

Opinion paper

Peri-implant soft tissue integration in humans – influence of materials: A study protocol for a randomised controlled trial and a pilot study results

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ABSTRACT

Background: Recently, there has been a growing interest in mucointegration as the formation of an early and long-standing soft tissue barrier seems essential for both the initial healing and long-term implant survival.

Aim: To develop an experimental method to characterize the mucointegration of different transgingival materials (titanium (Ti), polyetheretherketone (PEEK), polymethylmethacrylate (PMMA), zirconia (Zi), polymer infiltrated ceramic network (PICN), cobalt-chrome (Co–Cr), and lithium disilicate (LD)) in a human model.

Methods: The study is designed as a multi-part randomized controlled clinical trial. Ninety bone level Straumann implants will randomly receive an experimental, custom-made abutment to allow for the removal of the abutment together with the surrounding soft tissues using a punch biopsy device at 8 weeks of healing (10 per material). The specimens will be further processed for non-decalcified histology, followed by histomorphometric analysis. The same protocol will be used for additional 90 implants-abutments, but during harvesting, soft tissues will be separated from the abutment and processed for immunohistochemistry in order to study tissue inflammation and vascularization, while the abutments will undergo SEM analysis. Additionally, *in vitro* analyses, including SEM and profilometry, will be performed in order to characterize surface topography of all experimental materials.

Conclusion: The limited number of pilot samples presented herein indicate that the use of custom-made abutments in humans is a reproducible method to study peri-implant soft tissue integration. This further intensifies the rationale to compare different abutment materials, used as transgingival components in daily practice, under the same conditions.

1. Introduction

Osseointegration used to be the main concern regarding dental implant integration during the last decades [1–4]. As osseointegration can be successfully achieved with various implant systems nowadays, the interest has slowly shifted towards peri-implant soft tissue integration, i.e., mucointegration [5]. Indeed, the formation of an early and long-standing soft tissue barrier seems essential for both the initial healing and long-term implant survival [6–8]. Soft tissue-friendly prosthodontics have been deemed necessary in order to avoid

breakdown in the equilibrium that could lead to bacterial penetration, and consequently to peri-implant disease or even implant loss.

The findings from both *in vitro* and *in vivo* studies have suggested that physico-chemical material characteristics of the abutment may significantly influence the integration of the peri-implant soft tissues [9]. It was previously reported that abutment surface properties influence the adhesion, proliferation, and colonization of both cells and microorganisms [10], and are, therefore, considered the key influencing factors of a stable and healthy transmucosal seal. Although titanium has been the material of choice for abutments due to its biocompatibility and

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predictability demonstrated in many clinical studies and reviews [11–13], titanium-free abutments have been gaining popularity lately. With the invention and use of other more esthetic materials, as well as the esthetic expectations of the patients that have become clearly higher through the years [14], and their requests for faster treatments, there is a clear need to compare the existing materials in terms of its effects on both hard and soft peri-implant tissues. Consequently, many different materials are used as supragingival components that come in contact with the soft tissues around restored implants, either for immediate loading with provisionals crowns or for the definitive prosthodontic restorations.

Although valuable insights about several materials used supra-gingivally have been obtained from both *in vitro* and preclinical *in vivo* studies [15–23], considerable limitations inherent to these type of studies make the extrapolations of the results to a clinical setting difficult and rather unpredictable. However, there is limited clinical data comparing peri-implant soft tissue integration of the existing restorative materials and materials used for implant abutments. One of the first human studies reported that the peri-implant soft tissue formed at the experimental titanium one-piece mini-implants was of a character similar to that described in animal studies [24]. Similar findings were obtained by Tomasi et al. [11], who reported on the dimensional and qualitative characteristics of the mucosa around titanium abutments. Additional studies compared titanium and zirconia abutments [10,25,26] or titanium and PEEK abutments [27] in human, although histological assessment of healing was not always performed. There is little clinical data regarding other materials frequently used in daily practice for provisional crowns for immediate loading at implant placement, such as polymethylmethacrylate, for instance. Overall, the robust randomized control trials that could compare the bulk of the materials used by practitioners nowadays, in a proper clinical setting and under the same conditions, are still missing.

This project aims to develop the protocol to characterize the mucosal integration of seven different abutment materials (titanium (Ti), polymethylmethacrylate (PMMA), polyetheretherketone (PEEK), zirconia (Zi), cobalt-chrome (Co–Cr), and lithium disilicate (LD) in a human model. Additionally, Zi abutments with three different surface roughness will be tested.

In order to validate the experimental method and the sample size, a pilot study using titanium abutments was initially conducted.

2. Methods/design

2.1. Study design

The overall project will encompass 3 distinct, stand-alone randomized controlled clinical trials (Part I, II, III), which will be based on the same study design (explained further below; Table 1), but will involve different abutment materials:

PART I

- 1 Ti Grade 4
- 2 PMMA (Multilayer shaded PMMA discs, Dentsply Sirona, York, PA, USA)
- 3 PEEK (breCAM.BioHPP, Bredent, Senden, Germany)

PART II

- 1 Zi – machined - as delivered by the manufacturer (0.2 µm)
- 2 Zi – ultra-polished surface (0.05 µm)
- 3 Zi – rough surface (0.5–1 µm)

PART III

- 1 PICN
- 2 Co–Cr
- 3 LD

Each of these parts will include one preclinical study and 2 RCTs:

2.1.1. Raw abutment characterization – preclinical study

The surface topography of all materials to be used in the present project will be characterized using scanning electron microscopy (SEM) and profilometry.

The same study protocol will be used for PART I, PART II, and PART III.

2.1.2. Non-decalcified histology – RCT

The custom-made experimental abutments will be used to allow for the removal of the abutment together with the surrounding soft tissues using a punch biopsy device at 8 weeks of healing. The specimens will be further processed for non-decalcified histology. Additional TEM analysis will be performed for PEEK samples.

The same study protocol will be used for PART I, PART II, and PART III.

Table 1
Overall project & sample distribution.

N° of samples	Ti	PMMA	PEEK	PART I Total	Zi machined	Zi ultra- polished	Zi rough	PART II Total	PICN	Co-Cr	LD	PART III Total	T O T A L
Raw abutment characterization	3	3	3	9	3	3	3	9	3	3	3	9	27
Non-decalcified histology	10	10	10	30	10	10	10	30	10	10	10	30	90
TEM			10	10									10
SEM / IHC	10	10	10	30	10	10	10	30	10	10	10	30	90

Ti – titanium; PMMA – polymethylmethacrylate; PEEK – polyetheretherketone; Zi – zirconia; PICN – polymer infiltrated ceramic network; Co-Cr – cobalt-chrome; LD – lithium disilicate; TEM – transmission electron microscopy; SEM – scanning electron microscopy; IHC – immunohistochemistry.

