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ORIGINAL ARTICLE

FT-IR analysis of the Interface between Universal Scotchbond and Oral Mucosa: a preliminary *in-vitro* study

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ABSTRACT

BACKGROUND: The long-term success of implant therapy depends not only on proper osseointegration, but also on the healing of the epithelium and the quality of the biological seal on the abutment and on the implant neck. This study aims to evaluate the possible use of dentinal adhesives on the surface of the transmucosal path of dental implants in order to create a hermetic seal between keratinized epithelium and abutment.

METHODS: Four sections of 12 µm thickness were obtained from a sample of the oral mucosa. Scotchbond TM Universal Adhesive (3M ESPE, Seefeld, Germany) was carefully applied both to the samples and to the transmucosal path of titanium abutment (Win-Six, BioSAFin, Italy). The adhesives were polymerized. FT-IR analysis was performed on: 1) polymerized Scotchbond Universal Adhesive (3M ESPE, Seefeld, Germany); 2) the interface between the titanium abutment and the adhesive; 3) the interface between the adhesive and the mucosa; 4) the mucosa samples.

RESULTS: Comparing the spectra, it emerged that the adhesive has established chemical bonds both on titanium and on the keratinized mucosa, involving different types of chemical interactions.

CONCLUSIONS: The results of this *in-vitro* study are encouraging. In the future biocompatibility and comparative study with other adhesives will be required.

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KEY WORDS: Adhesives; Dental implants; Gingiva; Dental abutments.

Implant-supported prosthetic therapy is based on osseointegration.[1] In recent years, many authors have studied the possibility of increasing the amount of bone around the implants or accelerating bone formation by modifying the implant surface.[2-4] Memè *et al.*[5] reported that immediate loading was able to stimulate the osteoblastic response and therefore have a positive effect in the implant healing period. *In-vitro*[6] and *in-vivo*[7] studies have shown that static magnetic fields, produced by cover screws, could improve osteoblasts differentiation and, consequentially,

accelerate osseointegration process. On the other hand, several studies investigate new methods to enhance the biological response of the osteoblasts adjacent to the implants. New molecules, such as raloxifene, have shown to stimulate osteoblasts metabolic activity and differentiation.[2-4]

Peri-implant soft tissues play a fundamental role for the preservation of the underlying osseointegration.[8] The maintenance of an adequate and long-lasting biological seal has been the subject of many studies. In particular, in a study by Bambini *et al.*,[9] the thickness of the soft tissues was correlated with the exact position of the implant at the time of the first surgical phase, demonstrating once again how important the role of soft tissue in maintaining osseointegration and avoiding bone resorption around the first spire which would open the doors to peri-implantitis.

For an adequate process of osseointegration it is essential the absence of bacterial penetration through the soft tissue biological seal. Indeed, the junctional epithelium is able to adhere to the implant surface only in the absence of bacterial biofilm. Therefore, in this condition the epithelial cells are able to synthesize the proteoglycans necessary for attachment to the implant.[10-16] To ensure that the biological seal maintains its barrier function, it is fundamental that the cells of the junctional epithelium remain in an undifferentiated state, since this status allows them to produce the hemidesmosomal basal lamina which mediates their attachment to the implant.[17] In addition, the preservation of the undifferentiated layer also involves a softer bond between the basal cells and promotes leukocyte transmigration and facilitates the outward flow of crevicular fluid.[18]

In the case of pathological stimuli, such as the accumulation of plaque or repeated connection and disconnection maneuvers of the abutment, the phenomenon of apical epithelialization occurs along the surface of the dental implants.[12, 19-22]

Protection against apical epithelialization is necessary for the long-term maintenance of the implants themselves. In a study carried out by Kawahara *et al.* in 1998[18] the morphological protocol of the biological seal was demonstrated, which acts as a barrier against bacterial invasion and food residues in the gap at the implant-tissue interface.

