, the radiographic measurements of peri-implant bone changes were evaluated using the Intraclass Correlation Coefficient (ICC).

2.11 | Statistics

A power calculation (PASS 2019) showed that, with 10 implants in each material group (total 30 implants), an effect size of 0.60 for the length of the PIST could be discerned between groups by applying one-way analysis of variance; and assuming homogeneity of the group variances, a significance level of 5% and a power of 80%.

The 30 implants were randomized to the three materials (Ti, Re and PEEK) by 10 permuted blocks of size 3, assuming one implant per patient. At the end, there were 10 implants Ti, 10 implants Re, and 10 implants PEEK. The randomization was performed with R. Since a couple of patients had two implants, the two implants were necessarily allocated to different materials. This might have induced some correlation between measurements of the implants within the same patient.

Results were expressed as mean ± standard deviation (SD) for quantitative variables and as frequency tables for categorical findings. Group mean values were compared by one-way analysis of variance followed by Tukey's multiple comparisons

TABLE 1 | Profilometry characteristics.

	Total	Titanium	Resin	Peek	*p*
Number of implants	9	3	3	3	
Sa (μm)		0.39 ± 0.12	0.20 ± 0.06	0.54 ± 0.08	< 0.0001
Sq (μm)		0.49 ± 0.15	0.25 ± 0.07	0.67 ± 0.10	< 0.0001
Sku		2.54 ± 0.37	5.22 ± 1.21	3.22 ± 0.40	< 0.0001
Ssk		0.36 ± 0.26	0.69 ± 0.23	−0.20 ± 0.24	< 0.0001
Sdr (%)		2.06 ± 1.47	1.78 ± 0.87	2.18 ± 0.73	< 0.0001

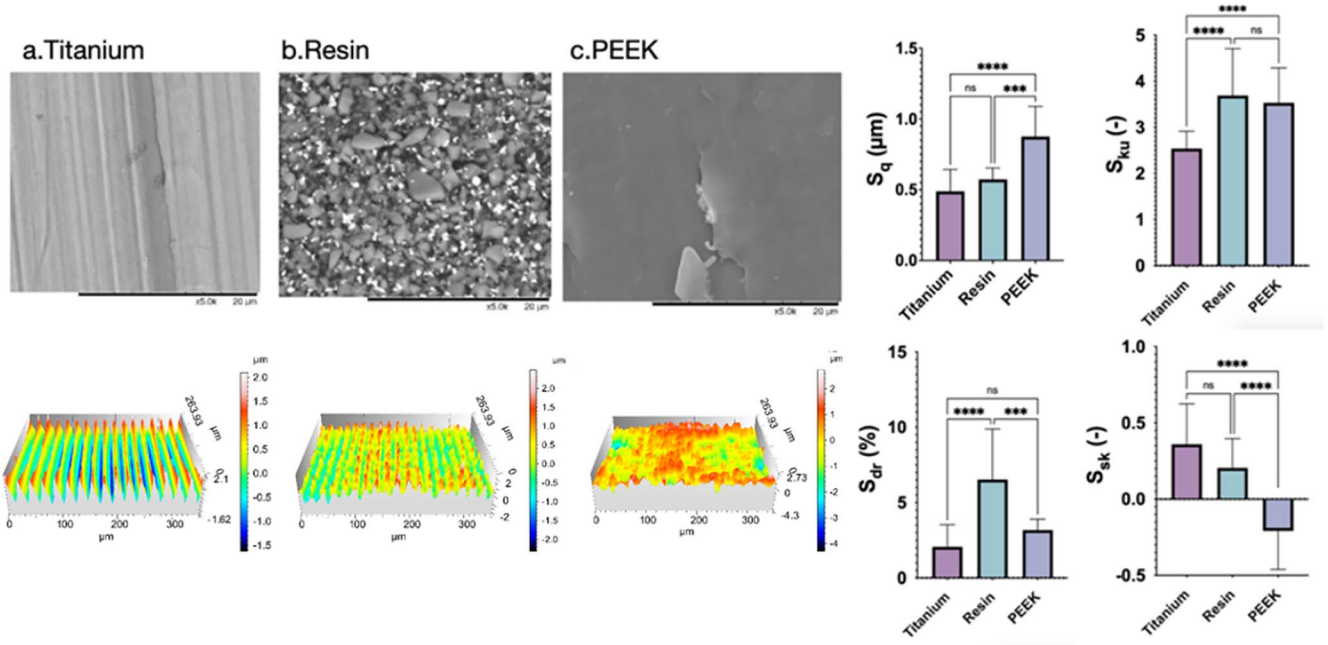

FIGURE 6 | SEM analyses (5.0 k) and comparative profilometry representing mean data of 6 analyzed points on each experimental abutment (3 Titanium, 3 Resin, and 3 PEEK) in terms of S_q, S_{ku}, S_{dr}, and S_{ds}.

considering all implants as independent. For proportions, comparisons were done by the classical chi-squared test. All results were considered significant at the 5% critical level ($p < 0.05$). Calculations were performed with SAS version 9.4 and R version 4.1.0 (R Foundation for Statistical Computing, Vienna) programs.7.