2.1.3. SEM/immunohistochemistry – RCT

The custom-made experimental abutments will be retrieved using punch biopsy device, but soft tissues will be separated from the abutment in order to evaluate the cell adhesion on the abutment surface using SEM, while the surrounding tissues will be processed for immunohistochemistry (IHC) in order to study proinflammatory markers.

The same study protocol will be used for PART I, PART II, and PART III.

Patient inclusion will be performed in 2 centers: University Hospital Liège, Liège, Belgium (for PARTS I, II, and III) and a private clinic “Dr. Happe und Kollegen”, Münster, Germany (for PART II). Overall, 180 participants will be enrolled (that is, 30 per each RCT and since each study PART contains 2 RCTs, 60 participants will be enrolled for each study PART; Table 1).

2.2. Study protocol

2.2.1. Raw abutment characterization

In vitro characterization of materials used for abutments will include evaluation of surface characteristics using SEM and Profilometry. A total of 27 samples will be characterized (9 per each study PART; Table 1).

2.2.1.1. SEM. The surface morphology of the abutments will be evaluated using an analytical benchtop scanning electron microscope (TM3030, Hitachi High-Technologies Europe GmbH, Krefeld, Germany). The samples will be mounted on aluminium stubs with conductive carbon tape and images will be taken with an accelerating voltage of 15 kV. Prior to imaging, the polymeric samples, such as PEEK and PMMA, will be sputter-coated with gold (Cressington 108 Auto, Cressington Scientific Instruments, Watford, UK). The images obtained will serve to describe materials' external morphology (texture) as well as the cleanness of a surface.

2.2.1.2. Profilometry. Profilometry measurements will be performed on an S Neox optical profiler from Sensofar (Spain) controlled with the SensoSCAN 6.3 software, also from Sensofar. Samples will be imaged with an EPI 50× objective using the confocal mode at six random non-overlapping positions with an area of 350.88 Å~ 264.19 μm² (1360 px Å~ 1024 px). Surface parameters will be obtained from image analysis and processing will be done using SensoMap Standard 7.3 (Sensofar, Digital Surf's Mountains Technology®, Spain).

2.2.2. Non-decalcified histology – RCT

2.2.2.1. RCT – study design. Thirty implants will be randomly allocated to one of the 3 experimental materials. Therefore, 10 abutments per condition will be available for further analyses.

2.2.2.2. Randomization. Experimental abutments will be randomly allocated so that no more than two different abutment materials are placed in the same patient. Randomization will be performed using a table containing multiple lines of random presets of six combinations in each line, formed with letters A, B, and C pertaining to the three different experimental abutments (e.g. B, BC, AC, A, C, AB, etc.), taking into account that a patient may receive more than one implant/abutment. For instance, if a patient were to receive one implant, based on the above example, he/she will

Receive abutment B; the next patient receiving one implant would receive abutment A; the next patient receiving two implants will receive abutments B&C, etc.

2.2.2.3. Inclusion criteria.

- Participants have to voluntarily sign the informed consent form before any study related action

- Patients aged 18 or over
- Patients with one or more missing teeth in the maxillary or mandible area, seeking implant therapy
- Men/women
- Patients in good systemic health (ASA I/II) and no contraindication for oral surgical interventions
- Patients requiring a replacement of missing teeth; the tooth at the implant site(s) must have been extracted or lost at least 12 weeks before the date of implantation
- At least 3 mm of fibrous mucosa in the bucco-lingual dimension
- Full mouth plaque score (FMPI) lower than or equal to 25%
- At least a diameter of 4 mm for the implant (regular d diameter)

2.2.2.4. Exclusion criteria.

- Autoimmune disease requiring medical treatment
- Medical conditions requiring prolonged use of steroids
- Use of Bisphosphonates intravenously or more than 3 years of oral use
- Infection (local or systemic) – patients with gingivitis or active local infection will undergo a medical treatment prior to the entrance to the study, and each individual will be evaluated for suitability; in case of a systemic infection, the evaluation will be based on medical anamneses, and if necessary, a patient will be referred to relevant medical tests
- Current pregnancy or breastfeeding women
- Alcoholism or chronic drug abuse
- Immunocompromised patients
- Uncontrolled diabetes
- Smokers
- Prisoners
- Implant's diameter under 4 mm (narrow implant)
- Conditions or circumstances, in the opinion of the investigator, which would prevent completion of the study or interference with analysis of study results, such as history of non-compliance, or unreliability

2.2.2.5. Local exclusion criteria.

- Sites treated with socket preservation techniques
- Untreated local inflammation

- Mucosal diseases or oral lesions
- History of local irradiation therapy in head-neck area
- Persistent intraoral infection
- Patients with bad oral hygiene
- Patients unmotivated for standard home-care

2.2.2.6. Materials.

- Implants

Regular Bone Level or Bone Level Tapered Implants featuring the CrossFit® connection (4.1 or 4.8 mm) will be used in the study (Straumann®, Straumann, Basel, Switzerland).

- Abutment materials

The CAD/CAM experimental abutments will be used as delivered by the manufacturer without any further surface modifications in the PART I and PART III of the project; in the PART II, Zi abutments will undergo in-lab surface modifications in order to achieve different surface roughness.

A dental manufacturing company ProScan (Zonhoven, Belgium) will produce the abutments using the above mentioned materials. A custom-made abutment design has been developed in order to allow sample harvesting using a guide that is screwed on the top of the experimental abutment at the time of the abutment retrieval (the retrieval of the abutment together with the ring of soft tissues attached to its surface in one bloc using punch biopsy) (Fig. 1). In cases when the material does not allow full abutments to be produced, Ti will be used as a base and the material will be used as a 'sleeve' that comes in contact with the soft tissues, as illustrated in Fig. 1d.

- Punch device

A circular punch device (Acu-Punch, Acuderm inc., Milan, Italy), 4 mm wide, will be forced apically so that it encompasses the punch guide of 3.8 mm in order to harvest the tissues surrounding the experimental abutment.