Epithelial cells have shown a higher adhesion strength to the titanium substrate than fibroblasts. Therefore, it can be assumed that the connective tissue can be more easily detachable from the implant surface. The adhesive force, in the intercellular bonds and with titanium, allows the epithelial cells to stick tenaciously to the surfaces of the implants, constituting a first factor in facilitating the apical epithelialization mechanism. The second factor of equal importance is represented by the greater growth that epithelial cells have been shown to have in the presence of plaque and in pathological conditions in general.[21]

Many studies have been carried out to improve the biological seal.[23-27] It is well known how different cellular behaviors such as adhesion, morphological changes, functional alterations and proliferation are greatly influenced by the properties of the various surfaces. Recent studies have shown how, with the introduction of zirconia as an abutment material, it is possible to reduce the amount of inflammatory infiltrate in peri-implant tissues.[28] In fact, further research has shown that the gene expression of anti-inflammatory cytokines such as IL-10 and TGF-β1 are higher near the tissues in contact with the zirconia abutments than those in titanium.[8] Furthermore, this ceramic appears to be able to accumulate a very low amount of bacterial plaque compared to titanium.[29] Some Authors suggested zirconium coating as titanium surface modification.[30] Even if the epithelial adhesion and proliferation was similar to machined titanium,[31] other studies have reported that zirconia surface coating was able to inhibit the biofilm formation.[32]

Recently, several studies have introduced bioactive molecules deposition on the transmucosal path of the implant surface to improve soft tissue mucosal seal. Werner[27] analyzed the behavior of epithelial cells exposed to a titanium surface with a laminin-5 coating. In fact, discontinuous hemidesmosomal systems were observed in the junctional epithelium in contact with the implant surface and in smaller numbers when compared with those located in the natural tooth-gingiva interface. This is due to a reduced expression of laminin-5 in the inner part of the basal lamina of the epithelial cells. Laminin-5 belongs to a family of polypeptide glycoproteins that are involved

in the structural scaffolding of epithelial tissues. Together with the α6β4 integrin, laminia-5 is involved in the assembly and maintenance of the integrity of the hemidesmosomes.

Using the implant surfaces coated with bioactive molecules, the behavior of gingival fibroblasts was also studied when they are in contact with the latter. In a study by Jin *et al.*[26] an Multilayer Polyelectrolyte Films (MPFs) was built on the titanium surface which in turn incorporated type I collagen molecules and hyaluronic acid derivatives. From this study it emerged that the surfaces functionalized with bioactive molecules promoted the initial fibroblasts proliferation attachment and compared to the control side with acid-etched titanium surfaces. Therefore, it could be assumed that the use of surfaces functionalized with bioactive molecules can represent a prolific line of research in the coming years to improve the seal offered by peri-implant soft tissues.

This study aimed to evaluate the possible use of enamel-dential adhesive in implant prostheses with the aim above all of verifying whether these substances, which are today used exclusively in restorative dentistry, can be applied on the surface of the transmucosal path of dental implants, in order to create a hermetic seal between keratinized epithelium and abutment. The application of the material could in return protect as well as ensure that bacterial colonization does not affect the junctional epithelium and the underlying connective tissue.

Materials and methods

Inclusion criteria

1. good general health;
2. high standards of oral hygiene;
3. absence of inflammation of the oral soft tissues;
4. absence of periodontal disease;
5. presence of third molar requiring extraction.

A 24-years-old male, who met the inclusion criteria, was enrolled in this study. The patient provided informed consent to the clinical procedure and the present study.

At the time of the extraction surgery of the dental element 1.8 a sample of oral mucosa was taken from the region of the maxillary tuber of the same quadrant. The area was selected in order to include the keratinized mucosa as much as possible.

The sample was then frozen in liquid nitrogen at a temperature of -185 °C. Then the sample was mounted on a cork support with Killik (embedding medium for cryostat) to allow the creation of cryostat sections. At the moment of sectioning of the sample, a cryostat associated with a microtome (Microm HM550 supplied by Bio-Optica) was used at a working temperature of -20 °C. After carrying out 4 control sections of 12 μm thickness of the oral mucosa, a dental adhesive (Scotchbond TM Universal Adhesive, 3M ESPE, Seefeld, Germany) was carefully applied to the sample, paying particular attention to spread it on the keratinized epithelial component.

As there is no protocol for applying the adhesive to soft tissues in the literature, it was decided to use the protocol used for the hard tissues of the tooth. Then, after applying the adhesive to the keratinized part of the sample for 20 seconds, we moved on to photopolymerization for 30 seconds. For this phase, an LED light source lamp was used whose emission band is between 250 and 400 mW and which determines the start of the polymerization by acting on the camphorquinone (one of the main initiators that is activated at a wavelength of 468 nm). At the end of the photopolymerization phase, 4 new cryostat sections of 12 μm of the sample were carried out.

