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Influence of Implant Component Materials on Peri-Implant Soft Tissue Healing: A Comparative Histological and Immunohistochemical Study in Humans

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ABSTRACT

Background: Recently, the importance of peri-implant soft tissue integration quality has been recognised as an essential factor in the long-term success of dental implant rehabilitation.

Aim: The aim of this study was to explore the influence of three materials commonly used in implant dentistry, namely titanium (Ti), dental adhesive resin (Re) and polyetheretherketone (PEEK), on the peri-implant soft tissues.

Methods: In this clinical randomised comparative study, 37 bone-level implants were placed, and experimental transmucosal healing abutments made of different materials were randomly assigned to each implant. These abutments were removed together with the surrounding soft tissues after 8 weeks. Immunohistochemical analyses were performed to determine the presence and localisation of different immune cells. In addition, clinical and radiographic data were collected and peri-implant bone remodeling was assessed.

Results: 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 ($p = 0.01$).

Conclusion: The use of resin material as a transmucosal healing abutment should be carefully considered as it was associated with a higher presence of inflammatory cells at 8 weeks post-implantation as well as superior bone remodeling compared to PEEK and Ti.

1 | Introduction

For a long time, osseointegration and peri-implant bone changes were considered the main factors in evaluating implant success. The phenomena of osseointegration and bone healing around implants are now well-known and widely described (Brånemark et al. 1969; Tonetti 1998).

More recently, the importance of a durable soft tissue integration around the transmucosal implant components was emphasised to play an important role in the long-term success of implant-supported rehabilitations as it acts as a biological barrier against bacterial infiltration (Schwarz et al. 2018). The mucosal interface (biological width) around the dental implant is composed in the coronal part of an epithelial adhesion

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rather similar to the junctional epithelium on natural teeth and apically, of a connective tissue interface where the collagen fibers adhere in parallel or circumferentially to the implant surface, contrasting with the perpendicular orientation of these fibers in natural teeth (Berglundh et al. 1991; Tomasi et al. 2014). The dimension of the biological width on implants is 4 mm on average and mature healing is achieved within 6–8 weeks (Berglundh et al. 2007; Salvi et al. 2015; Sculean, Gruber, and Bosshardt 2014). It was suggested that the quality and stability of the interface between the soft tissue and the implant/component surface are influenced by multiple factors such as transmucosal designs (Canullo et al. 2020; Valente et al. 2020), prosthodontics manipulations (Vatėnas and Linkevičius 2021) or abutment materials (Grenade et al. 2016, 2017; Sanz-Martín et al. 2018).

In order to respond to a growing aesthetic demand, different implant restoration protocols involving peri-implant soft tissue conditioning using composite resin have been suggested (Proussaefs 2016; Wittneben et al. 2013), and different types of materials such as dental resin adhesive (Re), Polyetheretherketone (PEEK), zirconia or gold are commonly used submucosally, in direct contact with the peri-implant soft tissue. However, to ensure proper mucosal integration and long-term stability, these different materials used submucosally should not induce an inappropriate host response. For example, gold abutments have long been considered the gold standard in terms of aesthetic results, however, they were abandoned due to their lack of biocompatibility since they do not allow the formation of a sealed soft tissue barrier (Abrahamsson et al. 1998). On the other hand, zirconia has been progressively used for implant abutments because of its interesting aesthetic and mechanical properties and numerous studies demonstrated its biocompatibility as long as it is not veneered with cosmetic ceramic (Thoma et al. 2016). Recently, a systematic review highlighted the equivalence of zirconia abutments to titanium in regard to soft tissue compatibility as well as the aesthetic superiority of zirconia especially for thin mucosal phenotypes (Linkevicius and Vaitelis 2015).

Despite this existing evidence, a recent EAO consensus report emphasised the need for further research into the specific biological interaction between abutment material and the soft tissues (Sanz et al. 2018). Indeed, a limited number of studies have focused on the biological impact of materials on their surrounding soft tissues. Different animal studies have suggested that the surface topography of the transmucosal part of the implant has an impact on soft tissue adhesion level (Canullo et al. 2021). Additionally, some authors analyzed the peri-implant soft tissue healing dynamic in humans and demonstrated mature healing at 8 weeks after implantation (Sculean, Gruber, and Bosshardt 2014; Tomasi et al. 2014). More specifically, immunohistological studies of inflammatory phenomena occurring on implants with clinical signs of peri-implantitis suggested different inflammatory mechanisms leading to bone loss (de Araújo et al. 2017; Bullon et al. 2004). Recently, it has been suggested that the inflammatory processes occurring around titanium and zirconia abutments are relatively similar, in contrast to the gold abutment, which revealed a more abundant inflammatory infiltrate and a higher amount of B cells, T cells and macrophages (Serichetaphongse et al. 2020).

To our knowledge, few studies have investigated the human host-related response of peri-implant soft tissues toward material such as dental resin or PEEK, often used submucosally for provisional crowns or custom-made healing abutment (Finelle et al. 2021; Lilet et al. 2022).

Although various studies have evaluated different peri-implant materials, most of them have only assessed the effect of titanium and zirconia (Cao et al. 2019; Hanawa 2020; Linkevicius and Vaitelis 2015; Naveau, Rignon-Bret, and Wulfman 2019). One single animal study investigated the histological attachment of PEEK when compared to titanium (Rea et al. 2017). Moreover, few studies have performed immunological analyses to demonstrate the impact of the materials on the soft tissues (Enkling et al. 2022; Serichetaphongse et al. 2020).

Therefore, the present clinical investigation was designed to evaluate the effect of different materials commonly used transmucosally in daily implant dentistry. The aim of this study was to characterise the immune host-related response of peri-implant soft tissue induced by three different materials: titanium, resin and PEEK. Additionally, clinical and radiological features were evaluated.

2 | Materials and Methods

The study protocol was approved by the Ethical Committee of the University of Liege, Belgium (N°B707201628072). The study was registered on clinicaltrial.gov (file number: NCT05843526) and performed according to Consolidated Standards of Reporting Trials (CONSORT) guidelines and European Medicines Guidelines for Good Clinical Practice. The Material and Methods were published in a previous article (Borie et al. 2020) and are reported briefly as follows.