2.2.2.7. Screening and consent. Prospective participants will be screened for enrolment in the study according to the criteria listed above. Only participants that comply with the inclusion criteria will be enrolled in the study. Potential participants at each study site will be provided with written information concerning the study, explaining the

study requirements, and possible risks. Study coordinators/investigators will ensure that potential participants understand the information provided, and will review requirements and potential risks.

Individuals agreeing to participate in the study will have to sign the informed consent form according to local regulations. All signed consent forms will be maintained in the investigator's file at the study site.

During the screening visit, patients will be examined clinically and they will undergo a cone beam CT scan with a cotton roll on the buccal side in order to be able to evaluate the buccal soft tissues thickness, to assess the bone dimensions in the area of interest, and to assure that they comply with the requirements of at least 6 mm in width (the bucco-lingual dimension) and at least 8 mm in height (the apico-coronal dimension). This is a routine test for patients seeking dental implant therapy. Previous bone regeneration, except sinus lift, is one of exclusion criteria.

The approval of the institutional Ethical Committee has already been obtained for PART I (B707201628072). All RCTs will be registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (<https://clinicaltrials.gov>).

2.2.2.8. Surgical procedure. All subjects will receive preoperative antibiotic (amoxicillin 2 g, or if allergic, clindamycin 600 mg). After local anaesthesia, if necessary, a crestal incision will be made above the treatment site, and mucoperiosteal flaps will be reflected to allow access to the site. Alternatively, a punch biopsy and a flapless approach for implant placement can be considered; the flap design decision-making will be entrusted to the surgeons.

The implantation procedure will be carried out according to a standard surgical protocol, and according to the manufacturer's protocol. All implants will have to reach an insertion torque higher than 15 Ncm. Implants will be randomly assigned to one of the study groups after flap opening. The abutment will be placed in a non-submerged approach and tightened at 10 N/cm for a period of 8 weeks in total. The mucoperiosteal flaps will be sutured with non-resorbable interrupted sutures. The abutment screw access channel will be closed with a layer of Teflon tape to isolate the screw head from the composite used to seal the access channel (Telio, Ivoclar Vivadent, Ellwangen, Germany) (Fig. 2).

A standard periapical x-ray will be taken, in order to index the level of the implant in the apico-coronal direction. The patients will be instructed to rinse twice daily with an aqueous solution of 0.2% chlorhexidine. In addition, analgesics (ibuprofen 400 mg, up to 4/d) will be prescribed for the following days according to individual needs. Patients will be also instructed to refrain from mechanical plaque removal in the area of implantation for 1 week. The sutures will be removed after 10–14 days. After suture removal, they will stop using mouthwashes and will be instructed to apply standard hygiene procedures.

2.2.2.9. Harvesting procedure. The specimens will be retrieved 8 weeks after implant placement, following local anaesthesia. The abutments

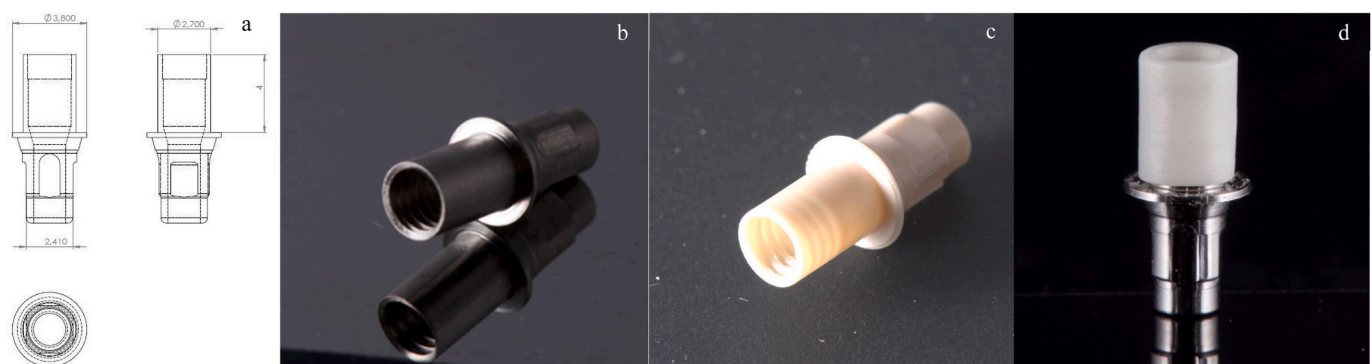


Fig. 1. A custom-made abutment design (a); a custom-made titanium abutment (b), PEEK abutment (c), and a custom-made zirconia abutment: zirconia 'sleeve' on a Ti base.