Parallel to this phase, the transmucosal path of a WINSIX® implant system, supplied by BioSAFin S.r.l., was also treated with the same adhesive.

Therefore, Scotchbond TM Universal Adhesive (3M ESPE, Seefeld, Germany) was applied to the transmucosal path of the abutment (grade 5 titanium) with the same protocol used for the mucosa and then light cured for the same duration.

Subsequently, the 4 control sections of the mucosa, the 4 sections of mucosa with the adhesive applied and the treated implant system were analyzed using the Fourier transform infrared spectroscopy (FT-IR) method[33-35] to assess whether or not there was the formation of chemical bonds in the abutment-adhesive interface and in the keratinized-adhesive oral mucosal interface.

General principles regarding Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IT) is a technique used to identify mainly organic materials by measuring the absorption of infrared radiation by the sample. The graph showing the transmittance of the sample as a function of time is called an interferogram. Since it is difficult to interpret, a mathematical operation is used, the Fourier Transform, which converts it into a spectrum obtaining the transmittance as a function of the wave number (the reciprocal of the wavelength). The resulting graph can be used to carry out a qualitative identification of the sample in question.

The spectrum of a material is made up of two fundamental regions:

1. the absorption bands in the range 4000-1500 cm^{-1} which are typically characteristic of the functional groups (*e.g.* OH, NH, etc.) and which allow to distinguish, for example, alcohols from amines from organic acids etc.;

2. the region between 1500 and 400 cm^{-1}, known as "fingerprints," as it highlights specific absorptions of each individual material or substance, allowing more precise identification within the family revealed by the previous absorption band.

The spectrum is also identified through the comparison with the available libraries of spectra of various substances (maps or correlation papers). FT-IR spectrometers can also be used in association with microscopes obtaining what is infrared microspectroscopy.[33-35]

Results

FT-IR analysis of the abutment/adhesive interface

In the first instance, the spectrum of the polymerized Scotchbond TM Universal Adhesive (3M ESPE, Seefeld, Germany) was obtained (Figure 1). The absorbance (logarithm of the inverse of the transmittance) is present on the ordinate axis (y), while the wave number is present on the abscissa axis (x). Subsequently, the interface between the titanium abutment (Win-Six, Biosafin, Italy) and the adhesive was analyzed.

The comparison suggests that at the interface there are interactions between titanium and polymerized adhesive (Figure 2).

In particular, the carbonyl band of the adhesive at 1738 cm^{-1} is doubled (1724 cm^{-1} and 1725 cm^{-1}) and falls at lower frequencies, proving that this group has interacted with Van der Waals bonds with the titanium surface (Figure 3).

The shift to lower frequencies (red shift) means that the dipole of the C=O group is involved in intermolecular interactions (Figure 4).

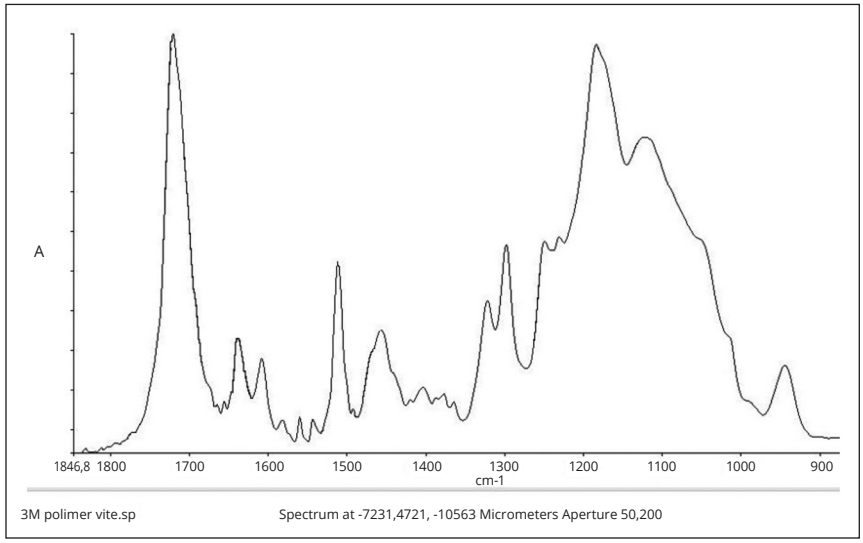

Figure 1.—Spectrum of the cured adhesive Scotchbond TM Universal Adhesive (3M ESPE, Seefeld, Germany).