2.1 | Abutment Design and Manufacturing

Experimental abutments were designed with a platform to facilitate peri-implant soft tissue harvesting (Figure 1A). Twenty-five pieces were manufactured in grade 5 Titanium (Ti), with 13 of them covered with Resin to constitute the second group, and 12 in PEEK (Figure 1B).

2.2 | Study Design

This randomised comparative study aimed to investigate the peri-implant soft tissue response of three different abutment materials implanted in patients. The primary endpoint was the scoring of the markers for inflammation. 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 included in the study. The participation was strictly voluntary and all enrolled subjects signed an informed consent agreement prior to inclusion.

A total of 37 bone level implants (Institut Straumann AG, Basel, Switzerland) were placed and experimental abutments made of grade 5 titanium (Ti), dental resin (Optibond FL, Kerr Dental), and polyetheretherketone (PEEK) were randomly allocated to each implant using a computer-generated block randomisation

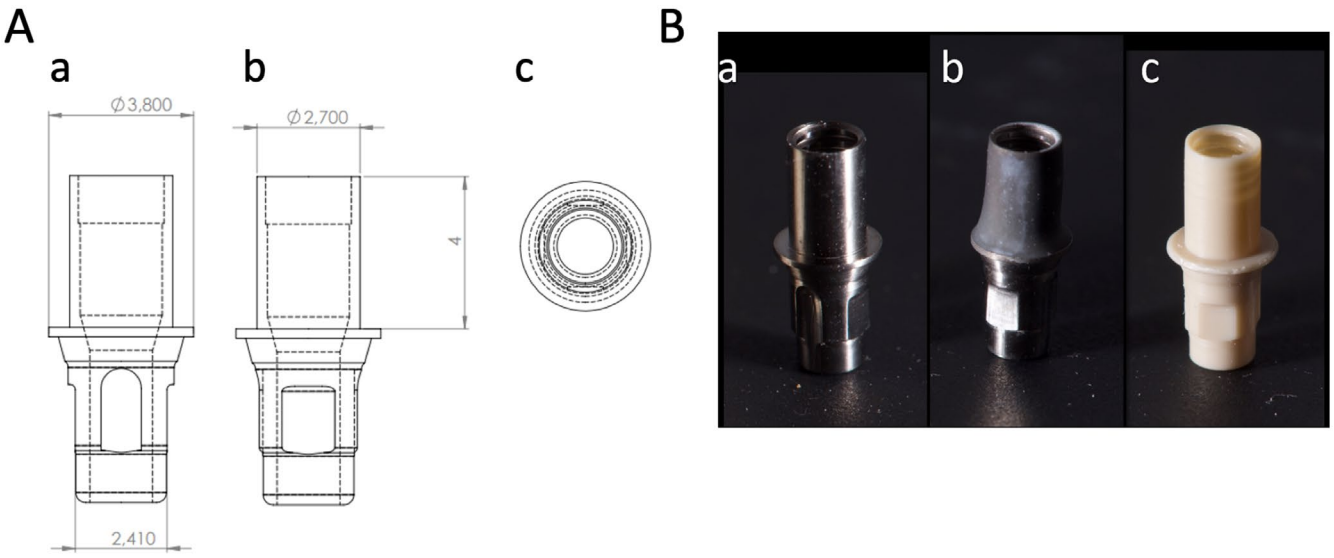

FIGURE 1 | Experimental abutment design and pieces. (A) Experimental abutment design, including diameters of the respective platform (millimeters): (a) Large platform used for soft tissue biopsy, (b) transmucosal part of the abutment and (c) internal implant connectors. (B) Experimental abutments: (a) titanium, (b) resin and (c) PEEK.

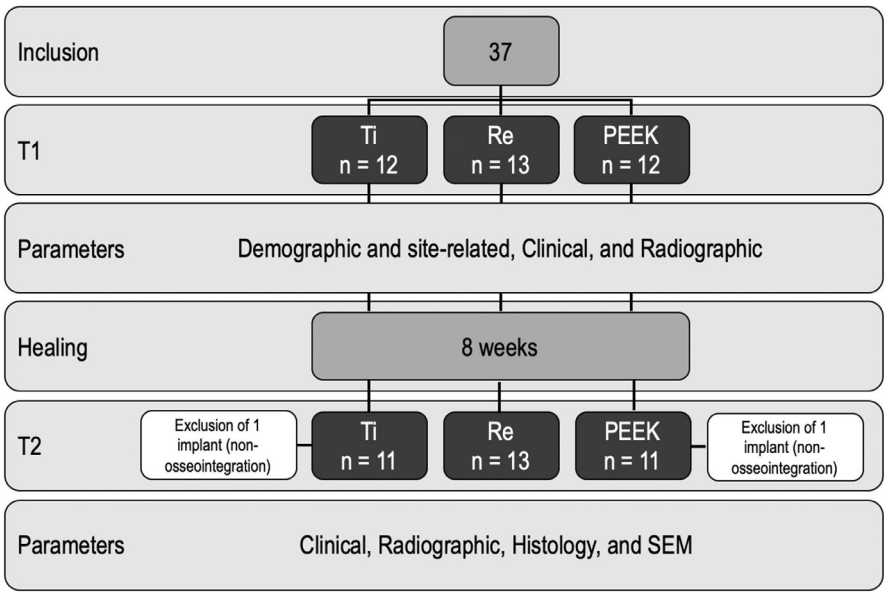

FIGURE 2 | Timeline and study design.

table. Concealment measures were taken and the surgeons were blinded until the abutment placement. Patient, site-related, clinical and radiographic data were collected at baseline and 8 weeks post-implantation. Experimental abutments with surrounding tissues were removed 8 weeks after implant placement, and the peri-implant soft tissues underwent standard paraffin-embedded histology and immunohistochemistry processing whereas the abutments' surfaces were examined by scanning electronic microscopy (SEM) to evaluate the presence of dental plaque (Figure 2).