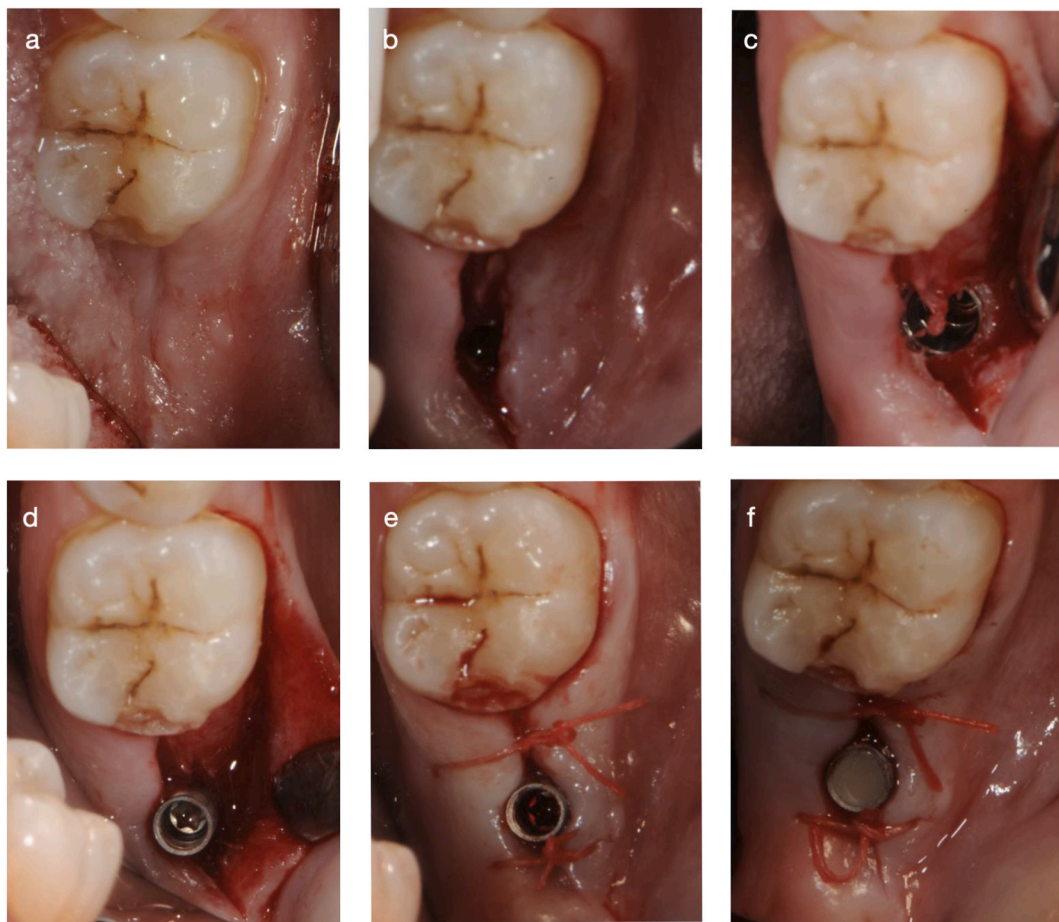


Fig. 2. Intra-operative images: surgical site (a), implant bed preparation (b), implant placement (c), custom-made abutment placement (d), suturing (e), closing of abutment screw access channel (f).

will be replaced by an SRA abutment (Straumann®, Straumann, Basel, Switzerland). During the removing procedure, a custom-made guide and then a punch biopsy device will be used to retrieve a circumferential biopsy so that peri-implant soft tissues around the abutment are harvested together with the abutment (Fig. 3).

2.2.2.10. Data collection. The following clinical measurements will be taken at the time of abutment connection and at follow-ups:

- Keratinized tissue height buccally and lingually – from the most apical point of the gingival margin to the mucogingival junction at the mid-buccal point (using a periodontal probe);
- Soft tissue thickness above the bone crest;
- Periodontal biotype at natural adjacent teeth (according to De Rouck et al. [28]);
- Peri-implant plaque index (PI);
- Presence of suppuration and any adverse events will be noted.

Clinical photography and intra-oral radiographs using the parallel technique will be taken at the time of the experimental abutment removal at 8-week follow-up.

2.2.2.11. Histology. The samples retrieved with a punch biopsy and containing the abutment and the surrounding soft tissue will be

processed for non-decalcified histology using polymethacrylate (PMMA). Briefly, after fixation for 2 days in a 4% formaldehyde solution, the samples will be dehydrated in graded series of ethanol followed by xylene. Thereafter, the samples will be embedded in polymethylmethacrylate (Merck). The resulting resin blocks will be cut vertically parallel to the abutment axis with a diamond-coated saw (VC-50, Leco) in a mesio-distal direction and once again in a bucco-oral direction. The sections will be ground to a final thickness of 150 µm (Pedemax-2, Struers) and stained with Toluidin Blue-Fuchsin. The sections will be scanned at high resolution with a Zeiss microscope (Axio Imager. M2, Zeiss).

2.2.2.12. Histomorphometry. These digital images are used to locate anatomical landmarks. Histometric measurements to determine the dimensions of the biological width (i.e. vertical distances of sulcus depth, epithelial component and soft connective tissue component) will be carried out by using an image analysis software (ZEN pro 2012, Zeiss).

2.2.2.13. Transmission electron microscopy (TEM). The specimens will be infiltrated with resin and 70 nm ultrathin sections will be cut with diamond knives mounted on a Leica EM UC6 microtome. The sections will be cut through the intact interface between PEEK abutments and the peri-implant soft tissues. The sections will be examined in a Supra 40 VP SEM equipped with a TEM detector at magnification $\times 1'000$ to $200'000$.

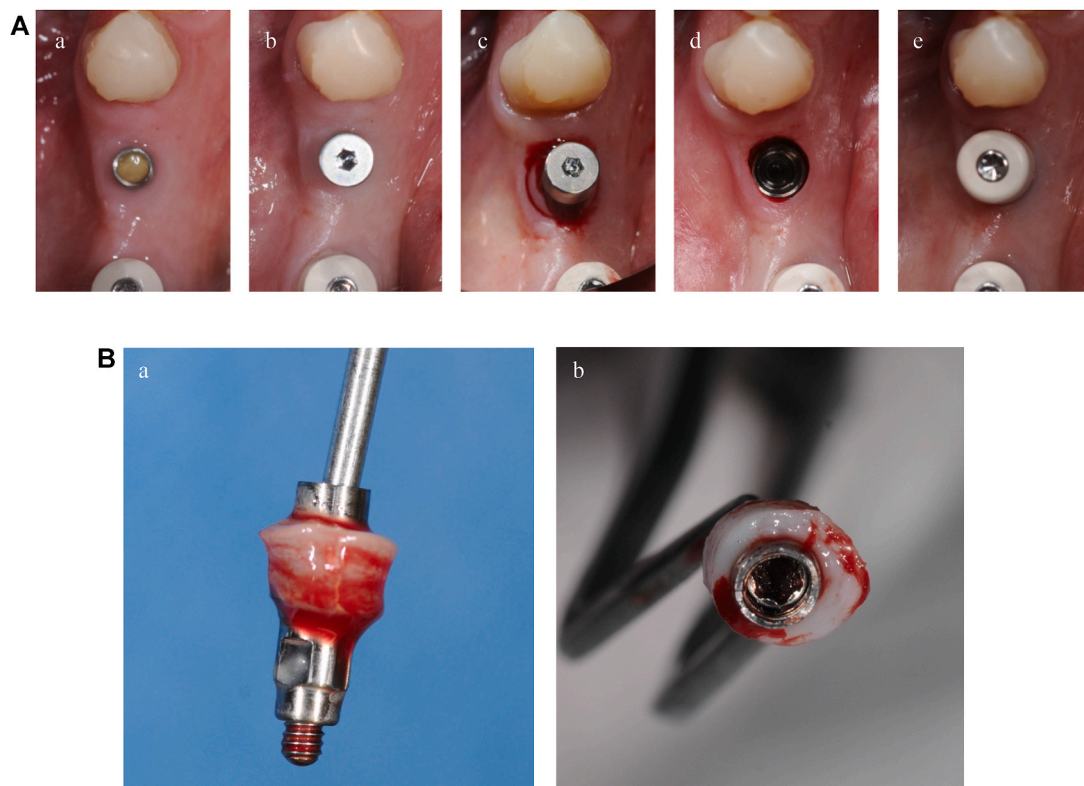


Fig. 3. a. Harvesting procedure (occlusal view): a custom-made abutment in place (a); a custommade guide corresponding to the diameter of the punch device is screwed on the experimental abutment in order to facilitate the harvesting procedure (b); tissues after using a punch biopsy device (c); SRA abutment (d) and protecting cap in place (e).