3 | Results

3.1 | Abutment Surface Characterization

Analysis of the different abutments revealed statistical differences for all examined surface parameters. PEEK and titanium surfaces were rougher ($S_a = 0.54\,\mu m$ and $S_a = 0.39\,\mu m$ and $S_q = 0.67\,\mu m$ and $0.39\,\mu m$ respectively), sharper ($S_{ku} = 3.22$) and with more texture ($S_{dr} = 2.19\%$ and 2.06%, respectively) compared to resin, presenting a smoother ($S_a = 0.20\,\mu m$ and $S_q = 0.25\,\mu m$), sharper ($S_{ku} = 5.22$) surface and less texture ($S_{dr} = 1.78\%$). These features are also visible in the high-resolution SEM images.

The complete data set is summarized in Table 1 and Figure 6.

3.2 | Demographics and Site-Related Data

During the inclusion phase, two implants (in two patients) failed to integrate; therefore, in order to include 10 abutments per group, 2 additional implants were included. Thus, a total of 30 samples in 28 patients were collected for analysis. Age, gender, implant position, and flap designs were homogenous between the different groups (Table 2).

3.3 | Histomorphometric Analyses

Out of the 120 sections, 3 were damaged or incomplete and were not analyzed (one section in each of the three experimental groups). In 11 sections, the apical portion was turned away or partially damaged (one in Ti group, four in Re group, and six in PEEK group) but measurements could be performed.

The mean overall dimensions of the supra crestal peri-implant soft tissue were 2.68 ± 0.51 mm, 2.66 ± 0.47 mm, and 2.32 ± 0.55 mm, respectively, for Ti, Re, and PEEK; no significant difference was observed between the materials regarding the total length of the peri-implant supra crestal soft tissue ($p = 0.22$).

TABLE 2 | Patients and site-related characteristics.

	Total	Titanium	Resin	Peek
Number of implants	30	10	10	10
Age (years)				
Mean ± SD	57 ± 10	57 ± 12	54 ± 9	60 ± 9
Gender (M/F)				
Male	13	5	4	8
Female	17	5	6	2
Implant position				
Maxilla	17	6	6	5
Mandible	13	4	4	5
Flap design				
Flap	24	9	9	6
Mini-flap	6	1	1	4

The mean sulcus depth yielded respectively 0.71 ± 0.69 mm, 0.74 ± 0.50 mm, and 0.68 ± 0.63 mm for Ti, Re, and PEEK; non-significant differences were found ($p = 0.97$).

The mean length of the epithelial interfaces was also not significant between the groups, with mean values of 1.82 ± 0.67 mm, 1.56 ± 0.47 mm, and 1.53 ± 0.40 mm, respectively, for Ti, Re, and PEEK ($p = 0.41$).

Finally, the mean connective tissue interface was 0.30 ± 0.29 mm for Ti, 0.36 ± 0.38 mm for Re, and 0.09 ± 0.10 mm for PEEK. Even though a significant difference was not demonstrated, a tendency for a shorter connective tissue portion on PEEK abutments was observed ($p = 0.09$). With the PEEK material, the CT interface represents only 3.9% of the overall PIST, while it reached 11.2% and 13.5%, respectively, for the Ti and Re abutments. Results are reported in Table 3, Figures 3 and 6.

3.4 | Descriptive Histology

After 8 weeks, the interface between the experimental abutments and the peri-implant soft tissue was characterized from coronal to apical by the sulcus, the junctional epithelium, and the connective tissue (Figures 3 and 4). This configuration was observed in all samples, but each component's length seemed to

TABLE 3 | Experimental abutments-related data (histological-histomorphometric analyses).

	Titanium	Resin	Peek	*p*
Number of implants	10	10	10	
Sulcus depth (mm)				
Mean ± SD % of total length	0.71 ± 0.60 26.5%	0.74 ± 0.50 27.8%	0.68 ± 0.63 29.3%	0.97
Range	0.08–2.41	0.23–1.87	0.10–2.01	
Epithelial adhesion (mm)				
Mean ± SD % of total length	1.82 ± 0.67 68.0%	1.56 ± 0.47 58.6%	1.53 ± 0.40 66.0%	0.41
Range	0.52–2.89	0.48–2.03	1.00–2.25	
Connective tissue (mm)				
Mean ± SD % of total length	0.30 ± 0.29 11.2%	0.36 ± 0.38 13.5%	0.09 ± 0.10 3.9%	0.09
Range	0.00–0.69	0.00–1.05	0.00–0.30	
Total length of the PIST excluding sulcus (mm)				
Mean ± SD	1.97 ± 0.77	1.91 ± 0.36	1.63 ± 0.44	0.33
Range	0.52–2.89	1.52–2.53	1.00_2.25	
Total length of the PIST including sulcus (mm)				
Mean ± SD	2.68 ± 0.51	2.66 ± 0.47	2.32 ± 0 0.55	0.22
Range	1.58–3.20	1.65–3.12	1.58–3.40	