2.3 | Inclusion Criteria

Patients were enrolled if they met the following criteria: aged 18 years or older and needing implant therapy, with one or more missing teeth in the posterior area of either maxilla or mandible, in good systemic health (ASA I/II), and a full mouth plaque score (FMPI) lower than or equal to 25%. The tooth at the implant site(s) had to be extracted or lost at least 12 weeks prior to implant placement, presenting at least 3 mm of keratinised mucosa in the bucco-lingual dimension with bone crest allowing at least a regular diameter implant of 4.1 mm.

2.4 | Exclusion Criteria

Patients were not enrolled in case of any of the following criteria present: autoimmune disease requiring medical treatment, medical conditions requiring prolonged use of steroids, use of bisphosphonates and 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), local or systemic infection. The site was excluded if it was previously treated with socket preservation techniques, presented untreated local inflammation, mucosal diseases, oral lesions, history of local irradiation therapy or had a persistent intraoral infection.

2.5 | Surgical Procedure

After local anesthesia, a full-thickness flap or a mini-flap was raised or, alternatively, a flapless approach was used to access the implant site. The decision regarding the flap design was made by the surgeon. The implantation procedure was performed according to the protocol provided by the manufacturer and a bone level implant of 4.1 or 4.8 mm in diameter (Bone level SLA, Roxolid, Institut Straumann AG, Basel, Switzerland) was placed with an insertion torque of at least 15 Ncm. Following predetermined randomised allocation, an experimental abutment, described in Figure 1, was then placed in a non-submerged approach and tightened at 15 Ncm. When needed, simple interrupted sutures with absorbable materials were performed directly after abutment placement, and the abutment screw channel was filled with Teflon tape and closed with composite (Telio, Ivoclar Vivadent, Ellwangen, Germany).

Thereafter, a non-standardised periapical radiograph was taken using the parallel technique. Regarding post-surgical care, the patients were advised to use chlorhexidine-based mouthwash (0.2%) for 10 days and analgesics (ibuprofen 400 mg, up to 4×/d), if necessary. They were also instructed to avoid brushing in the implant site for 1 week after surgery and to apply standard hygiene procedures once the sutures are removed (10 days post-surgery).

2.6 | Harvesting Procedure

After a healing period of 8 weeks, the experimental abutment along with the surrounding soft tissue was collected according to the following procedure (Figure 3). After removal of the composite and teflon tape to access the screw channel (Figure 3a), a custom-made guide was placed on the experimental abutment (Figure 3b). A local anesthesia was performed and a 4.5 mm in diameter punch biopsy device was used to harvest a mucosal ring of 1 mm of thickness around the abutment (Figure 3c). The custom-made guide was then unscrewed and a bur mark was used as a landmark to identify the buccal side of the abutment, allowing the preservation of its orientation for SEM analyses. The abutment and the surrounding soft tissues were then harvested and gently separated using sterile dental tweezers (Figure 3d). Soft tissue samples were directly placed in eppendorfs containing 4% formaldehyde solution and transferred to a PBS solution after 2 h. The experimental abutments were processed for SEM analysis. The implant screw channel was rinsed with a saline solution and the experimental abutment was replaced by an abutment (RC Screw-Retained abutment, Institute Straumann AG, Basel, Switzerland) or a conventional healing abutment (RC Healing abutment, Institute Straumann). No sutures were performed.

2.7 | Clinical Parameters

Dimension of keratinised mucosa and soft tissue height at the surgical site were measured at the time of implant placement and 8 weeks later. These parameters were collected using a periodontal probe (Perio Probe 1–15, U Friedy, USA). The quantity of keratinised mucosa was measured from the incision line to the mucogingival junction line in the lingual and buccal aspects. The mucosal height was recorded at four sites perpendicular from the crestal bone level to the mucosal margin. Plaque index was also recorded in a binary manner at the time of the biopsy. Probing depth and bleeding on probing were not collected in order to minimise the risk of soft tissue damage.

2.8 | Radiographic Analysis

The peri-implant bone levels were assessed at baseline and 8 weeks after implant placement on periapical radiography (Figure 4) by two independent and calibrated examiners (postgraduate students in Periodontology). Before the radiographic assessment, the two examiners performed two calibration sessions at 1-week intervals, after which resulting measurements were critically discussed. The radiographs were non-standardised and performed using the parallel technique. The receptor type was a phosphor plate. Thereafter, the radiographs were calibrated using the known distance between the implant treads using the software Image FIJI (Schneider, Rasband, and Eliceiri 2012). With the same software, the linear distance between the implant shoulder of the bone-level implants and the first bone-to-implant contact (DIB, mm) was measured at the mesial and distal aspects (Buser et al. 2009). Final DIB values were recorded as the average of the obtained mesial and distal values. Also, the radiographical peri-implant bone loss

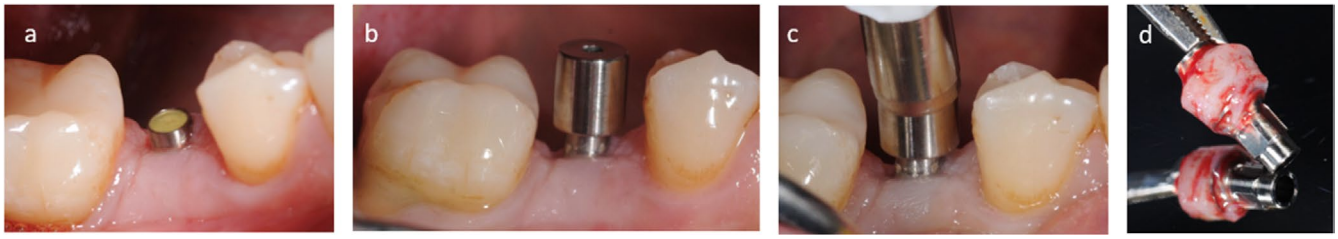

FIGURE 3 | Harvesting procedure (lateral view): (a) Custom-made abutment in place, (b) custom-made guide corresponding to the diameter of the punch device, (c) use of the biopsy punch to remove a standardised amount of soft tissue and (d) soft tissue and custom abutment harvesting.