The images obtained will serve to describe the adherence mechanisms of both the junctional epithelium and the peri-implant connective tissue to the abutment surfaces.

2.2.3. SEM/immunohistochemistry – RCT

2.2.3.1. RCT – study design. Thirty implants will be randomly allocated to one of the 3 experimental materials. Therefore, 10 abutments per condition will be available for further analyses.

The same surgical protocol as described for B| Non-decalcified histology will be applied up to the harvesting procedure.

2.2.3.2. Harvesting procedure. Once the experimental abutment is retrieved using the punch device, the tissue biopsy will be detached from the abutment by pulling the ring out with a micro tweezer instrument. The biopsy will be subjected to immunochemistry (IHC). Additionally, the experimental abutment will be subjected to SEM in order to evaluate the cell adhesion on the abutment surface (Fig. 4).

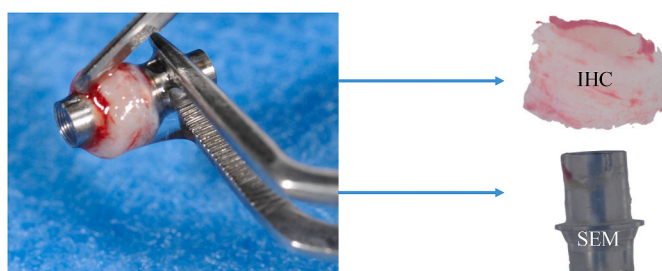


Fig. 4. Soft tissues and the experimental abutment will be separated and subjected to IHC and SEM, respectively.

2.2.3.3. SEM analysis. The abutments will be fixed in a 2.5% glutaraldehyde in distilled water during 1–2 h at 0–4 °C. Thereafter, the samples will be rinsed with distilled water during 10–20 min at 0–4 °C. A second fixation will be performed with 1–4% osmium tetroxide in distilled water during 1–2 h at 0–4 °C. Thereafter, the samples will be rinsed with distilled water during 10–20 min at 0–4 °C and then dehydrated during 10 min in crescent ethanol bath (25%, 50%, 70–75%, 90–95%, 100%) at 0–4 °C. For the analysis, the abutments will be mounted on specimen stub with silver paste and coated with gold/palladium alloy. A qualitative analysis will be performed, and if possible, an evaluation of the percentage of surface covered with cells will be performed.

2.2.3.4. IHC analysis. The soft tissue samples will be immediately fixed in a PLP solution (0.01 M periodate, 0.75 M Lysine, 2% paraformaldehyde) (McLean & Nakane, 1974, Rosendren et al., 1994) for 3 h at room temperature. Thereafter, the samples will be rinsed in 10% sucrose-phosphate-buffered saline overnight. Fixed soft tissue biopsies will be embedded and frozen in 2-methylbutane in liquid nitrogen and stored at –70 °C until sectioning. Frozen sections will be cut and fixed in cold acetone and stored at –70 °C. Endogenous peroxidase activity will be blocked by incubation with 0.3% H₂O₂. The immunoincubation will be performed with the following antibodies:

- CD3: T-cells
- CD20: B-cells
- CD68: monocytes/macrophages
- CD34: blood vessels

All sections will be analysed with light microscopy (×2, ×16, ×40, ×100; Olympus IX 81 Shinjuku, Tokyo, Japan) in order to locate the inflammatory cells present and blood vessels. Each antibody will be assessed in each soft tissue sample and will be categorized following this classification:

- (–) no labelled cells.
- (+) a few labelled cells.
- (++) a focal infiltration of labelled cells.
- (+++) an intense infiltration of labelled cells throughout the tissue.

2.2.4. Statistical methods

Power analyses has been performed to define the population sample. Descriptive statistics will be performed using means of absolute and relative frequency and medians and means. The analysis of the outcome measures will be performed using appropriate statistical tests according to the distribution patterns. The calculation will be performed using SAS (version 9.3 for Windows).

3. Results of the pilot study (titanium abutments)

3.1. Raw abutment characterization

3.1.1. SEM

The regular pattern produced by the machining process could be observed in the titanium abutments (Fig. 5).

3.1.2. Profilometry

The results obtained for amplitude parameters like root-mean-square deviation, (Sq) skewness (Ssk), and kurtosis (Sku), and hybrid parameters such as the density of summits (Sds) and the developed interfacial area ratio (Sdr) are presented. Additionally, we also present a set of functional indices like the core fluid retention, Sci, the surface bearing index, Sbi, and the valley fluid retention, Svk in order to clarify the possible correlation between the surface properties of the abutments and tissue integration.

All results were obtained from the measurements performed at six randomly distributed spots on three different abutments (N = 18) and the values are presented as the mean \pm SD (Table 2a,b,c).

3D reconstruction was also performed in order to demonstrate the specific topography and surface characteristics of the experimental abutment (Fig. 6).

3.2. Non-decalcified histology & SEM/immunohistochemistry

3.2.1. Demographics and site-related data

All patients were included at the Department of Periodontology and Oral Surgery, Faculty of Medicine, University of Liège, Belgium. Nine patients were included in this pilot study; 4 (44.4%) were females and 5 (55.6%) were males, with a mean age of 58.8 years (range: 35–77 years). None of the participants were smokers (Table 3).

A total of 10 implants were inserted into the surgical sites, out of which 8 in the premolar position. Regarding flap design, none of the implants were placed using a flapless approach. Bone quality was type 2 or 3 in all but one case. All inserted implants had a diameter of 4.1 mm

Table 2a

Amplitude parameters.

Sq (μm)	Ssk	Sku
0.49 ± 0.15	0.36 ± 0.27	2.54 ± 0.37

Sq – root-mean-square deviation; Ssk – skewness; Sku – kurtosis.

Table 2b

Hybrid parameters.

Sdr (%)	Sds (μm^{-2})
2.06 ± 1.47	0.03 ± 0.01

Sdr – interfacial area ratio; Sds – density of summits.