vary, especially for the connective tissue portion. In 11 sections, an epithelial interface was found all the way apically to the implant neck (four in Ti group, three in Re group, and four in PEEK group). Inclusion of bone fragments was also observed in three sections (one in Re group, two in PEEK group).

3.5 | Semi-Quantitative Histological Analyses

Submucosal biofilm was found in half of the sections for the Ti and Re groups and in a third of the sections for the PEEK group. However, no statistical differences between the three groups were observed. Supramucosal biofilm was found in most of the sections in the Re groups (86.6%), which was significant ($p = 0.026$) compared to the two other groups (66.6%) (Table 4). Inflammation scores did not differ significantly among the three materials studied (Table 4). Nevertheless, the level of inflammation remained low, with most of the abutments displaying a score of 0 or 1. Only 5% of the PEEK and Re sections presented a score of 2.

TABLE 4 | Biofilm and inflammation.

	Titanium	Resin	Peek	*p*
Submucosal biofilm, plaque presence per				
Total of surfaces	20/39	18/39	13/39	0.26
Percentage of covered surface	51.2%	20.6%	33.3%	
Mesial sites	5/10	5/9	3/10	0.52
Distal sites	5/10	6/10	2/10	0.18
Vestibular sites	6/9	3/10	4/10	0.28
Lingual sites	4/10	4/10	4/9	0.67
Supragingival Biofilm, Plaque presence per:				
Total of surfaces	26/39	35/39	26/39	0.026
Percentage of covered surface	66.6%	89.6%	66.6%	
Mesial sites	7/10	8/9	8/10	0.62
Distal sites	8/10	10/10	5/10	0.029
Vestibular sites	6/9	8/10	8/10	0.76
Lingual sites	5/10	9/10	5/9	0.14
Mean grade of inflammation (scoring 0–3)				
Mean	0.33	0.56	0.52	0.12
Score 0	51.3%	30.8%	41.0%	
Score 1	48.7%	64.1%	53.9%	
Score 2	0%	5.1%	5.1%	
Score 3	0%	0%	0%	

3.6 | Radiographic Analyses

The radiographical peri-implant bone loss measurements were assessed by the ICC and 95% confidence interval, with respectively 0.995 (95% CI: 0.992–0.997) and 0.991 (95% CI: 0.987–0.996) at baseline and 8 weeks. Analysis of the radiographs did not reveal any difference between the groups at the time of implant placement. After 8 weeks of healing, the average peri-implant bone loss was similar between groups ($p = 0.29$). The complete data set is summarized in Table 5.

4 | Discussion

In this comparative histological study in humans, we aimed to characterize the peri-implant supracrestal soft tissue (PIST) interface in response to different abutment materials (titanium, resin, and PEEK). Integration of the peri-implant soft tissue was observed for all tested materials, with no statistically significant differences in the dimensions of the sulcus, junctional epithelium, and connective tissue interface across the groups.

Despite limited evidence on the interactions between abutment materials and peri-implant soft tissue, this interface remains a critical topic in implant dentistry [19]. To our knowledge, this is the first study to explore the human histomorphology of the interface between peri-implant soft tissue and resin transmucosal components.

4.1 | Histomorphometry

The mean dimension of the harvested PIST was 2.6 mm, comprising a sulcus depth of 0.81 mm, epithelial adhesion of 1.63 mm, and a connective tissue compartment of 0.2 mm. These values are below the commonly accepted range of 3–4 mm described for peri-implant supracrestal soft tissue

TABLE 5 | Peri-implant bone level changes from baseline to abutment removal (8 weeks).

	Titanium	Resin	Peek	*p*
Bone level changes (mm)				
Number of implants	10	10	10	
Mean ± SD	0.46 ± 0.33	0.27 ± 0.37	0.25 ± 0.35	0.29

[4, 7, 43]. However, the existing data on PIST dimensions is primarily based on animal models, with limited human data available.

In human, Tomasi et al. reported a supracrestal soft tissue length of approximately 3.6 mm at 8 weeks around titanium abutments, with a connective tissue interface of 1.7 mm, aligning with preclinical data [6, 44]. While the mean junctional epithelium length in our study aligns with their findings, the connective tissue portion was significantly shorter than the values reported by Tomasi et al. This discrepancy may stem from partial harvesting during biopsy, as tissues were collected above the implant neck of bone-level implants, avoiding complete extension to the bone for ethical considerations. Additionally, the measurements did not include horizontal portions of the soft tissue around the abutments. The missing part of the connective tissue portion should be considered one major factor in underestimating PIST and connective tissue dimensions.