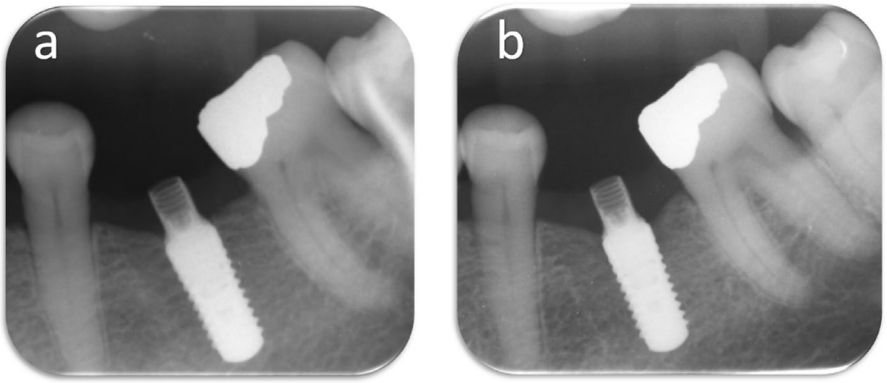

FIGURE 4 | Parallel radiography procedure: (a) After surgery and placement of custom-made abutment in place and (b) at the 8 weeks control, before removing the abutment.

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.

2.9 | Immunological Sample Preparation

After being harvested, the cylindrical peri-implant soft tissue samples were cut and opened. Samples were then included and embedded in paraffin wax with particular attention to maintaining their orientation. The subsequent blocks were then cut into serial 5 μm thick sections following the longitudinal axis and using a microtome (Leica RM2245, Leica, Germany) with the implant side on the left and the external side on the right. Sections were then deparaffinised and rehydrated through graded xylene and alcohol baths (3 baths of xylene, one bath of Isopropanol 80%, one bath of Isopropanol 60%, and two baths of demineralised water). A first series of sections was stained with haematoxylin–eosin for plasmocytes and polymorphonuclear cells (PMNs cells) exploration. The following serial sections were used for specific immunohistochemical staining (anti-CD3 [790–4341], anti-CD20 [790–4441], anti-CD68 [790–2931], and anti-CD34 [790–2927], Roche, Basel, Switzerland). The sections were heated in EDTA buffers for antigen retrieval in a pressure cooker for 11 min (except for anti-CD34), blocking non-specific binding with protein block serum-free (Dako) for 10 min and incubated with anti-CD3 (1:250), anti-CD20 (1:500), anti-CD68 (1:500) or anti-CD34 (1:250) primary antibodies for 1 h at room temperature. Finally, antibodies were visualised using diaminobenzidine (Dako) and the sections were counterstained with haematoxylin. Positive and negative controls were performed for each antibody.

2.10 | Histological Analyses

For the blood vessel quantification, CD34-stained sections were scanned into digital images with the NDP NanoZoomer Digital pathology digital slide scanner (Hamamatsu Photonics, Japan). Using the ImageJ software (Schneider, Rasband, and Eliceiri 2012), a semi-automated procedure was developed to easily quantify the blood vessel density in the stroma (number of blood vessels per mm^2) as well as the percentage of stromal surface occupied by the blood vessels.

The presence of plasmocytes, PMNs, T lymphocytes, B cells and macrophages in the stroma was semi-quantitatively scored by a senior anatomopathologist, who was calibrated and blinded to the sample origin, using the H&E, anti-CD3, anti-CD20, and anti-CD68 stained sections (Figure 5). The epithelial invasion of these cells, except for plasmocytes, was also described. A score was assigned to each sample using the scoring system as follows: 0 = no detectable cells, 1 = Low presence of cells, without cluster; 2 = Mild presence of cells, without cluster; 3 = Numerous cells or clusters.

2.11 | Plaque Evaluation by Scanning Electron Microscopy (SEM)

Additionally, the quantity of plaque was evaluated semi-quantitatively on the retrieved experimental abutment using scanning electronic microscopy (TM3030, Hitachi High-Technologies Europe GmbH, Krefeld, Germany). After being gently separated from the peri-implant soft tissue with sterile dental tweezers, abutments were incubated for 2 h in 2.5% glutaraldehyde, rinsed with cacodylate buffer, incubated for 1 h in 1% OsO4, rinsed with pure water, and dehydrated in ethanol at 4°C. Then, each sample was fixed on an adhesive plaque and coated with gold. Images of the buccal and lingual aspects were used at low magnification (x50) and divided into three regions of interest: coronal, middle and apical. Each region was analyzed with a magnification between ×1000 and ×2600 to allow the identification of dental plaque or other biological components. Representative pictures of the 3 groups of abutments are presented in Figure 7. Each section was semi-quantitatively scored using a scale from 0 to 4, as follows: 0 = absence of plaque on the surface; 1 = superior to 0 and inferior or equal to 25% of plaque covering the surface; 2 = superior to 25 and inferior or equal to 50% of plaque covering the surface; 3 = superior to 50% and inferior or equal to 75% of plaque covering the surface; 4 = superior to 75% and inferior or equal to 100% of plaque covering the surface.

2.12 | Statistical Analysis

A sensitivity power analysis using the G*Power software (Version 3.1.9.3), allowing the calculation of an effect size according to a given sample size, indicated that, with 11 abutments per group, a difference in scoring of 1 (out of a scale varying from 0 to 3),