Table 2c

Functional indices.

Sci	Sbi	Svk
1.70 ± 0.14	0.54 ± 0.20	0.08 ± 0.01

Sci – core fluid retention; Sbi – surface bearing index; Svk – valley fluid retention.

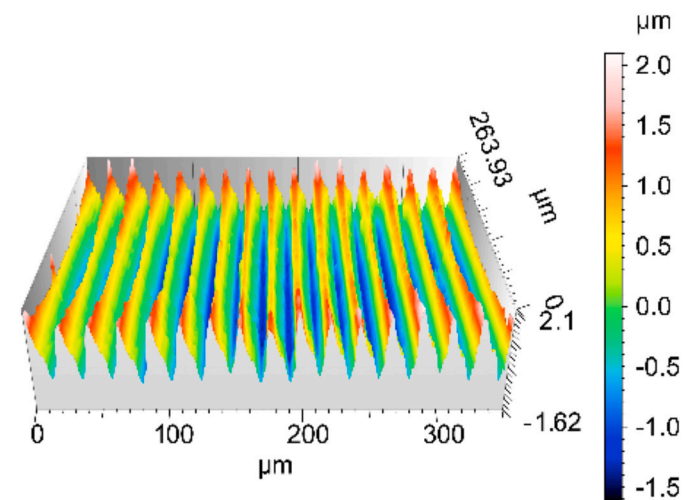


Fig. 6. Representative profilometry image showing the 3D reconstruction obtained for titanium.

and although the length varied, the minimum was 8 mm (Table 4).

Similar values were recorded preoperatively and postoperatively for both keratinized tissue height (buccally and lingually) and soft tissue thickness above the bone crest, and no statistically significant differences were found (Table 5).

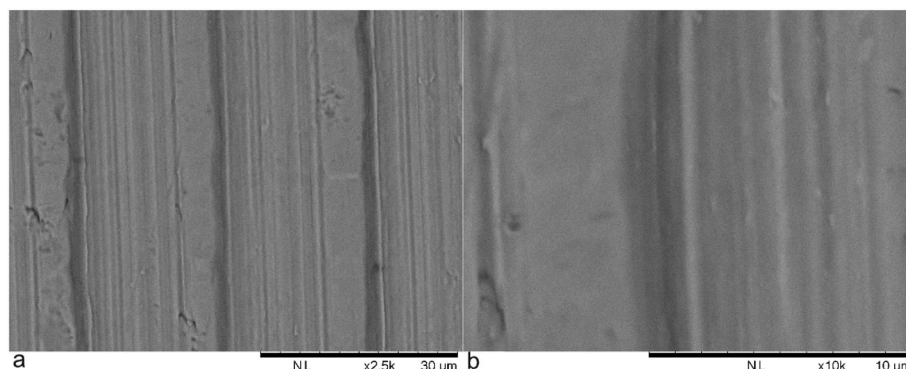


Fig. 5. Representative SEM micrographs of titanium obtained with a 2.5 k magnification (a) and 10 k amplifications (b).

Table 3
Characteristics of the study participants.

Number of subjects	9
Number of implants	10
Mean age of subjects (range)	58.8 ± 15.3 (35–77)
Gender (M/F)	5/4
Smokers (yes/no)	0/9

Table 4
Patient and site-related characteristics.

	N/%
Number of implants	10
Implant position	
1st premolar	3 (30.0)
2nd premolar	5 (50.0)
1st molar	2 (20.0)
Flap design	
Flap	10 (100.0)
Flapless	0 (0.0)
Bone quality	
1	0 (0.0)
2	3 (30.0)
3	6 (60.0)
4	1 (10.0)
Implant length (mm)	
8	6 (60.0)
10	3 (30.0)
12	1 (10.0)
Implant diameter (mm)	
4.1	10 (100.0)

Table 5
Soft tissue - clinical measurements.

	Surgery	Abutment removal (8 w)
Keratinized tissue height		
Buccal	3.2 ± 0.6 [2–4]	3.4 ± 0.5 [3–5]
Lingual	5.9 ± 2.7 [3–12]	6.0 ± 3.3 [3–14]
Soft tissue thickness	4.3 ± 2.3 [3–7]	4.3 ± 2.3 [3–7]

3.3. Non-decalcified histology

The interface between the abutment and the peri-implant soft tissues was characterized by a well-defined barrier epithelium coronally and a connective tissue apically to the junctional epithelium (Fig. 7); in a few cases, epithelial adhesion continued until the implant neck. Regarding

the dimensions of these mucosal components, the following assessments were performed: sulcus depth, total mucosal height, epithelial adhesion length, and connective tissue adhesion length. The results are reported in Table 6.

3.4. SEM/immunohistochemistry (IHC)

3.4.1. SEM

Overall, epithelial cells, connective tissue fibers, some erythrocytes, plaque, calculus, and inorganic compounds could be identified on SEM images (Fig. 8). The number of remaining cells differed from one abutment to another, but some epithelial cells were found on all of them. The connective tissue fibers were found on the apical parts, but on a few abutments, the fibers were not found and the apical portion of the abutment was mainly acellular (only some inorganic particles were noted). The amount of plaque/calculus varied in quantity and it was localized mainly in the coronal parts of the abutments.

3.4.2. IHC

CD34-positive cells were found evenly distributed in connective tissue of all samples. An intense infiltration of HLA-DR-positive cells in the epithelium (Langerhans cells) was noted in all samples. On the other hand, only mild to moderate presence of inflammatory cells was observed in the connective tissue (Fig. 9).

4. Discussion

This study is designed to directly analyse, in a human model, different materials used as supragingival components in daily implant dentistry. Though a few clinical studies have attempted to test some of the materials used (mainly titanium and zirconia) [10,12,29], the main limitations of prior studies is that they focused on one or two materials and/or the study design was not a randomized clinical trial and/or the analyses performed did not allow for the direct observation of the interface between the abutment material and soft tissues (i.e. instead of histology, other surrogate outcomes have been used). The method and

Table 6
Results from the histometric linear measurements.

	Mean	SD	Min	Q1	Median	Q3	Max
Sulcus	0.61	0.44	0.07	0.25	0.56	0.88	1.38
Epithelium	2.68	0.96	1.00	2.21	2.60	3.23	4.06
Conn. tissue	1.06	0.94	0.00	0.09	1.12	1.71	2.46

Data are presented in mm.