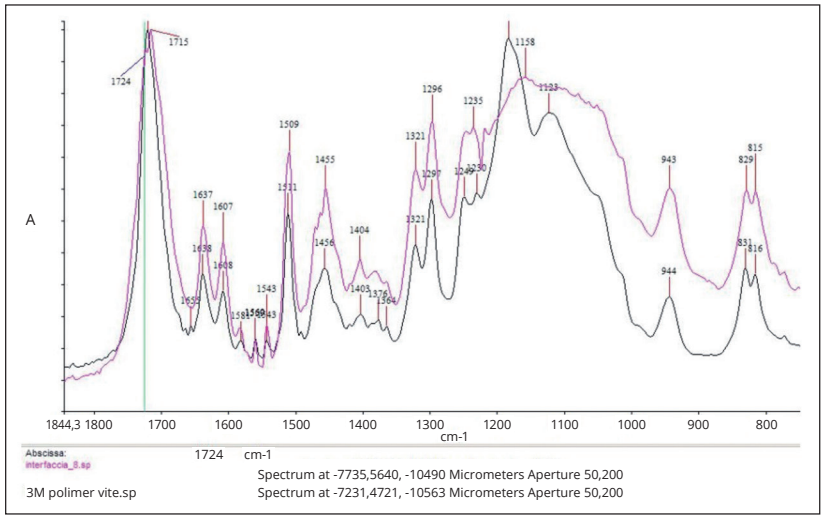

Figure 2.—Comparison between interface spectrum and polymerized adhesive spectrum.

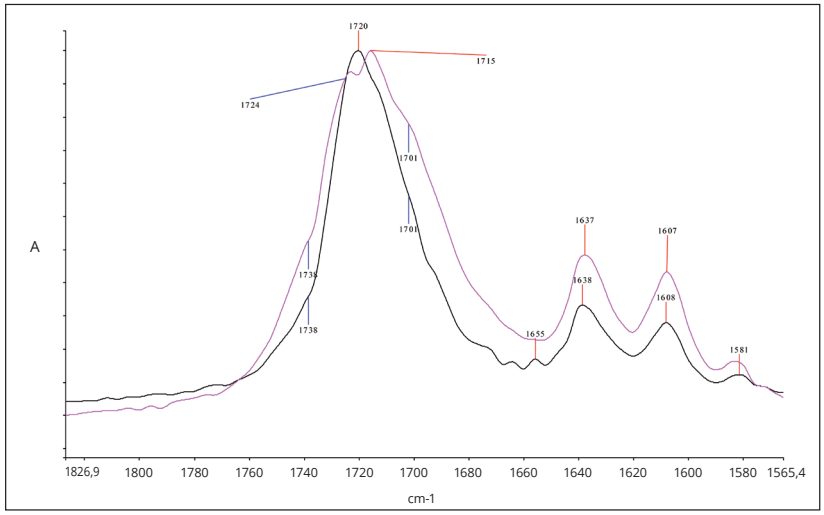

Figure 3.—Splitting of the carbonyl band.

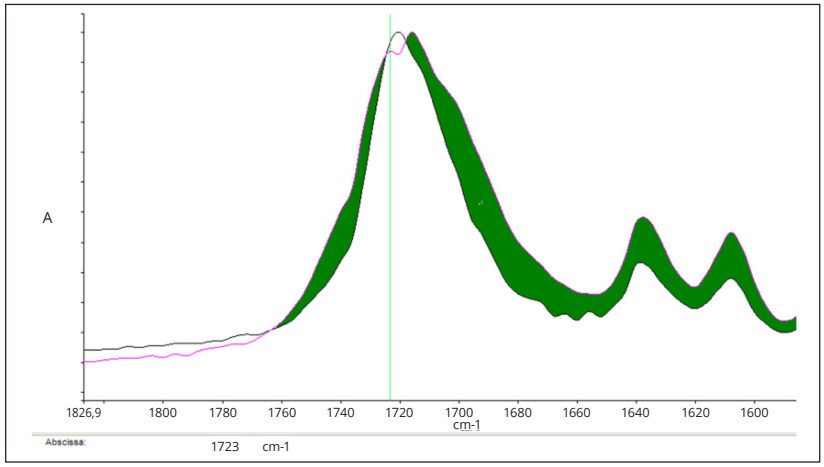

Figure 4.—Shift of the spectrum to lower frequencies.