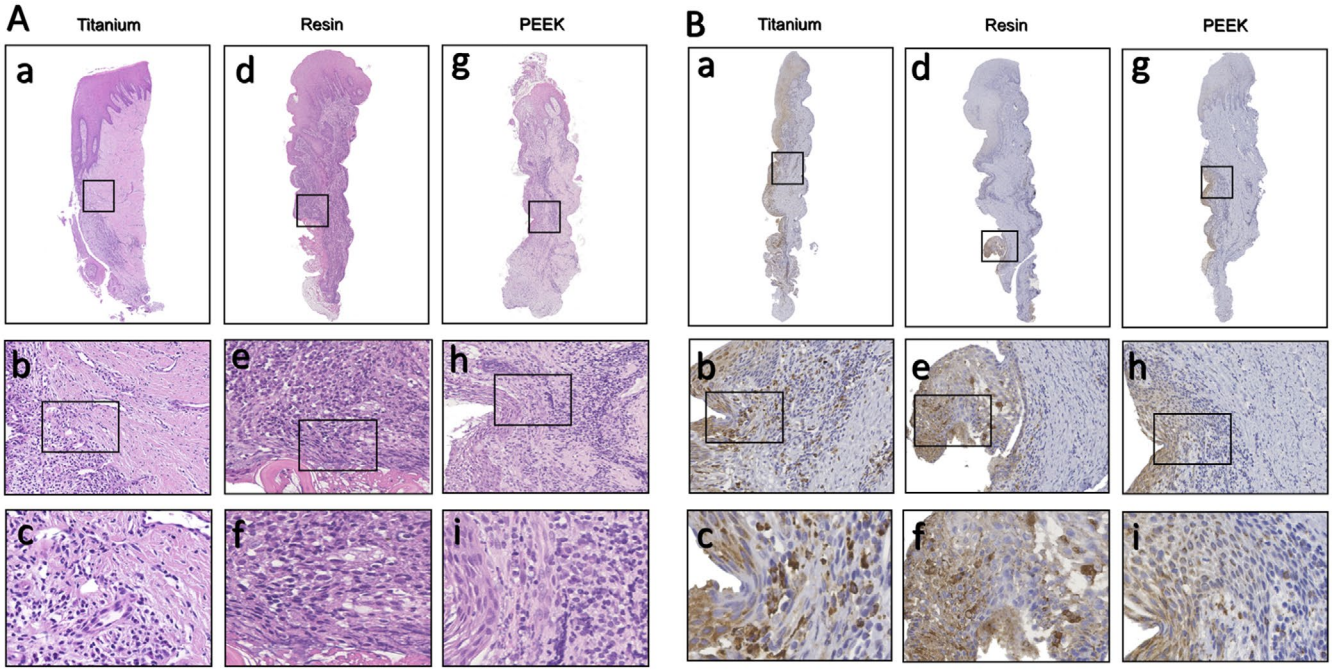

FIGURE 5 | Histological section of titanium, resin and PEEK abutments. (A) Haematoxylin–eosin staining: (a, b and c) Titanium low, 20× and 40× magnification; (d, e and f) resin low, 20× and 40× magnification; (g, h and i) PEEK low 20× and 40× magnification. (B) Anti-CD68 immunohistochemistry: (a, b and c) Titanium low, 20× and 40× magnification; (d, e, and f) resin low, 20× and 40× magnification; (g, h and i) PEEK low 20× and 40× magnification.

for at least one of the 5 studied markers (Plasmocytes, PMNs, B Cells, T cells, Macrophages), could be detected between the groups, with a power of 95%, a significance level of 5%, and a standard deviation of 0.65. Four additional abutments were included in case of technical failure. Under the null hypothesis, there is no difference in the level of inflammation between the 3 groups for none of the markers.

Results are presented as means and standard deviation (SD) for quantitative variables and as frequency tables for qualitative variables. Normality was assessed by the Shapiro–Wilk test. Comparisons between groups were made using the analysis of variance test (ANOVA) or Kruskal–Wallis test (non-parametric) for quantitative variables and the chi-square test for qualitative variables. The change in a quantitative variable between two times is studied by the paired Student's *t*-test or by the Wilcoxon signed ranks test. Generalised linear mixed models (GLMM) were used to analyse the effect of time, group and zone. Results are considered significant at the 5% uncertainty level ($p < 0.05$). Calculations were performed usingSAS version 9.4.

3 | Results

3.1 | Demographics and Site-Related Data

Out of the 37 implants initially included in the present study, two implants failed to osseointegrate and were therefore excluded. A total of 35 soft tissue samples were collected for immunohistological observation. Age, gender, as well as surgical parameters, were homogenous between the different groups as reported in detail in Table 1.

3.2 | Clinical Parameters

The analysis of clinical parameters (Table 2) showed a similar clinical full mouth plaque scores ($p = 0.77$). The evolution of the keratinised tissue height was similar between the groups at the buccal ($p = 0.37$) and the lingual ($p = 0.51$) aspects. Moreover, the soft tissue thickness was similar between the groups at the different measurement sites (buccal: $p = 0.28$, lingual: $p = 0.88$, mesial: $p = 0.69$, and distal: $p = 0.08$). The GLMM indicated no significant effect of the zone ($p = 0.20$) or the group ($p = 0.74$) but a significant effect of the time ($p = 0.01$) and the interaction between time and group ($p = 0.02$). There was a significant increase in the soft tissue thickness over time for the Re ($p < 0.0001$) and PEEK ($p = 0.01$) groups whereas for the Ti group soft tissue thickness was stable over time ($p = 0.58$).

3.3 | Radiological Analysis

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 higher in the Re group (0.82 ± 0.10 mm) than in the other two groups ($p = 0.04$). The complete data set is summarised in Table 3.

3.4 | Histological Descriptive Analysis

All the samples were characterised by a portion of epithelium and an underlying stroma. The thickness of epithelia varied from about 10 to 20 cell layers with some samples characterised by infiltrating inflammatory cells. Most of the connective

TABLE 1 | Patients and site-related characteristics.

	Titanium	Resin	PEEK	*p*
Number of samples	11	13	11	
Age (years) Mean ± SD	67.7 ± 8.9	58.8 ± 12.5	60.6 ± 12.3	0.17
Gender (M/F) *n* (%)	5 (45.5)/6 (54.5)	5 (38.5)/8 (61.5)	6 (54.5)/5 (45.5)	0.73
Implant position *n* (%)				
Maxilla	8 (72.7)	5 (38.5)	5 (45.5)	0.22
Mandible	3 (27.3)	8 (61.5)	6 (54.5)	
Flap design *n* (%)				
Flap	9 (81.8)	5 (38.5)	5 (45.5)	0.26
Mini-flap	2 (18.1)	7 (53.8)	5 (45.5)	
Flapless	0 (0.0)	1 (7.7)	1 (9.1)	

Note: Statistical tests: ANOVA, Chi-Square.

tissues were dense, rich in fibres and inflammatory cells were present with a diffuse or clustered distribution.