Schwarz et al. reported PIST lengths ranging from 2.35 to 2.51 mm, predominantly composed of junctional epithelium (1.88–1.96 mm) with a minimal connective tissue portion (0.43–0.55 mm) using a similar experimental model and biopsy method [45]. Glauser et al. also noted a predominance of epithelial junctions, with a connective tissue interface of approximately 0.6 mm [44]. Differences in connective tissue dimensions across studies may reflect variations in measurement methods, biopsy techniques, and abutment designs. Narrow abutments, for instance, enhance soft tissue dimensions compared to wider abutments [14]. Convergent abutment profiles also encourage axial development of peri-implant connective tissue [38]. The narrow design of the experimental abutments in this study may partially explain the observed variations in the supracrestal structure.

4.2 | Abutment Surface Characteristics

The experimental abutments used in this study displayed distinct surface topographies. Titanium abutments had the smoothest surfaces, with lower Sku values compared to PEEK and resin. PEEK exhibited sharper peaks and deeper valleys, while resin had the highest Sdr value (5.51%), reflecting a more complex texture.

Surface topography variations can influence tissue responses. Textured surfaces are associated with shorter epithelial attachment and longer connective tissue zones [44, 46]. Recent studies have also shown that anodized abutments result in a larger epithelial adhesion compared to other surfaces [47]. In this study, the limited connective tissue portion observed with PEEK abutments may be attributed to their smoother surface compared to resin and titanium abutments.

4.3 | Biofilm Adhesion and Inflammation

Semi-quantitative analysis revealed higher supragingival plaque accumulation on resin abutments compared to titanium and PEEK. However, this did not affect PIST inflammation grading or health within the short observation period. Textured surfaces are known to facilitate plaque accumulation and increase the risk of peri-implant diseases, whereas polished surfaces aggregate less plaque [3, 48]. Surface roughness Sa has previously showed to be positively correlated with oral biofilm adhesion, and less pronounced on flat and grooved than on irregular surface [46].

Although no significant differences in inflammation levels were observed between the materials in this study, resin-based materials have been associated with monomer release and related cytotoxicity, potentially intensifying inflammation [49, 50]. A previous study using a similar model demonstrated greater macrophage recruitment and an intensified inflammatory response around resin abutments [35]. The lack of observed differences here may relate to the type of histology (ground section) and evaluation method (subjective scoring).

One more limitation could be related to the short time of follow-up in the present study for the establishment of stable PIST. Early time points provide valuable information on healing and tissue attachment but might not reflect long-term soft tissue adaptation.

4.4 | Radiographic Data

No significant differences in bone remodeling were observed across the tested materials. Despite the shorter PIST height at 8 weeks, marginal bone levels remained stable, suggesting that early bone remodeling may depend on factors beyond PIST dimensions. This finding contrasts with previous observations [35].

4.5 | Study Limitations

The study has a few limitations. The interpretation of radiographic data suffers from a lack of standardized imaging, which may have affected accuracy. Calculations were done as if implants were independent (one implant per patient) but a couple of patients had two implants, inducing a correlation of measurements within these patients and potentially overlooking patient clustering effects [51]. Given that only two patients (6.7%) had two implants of different materials, the impact on the final outcomes was considered negligible. With respect to the total sample size and the number of hypotheses tested on the same material, p values should be considered with caution. Finally, it should be acknowledged that the study may be underpowered for some of the secondary outcomes, and that a lack of statistically significant differences does not establish equivalence.

5 | Conclusion

Within the limitations of this study, the composition and structure of peri-implant soft tissue at 8 weeks were comparable across the tested materials. The peri-implant supracrestal soft tissue was predominantly epithelial, with limited connective tissue portions. Resin abutments demonstrated higher plaque accumulation compared to titanium and PEEK, though without impacting PIST health or inflammation in the short term. The

long-term effects of these materials on peri-implant tissue warrant further investigation.

Author Contributions

Conceptualization: F.L., G.L., M.B. Data Curation: M.B., L.L., G.L. Formal analysis: D.V.H., M.B. Funding acquisition: F.L. Investigation: L.L., M.B., D.V.H. Methodology: F.L., D.V.H., G.L. Project administration: F.L., D.V.H. Resources: F.L., D.V.H., G.L. Software: D.V.H., L.L., M.B. Supervision: F.L. Validation: F.L., D.V.H. Visualization: L.L., M.B., D.V.H. Writing – original draft: L.L., M.B., D.V.H., F.L. Writing, review and editing: D.V.H., M.B., L.L., F.L.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** CONSORT-2010-Checklist.