The same applies to the asymmetrical (about 1240 cm^{-1}) and symmetrical (about 1000 cm^{-1}) stretches of the phosphate group (10 MDP), where the spectral profiles of the adhesive are very different from those of the adhesive on the titanium abutment (Win-Six, BioSAFin, Italy) (Figure 5). Therefore the area of the adhesive/abutment interface is the seat of adhesion forces involving Van der Waals interactions and micromechanical adhesion forces.

FT-IR analysis of the keratinized/adhesive mucosal interface

An image of the sample treated with the adhesive was initially acquired through infrared imaging where it is possible to appreciate the mucosa, the adhesive, and the interface between the two (Figure 6).

The spectra of the mucosa, of the polymerized adhesive and of the interface were then acquired in two different points (Figure 7).

The spectrum of the connective tissue underlying the keratinized epithelium was also acquired. In fact, by comparing the spectra of the two tissues, variations at 1662 cm^{-1} (keratinized mucosa; red spectrum) and 1631 cm^{-1} (connective; black spectrum) can be seen to testify the different protein content.

In the spectrum of the adhesive (in green in the online version) there are: the carbonyl band at 1730 cm^{-1} and the phosphate bands at about 1240 cm^{-1} and 1100 cm^{-1}, the latter as convoluted bands and characteristics of these polar groups.

The spectrum of the mucosa (in red in the online version) shows, like any biological material, the amide bands I and II (at 1660 cm^{-1} and 1550 cm^{-1}) and absorptions at about 124° and 1100 cm^{-1} attributable, among other things, to the nucleic acid component.

In the two interface spectra (in blue and gray in the online version) it is possible to appreciate with reasonable certainty the absorptions of both the polymer and the mucosa with the following modifications:

1. the carbonyl group has shifted from 1737 cm^{-1} to 1723 cm^{-1}, testifying to intermolecular interactions of this group with the organic part of the mucosa. At the same time, the spectral profile of amide I and II is modified as a result of variations in the secondary structure of proteins.

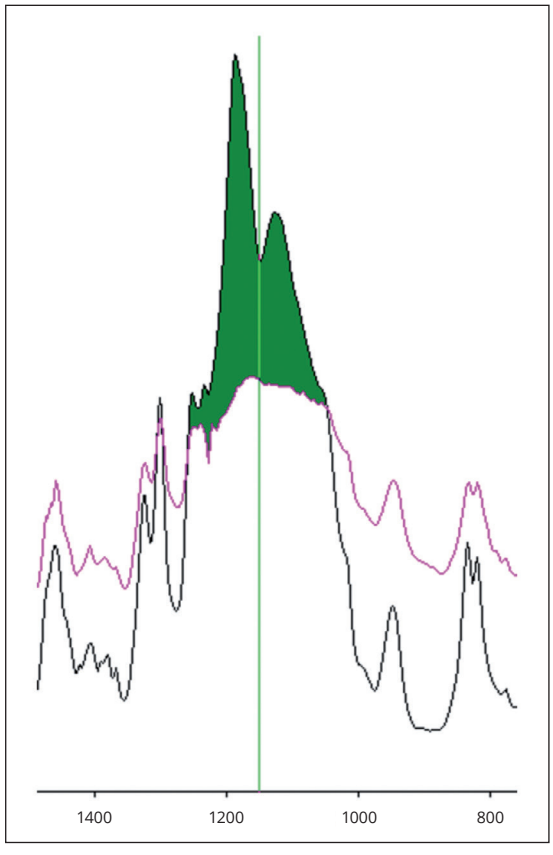

Figure 5.—Asymmetrical and symmetrical stretching of the phosphate group.

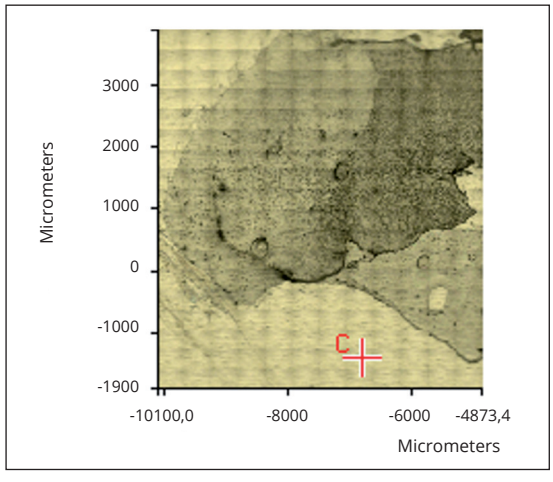

Figure 6.—Imaging of the sample (right) and of the adhesive (left).