3.5 | Histological Semi-Quantitative Analysis

The analysis of the H&E sections showed a higher neutrophil infiltration in the epithelium of the Re samples compared to the Ti group ($p = 0.03$). However, the number of plasma cells and neutrophils in the connective tissues was similar between the groups ($p = 0.43$ and $p = 0.90$ respectively) (Figure 5A).

The anti-CD3 staining demonstrated a similar occurrence of T lymphocytes in the connective tissues and in the epithelium between the different groups ($p = 0.86$ and $p = 0.65$ respectively). Similarly, anti-CD20 labelling in the epithelia was similar in all groups ($p = 0.67$). However, in the connective tissue, the amount of B lymphocytes tended to be higher in the Re group, without being statistically significant ($p = 0.07$). Interestingly, anti-CD68 labelling revealed a higher amount of macrophages in the connective tissues in the Re group compared to the Ti group ($p = 0.04$; Figure 5B).

Anti-CD34 labelling allowed the quantification of blood vessels. The ratio of blood vessel area to total area was similar between all groups ($p = 0.73$). Similarly, the density of blood vessels (nb/mm^2) was also comparable ($p = 0.88$; Figure 6).

The complete dataset is summarised in Table 4.

3.6 | Plaque Analysis by SEM

One abutment was significantly damaged during removal and was therefore excluded. Electron microscopic analysis of the experimental abutments revealed a comparable biofilm scores between the three groups ($p = 0.20$; Table 5). The GLMM revealed no difference between groups, but indicated a significant effect of the zone ($p < 0.0001$). The plaque accumulation was significantly more important in the coronal third and middle third than for the apical third ($p < 0.01$) while there was no difference between coronal third and middle third ($p = 0.09$).

4 | Discussion

In this randomised comparative study, the human host-related response towards transmucosal abutments composed of Ti, Re, and PEEK was compared for the first time. A stronger non-specific immune response (neutrophils and macrophages) was associated with the experimental Re abutments when compared to Ti and PEEK. Additionally, the implants that received the Re components were associated to a higher peri-implant bone remodeling.

Unfortunately, the literature on the specific biological interaction between abutment material and the soft tissues is scarce. Initially, Abrahamsson et al. demonstrated in animal studies the limited capacities of the peri-implant soft tissues to establish a functional connective attachment around gold abutments, resulting in bone remodeling and peri-implant mucosal recession (Abrahamsson et al. 1998). A few authors have also explored the inflammatory reaction that occurs during the tissue healing process after implant placement in patients. The healing response of peri-implant soft tissue with titanium abutments was investigated from baseline up to 12 weeks (Tomasi et al. 2016). The immunohistochemical analysis showed a progressive decrease in the amount of lymphocytes and blood vessels, leading to a resolution of the inflammation after a period of 8 weeks, which is comparable to the titanium group of the present study. More recently, Serichetaphongse and coworkers aimed to evaluate the peri-implant soft tissue integration at 8 weeks with titanium, zirconia and gold abutments and they found higher amounts of T and B lymphocytes as well as macrophages in the gold group when compared to titanium or zirconia (Serichetaphongse et al. 2020).

Among the factors impairing peri-implant soft tissue health, the impact of dental plaque has been widely demonstrated (Jepsen et al. 2015; Schwarz et al. 2018). Indeed, various authors have emphasised the higher tendency of biofilm

TABLE 2 | Clinical parameters, including full mouth plaque scores and soft tissue variations.

	Titanium	Resin	PEEK	*p*
Number of samples	11	13	11	
Scoring of plaque *n* (%)				
0	8 (72.7)	11 (84.6)	10 (90.9)	
< 25	2 (18.2)	1 (7.7)	1 (9.1)	0.77
> 25	1 (9.1)	1 (7.7)	0 (0.0)	
Keratinised tissue height (mm)				
T2–T1 Mean ± SD				
Buccal	−0.05 ± 1.82	−0.46 ± 1.71	0.14 ± 1.07	0.37
Lingual	−0.33 ± 0.58	0.43 ± 1.51	0.00 ± 1.10	0.51
Soft tissue thickness (mm)				
T1 Baseline mean ± SD				
Buccal	3.15 ± 0.47	2.69 ± 0.48	3.14 ± 0.45	0.08
Lingual	3.15 ± 0.67	2.69 ± 0.48	2.86 ± 0.55	0.26
Mesial	3.25 ± 0.54	2.69 ± 0.48	3.23 ± 0.41	0.02
Distal	3.10 ± 0.39	2.73 ± 0.53	3.05 ± 0.35	0.23
T2 8 weeks mean ± SD				
Buccal	2.95 ± 0.96	3.23 ± 1.25	3.27 ± 1.10	0.72
Lingual	3.55 ± 0.76	3.19 ± 1.11	3.45 ± 1.27	0.49
Mesial	3.60 ± 0.97	3.69 ± 1.60	3.82 ± 1.72	0.96
Distal	2.95 ± 0.69	3.96 ± 1.88	3.64 ± 1.31	0.27
Soft tissue thickness (mm)				
T2–T1 mean ± SD				
Buccal	−0.20 ± 0.76	0.54 ± 1.28	0.14 ± 1.00	0.28
Lingual	0.40 ± 0.94	0.50 ± 1.23	0.59 ± 0.89	0.88
Mesial	0.35 ± 0.88	1.00 ± 1.68	0.59 ± 1.60	0.69
Distal	−0.15 ± 0.53	1.23 ± 2.00	0.59 ± 1.04	0.08

Note: Statistical tests: Chi-Square, Kruskal–Wallis.