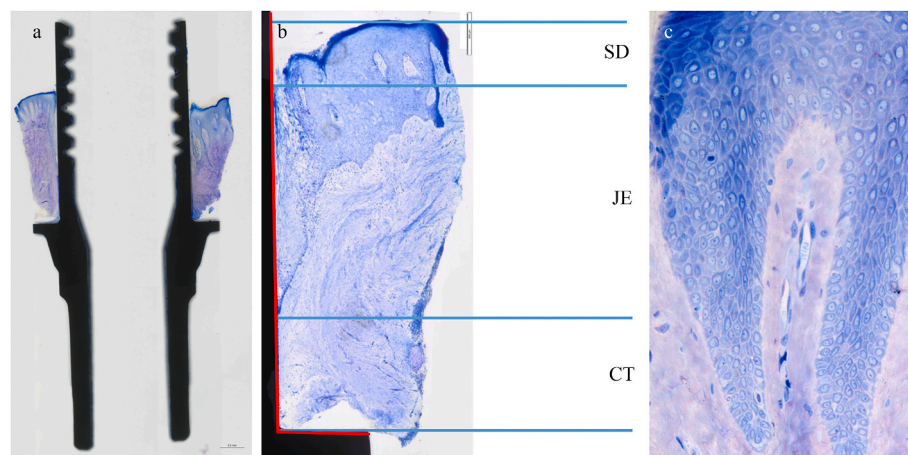


Fig. 7. Non-decalcified histology: soft tissue adhesion on the surface of the abutment harvested and processed together (a); clearly distinguishable epithelial and connective tissue (b); measurements include sulcus depth (SD), epithelial (JE) and connective adhesion length (CT); higher magnification (c).

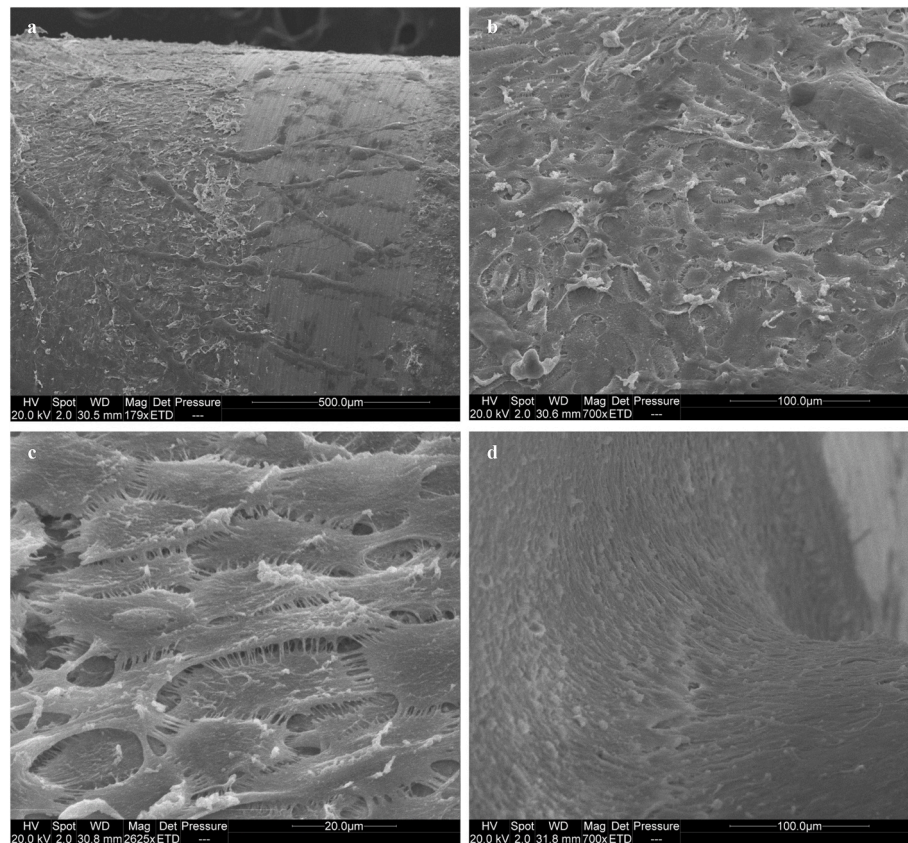


Fig. 8. Representative SEM micrograph of titanium abutment obtained after soft tissue removal from the abutment, showing soft tissue remnants on the surface of the abutment (a & b); on higher magnifications, the presence of epithelial cells (c) and connective tissue (d) could be observed.

design described herein is unique in that it aims to evaluate all major materials used for supragingival components in a clinical setting under the strict conditions of an RCT. Moreover, the analyses planned are aiming to encompass several different aspects, from the abutment *in vitro* characterization to the assessment of healing outcomes through histology and direct visualization of the abutment-soft tissue interface, and further soft tissue characterization in terms of inflammation and vascularization.

Preclinical raw abutment characterization has been added to the planned clinical studies as previous studies have demonstrated that the roughness of surface influenced the cellular adhesion and the shape of cells, such as gingival fibroblasts [30]. Smoother surface are also considered to be less prone to bacterial adhesion and a surface roughness threshold of 0,4 μm was found to be favorable for microbial adhesion [9]. As the material used for abutment components may influence the adhesion and colonization of microbial species [31], profilometry and SEM analyses planned herein, performed for each material used, will allow for both surface characterization and comparison between the materials.

Non-decalcified histology and the subsequent histomorphometric analyses will allow the assessment of soft tissue dimensions and peri-implant soft tissue barrier. The same protocol and study designs ensures that the main influence on biological width and abutment-soft tissue interface will be that of the abutment material applied, which will allow inter-material comparison. Our pilot results on titanium abutments, which demonstrated a longer junctional epithelial and shorter connective tissue length, are in accordance with several previous studies [10,24,32]. However, several other materials have not been assessed previously in this regard and this study will provide much needed information relative to their potential to ensure an adequate mucosal attachment.

Immunohistochemical analyses will be performed to assess the level of soft tissue inflammation and cellular and vascular densities in the soft tissue surrounding the abutments. Tomasi et al. [12] reported on the tissue interactions with titanium surface, but again, the comparison between the different materials is missing in the literature and this study has a potential to provide some additional insights in this regard.

The present pilot study provided valuable information in regards to optimal main study design and the following final sample size calculation, namely the need to separate initially planned single RCT into two RCTs and the subsequent doubling of number of subjects that need to be enrolled in each study for adequate power, using the length of biological width/epithelium and the percentage of positive cell markers as primary endpoints for the first and second RCT, respectively.