accumulation on resin components compared to titanium (Cazzaniga et al. 2015; Kanao et al. 2013). However, in the present study, the clinical plaque index and the quantity of dental plaque analysed by SEM on the experimental abutments were similar between the groups. Therefore, the differences in inflammation scores cannot be attributed to plaque accumulation and this suggests that the higher inflammatory cells scores found in the peri-implant soft tissues in contact with the resin might be caused by the material. Thus, inflammation in the Re group might be attributed to the release of specific monomers, a phenomenon previously described in vitro and in vivo by several authors (Schedle et al. 1998; Wataha et al. 1994). Indeed, it has been demonstrated in various in vitro studies that the cytotoxicity of composites and dental adhesives tended to increase during the days following polymerisation, and can persist up to 8 weeks (Ausiello et al. 2013; Bouillaguet et al. 2002; Şişman et al. 2016). Several studies have also reported the cytotoxicity of dental adhesives on different periodontal cell types such as fibroblasts, alveolar odontoblasts and macrophages (D'Alpino et al. 2017; Pagano et al. 2021; Porto et al. 2011). Despite the differences in composition, especially in terms of charges, the cytotoxic effect of composite resins and adhesives is similar and is induced by the release of monomers (Bapat et al. 2021). In a recent histological study in humans, assessing the toxicity of composite restorations on the surrounding soft tissues, the authors reported the presence of inflammatory infiltrates mainly composed of macrophages, lymphocytes, mast cells, and, to a smaller extent, neutrophils (Bertoldi et al. 2020). This local immune response was attributed to the continuous release of different monomers contained in the adhesives and composites, such as triethylene glycol dimethacrylate (TEGDMA), urethane acrylate methacrylate (UDMA), glycerol phosphate dimethacrylate, known to induce lipid peroxidation of microsomes and solubilisation

TABLE 3 | Peri-implant bone loss between baseline and 8 weeks.

	Titanium	Resin	PEEK	*p*
Bone level (mm)				
T1 baseline mean ± SD				
Mesial	0.19 ± 0.43	0.15 ± 0.38	0.03 ± 0.10	0.76
Distal	0.44 ± 0.57	0.21 ± 0.33	0.09 ± 0.29	0.16
T2 8 weeks mean ± SD				
Mesial	0.30 ± 0.72	1.51 ± 1.84	0.40 ± 0.42	0.13
Distal	0.23 ± 0.46	1.39 ± 1.46	0.34 ± 0.51	0.06
Average bone loss (mm)				
T2–T1 mean ± SD	0.07 ± 0.18	0.82 ± 0.10	0.27 ± 0.30	0.04

Note: Statistical tests Kruskal–Wallis, Dwass-Steel-Critchlow-Fligner.

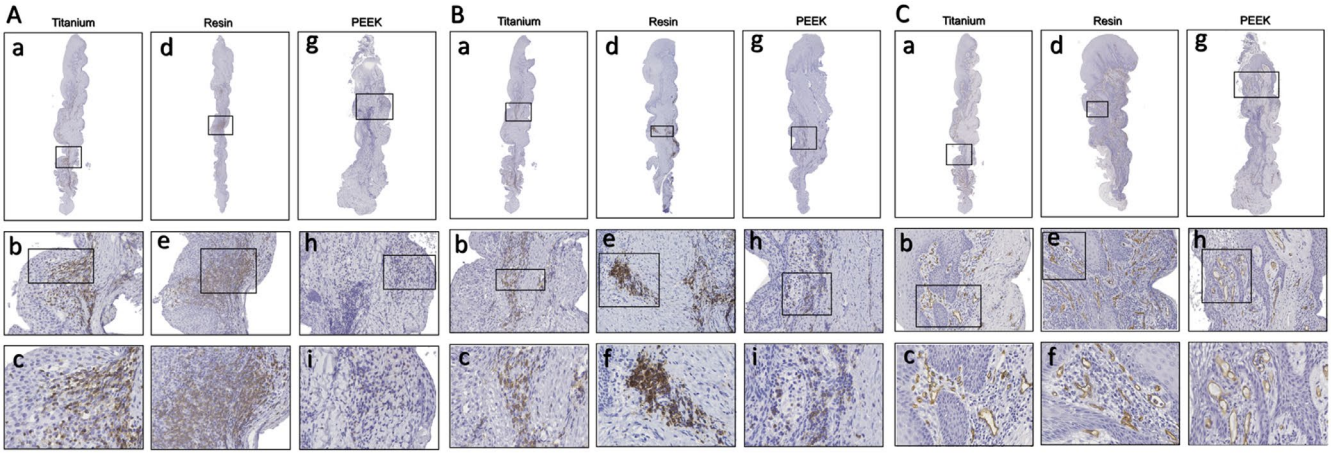

FIGURE 6 | Histological section of titanium, resin and PEEK abutments. (A) Anti-CD3, (B) Anti-CD20 and (C) Anti-CD34 immunohistochemistry: (a, b and c) Titanium low, 20× and 40× magnification; (d, e and f) Resin low, 20× and 40× magnification; (g, h and i) PEEK low 20× and 40× magnification.

of the lipid bilayer of cell membranes (Geurtsen 2000). The increased inflammatory reaction observed in the resin group in the present study may be explained by a similar phenomenon. The higher proportion of macrophages ($p = 0.04$) found in the connective tissues of the Re samples may also question the biocompatibility of transmucosal resin as these cells have an important role in the phagocytosis of pathogens or foreign bodies (Murray and Wynn 2011). Several animal studies, using different models, have revealed the importance of macrophages in tissue regeneration (Aurora et al. 2014; Huang et al. 2013) and the polarisation of macrophages has been emphasised (Cassetta, Cassol, and Poli 2011; Murray et al. 2014; Sica et al. 2015). Type M1 macrophages are pro-inflammatory cells and interfere with tissue healing due to their oxidative activity. It has been shown that M1 macrophages are responsible for osteolytic activity in periodontal and peri-implantitis lesions (Zhou et al. 2019). In contrast, M2 macrophages are anti-inflammatory and play a role in the repair of damaged tissues (Bashir et al. 2016; Murray et al. 2014; Sica et al. 2015; Wang, Liang, and Zen 2014). Unfortunately, the method used in the present study did not allow to identify the type of macrophages present in the peri-implant soft tissue, which represents an important limitation of the present study. We believe that this aspect should be further investigated.

According to the present results, the use of resin, such as flow composites for transmucosal implant provisional crowns may be questioned, especially on bone-level implants, as they showed a lower level of biocompatibility when compared to Ti or PEEK and lead to an increase early peri-implant bone loss. The increase peri-implant soft tissue thickness from baseline to 8 weeks might be the results of a localised inflammation and/or the consequence of peri-implant bone remodeling.

However, the present study suffers from several limitations and the results should be interpreted cautiously. From a statistical perspective, the implant was used as the statistical unit, although patient clustering could potentially influence implant outcomes (Chrcanovic et al. 2017). However, the effect of patient clustering is unlikely to significantly impact the results, as only two patients received more than one implant. Although the amount of plaque was similar in the 3 groups, clinical signs

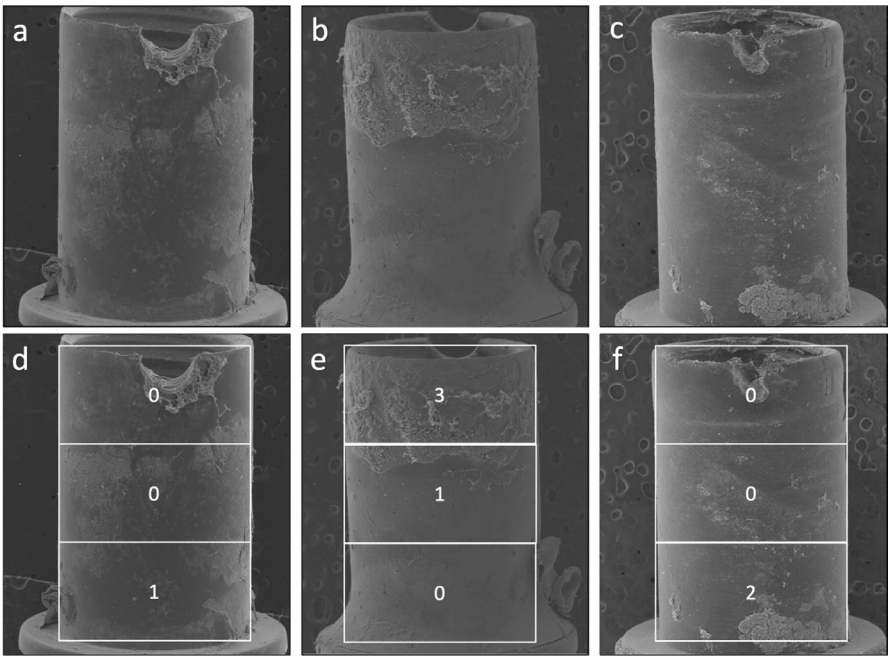

FIGURE 7 | Representative scanning electron microscope of Ti, resin and PEEK abutments. (a) Ti, (b) resin, (c) PEEK and (d, e and f) respective semi-quantitative analyses of three regions of interest: Cononal, middle and apical.

TABLE 4 | Histological scoring of CD3+, CD20+, CD68+ cells, neutrophils, plasmocytes and blood vessels quantification.

	Titanium	Resin	PEEK	*p*
Connective tissue mean ± SD				
$CD3^+$	1.55 ± 0.69	1.62 ± 0.65	1.64 ± 0.51	0.86
$CD20^+$	1.27 ± 0.91	2.00 ± 0.82	1.27 ± 1.01	0.07
$CD68^+$	1.18 ± 0.60	1.85 ± 0.56	1.82 ± 0.75	0.04
Neutrophils	1.18 ± 0.75	1.23 ± 0.73	0.91 ± 0.54	0.43
Plasmocytes	1.64 ± 0.81	1.46 ± 0.78	1.64 ± 0.81	0.90
Epithelium mean ± SD				
$CD3^+$	1.00 ± 0.78	1.23 ± 0.73	1.27 ± 0.65	0.65
$CD20^+$	0.18 ± 0.41	0.23 ± 0.44	0.09 ± 0.30	0.67
$CD68^+$	0.36 ± 0.67	0.38 ± 0.65	0.27 ± 0.47	0.96
Neutrophils	0.45 ± 0.69	1.38 ± 0.96	1.09 ± 0.70	0.03
Blood vessels area (%)	5.56 ± 3.33	4.74 ± 3.00	4.65 ± 1.74	0.73
Blood vessels density (Nb/mm^2)	183.7 ± 87.8	180.3 ± 110.4	160.6 ± 49.7	0.88

Note: Statistical tests: Kruskal–Wallis, Dwass-Steel-Critchlow-Fligner.
Abbreviations: $CD3^+$, T lymphocytes; $CD20^+$, B lymphocytes; $CD68^+$, macrophages.

of inflammation such as bleeding on probing as well as pocket depth could not be measured and it remains uncertain whether the histological signs of inflammation relate directly or indirectly to the materials or peri-implant pathogens. Another limitation of the present method relates to the semi-quantitative scoring of the sections; a computer-based full quantitative assessment may provide more accurate results. Moreover, the use of a specific marker for neutrophils and plasmocytes (anti-MPO and anti-CD138) would also have been preferable in terms of accuracy to the use of conventional HE staining. Finally, radiographs were not standardised and it can lead to substantial errors during the reading. To increase the accuracy of the evaluation and normalise the measurement, each radiograph was calibrated using the known distance between implant treads. Moreover, to mitigate reading bias, two examiners performed the measurements.

TABLE 5 | Plaque index evaluated by SEM analysis.

	Titanium	Resin	PEEK
Plaque index mean ± SD			
Numbers of abutments	11	12	10
Coronal third	1.55 ± 1.21	2.58 ± 1.38	2.10 ± 1.37
Middle third	1.09 ± 1.14	1.67 ± 1.30	2.30 ± 1.64
Apical third	0.45 ± 0.69	0.33 ± 0.65	0.70 ± 0.95

5 | Conclusion

Within the limitations of this study, the following conclusions can be made:

- The trans-mucosal use of resin on implant components induced a higher macrophage and neutrophil activity and higher peri-implant bone loss compared to Ti or PEEK abutments.
- Using resin material as a transmucosal healing abutment should be carefully considered.
- Further investigations are needed to understand the impact of the resin on the host immune response.

Author Contributions

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

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Research data are not shared due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.