Our preliminary results from a pilot study on titanium abutments indicate that the methods described herein—from abutment design to its removal—render all planned analyses feasible. Experimental abutment failure was not observed and it was demonstrated that it could be successfully used for soft tissue sampling. Surgical parts of the protocol as well as the follow-ups were uneventful and the harvesting method, with the use of a custom-made guide over the experimental abutment, was straightforward, reliable, and reproducible. Furthermore, the proposed approach is minimally invasive for patients as the experimental abutment of a small diameter had been specifically designed to allow for a punch biopsy to be taken and soft tissues to heal properly thereafter. Therefore, the healing is not hampered by this procedure and, at the same time, the harvested specimens allow for the interface between the experimental abutment and soft tissues to be directly assessed and analysed.

A few obstacles encountered during the pilot study were mostly of technical nature and were easily overcome; however, the use of HLA-DR antibody did not prove to be of adequate value as it was not possible to

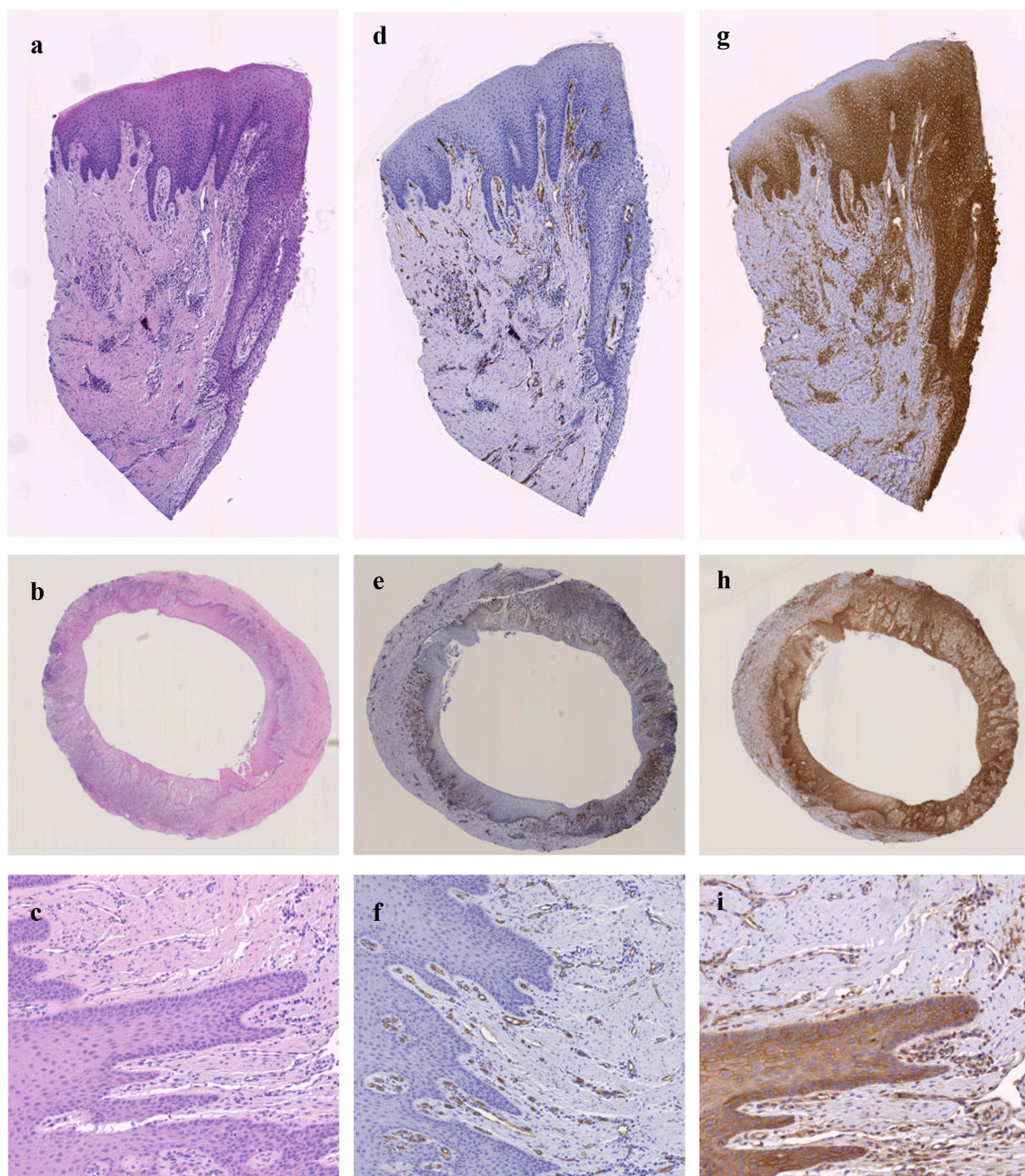


Fig. 9. Histological (HE; a, b, c) and immunohistochemical images (CD34 – d, e, f; HLA-DR – g, h, i). Horizontally, axial sections (a, d, g), transversal sections (b, e, h), and high magnification sections (c, f, i) are shown. Both epithelial and connective tissue can be observed in all images.

perform a quantitative analysis in the samples in which HLA-DR marker was used. Additionally, HLA-DR is not a highly specific marker and it has been therefore decided to exclude this marker from further analyses and to instead use separate markers for different cell types in the main study (as explained in the present Methods/Design section), so that the presence of different cells in the connective tissues can be quantified.

Overall, the pilot study helped us validate the study design and fine-tune the protocols used.

One of the limitations of the present study is that it focuses on early stages of healing and due to the already substantially complex and challenging study design, different time points were not considered. The present time point was chosen based on other clinical studies which

reported the formation of mature tissue at 8 weeks of peri-implant soft tissue healing [32,33].

The ultimate goal of this study is to provide more information on the effects of different materials on peri-implant soft tissue integration, which would be helpful in daily decision-making when it comes to choosing the optimal material(s) for either provisional crowns or definitive restorations. Helping practitioners make good choices at this stage will likely have influence not only on initial healing, but also on long-term implant survival.

5. Conclusion

The preliminary results on titanium abutments, based on the study protocol presented herein, show that the usage of custom-made abutments in human model is a reproducible method to study peri-implant soft tissue integration. This further intensifies the rationale to compare different abutment materials used in daily practice under the same conditions.

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Declaration of Competing interest

None.

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