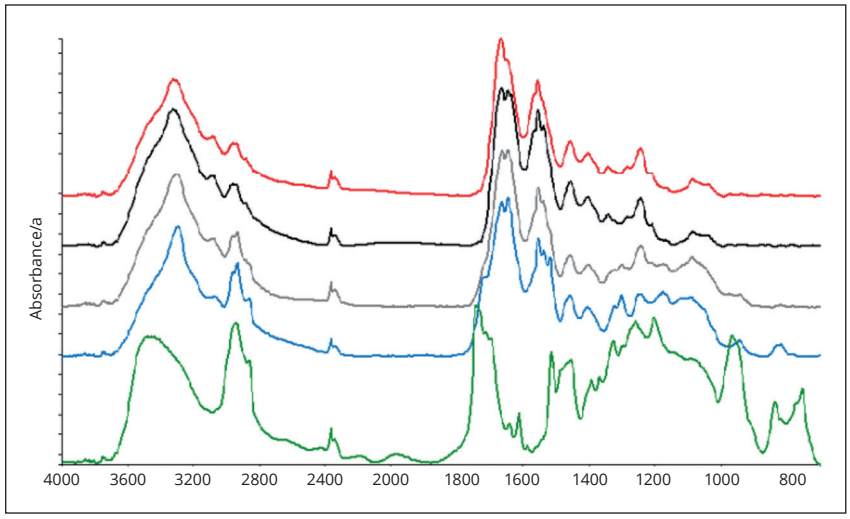

Figure 7.—Different spectra acquired: the keratinized mucosa in red, the connective tissue in black, the interface in gray and blue in two different points, the polymerized adhesive in green (colors in the online version).

2. the spectral profile of the asymmetrical and symmetrical stretches of the phosphates (1240 cm^{-1} and 1000 cm^{-1} approximately) is modified with the formation of new bands and changes in intensity of those attributable to the same vibrational modes. The phosphate band at about 970 cm^{-1} is also shifted to lower frequencies confirming the interactions of the phosphate groups of the polymer.

Discussion

Implant dentistry have reached great clinical successes over the past 20 years. However, the long-term success of dental implants does not depend only on proper osseointegration. In fact, a good integrity of the peri-implant mucosa at the level of the transmucosal path is fundamental for therapeutic success. In order to prevent bacterial colonization, the formation of a long-lasting and effective soft tissue barrier is of fundamental importance.

The soft tissue barrier around the implants has been described by several studies.[10, 12, 36, 37] The peri-implant biological width consists of an epithelial barrier of about 2 mm and an adhesion zone of connective tissue of the size 1-1.5 mm.[10] It has been shown that various conditions could affect it. The repeated disconnection /reconnection of the abutment[38] and the presence of bacterial plaque and associated inflammatory infiltrate[39] are two of the main causes of the apical retraction of the biological width. In particular, bacterial plaque appears to be the main factor in triggering the apical epithelialization process,[38, 40] responsible for the consequent resorption of the bone crest, compromising implant success.

Recently, several solutions have been suggested to improve the soft tissue barrier at implant sites, preventing the infiltration of both bacteria and their products.[9] The scientific research have mainly focused on surface treatment of the transmucosal path of the implant, which is in direct contact with the peri-implant tissues.[8] In fact, it is well known how different cellular behaviors such as adhesion, morphological changes, functional alterations and proliferation are greatly influenced by the properties of the various surfaces.[23] In more detail, these studies showed that smooth surfaces are able to promote the adhesion of epithelial cells. On the contrary, rough surfaces favor the formation of an initial network of fibrin and increase the stability of the connective tissue that acts as a barrier against epithelialization apical.[23]

Several studies have modified the transmucosal implant surface with bioactive molecules deposition process. Werner *et al.*[27] and Jin *et al.*[26] introduced laminin-5 and collagen and hyaluronic acid derivatives, respectively. The results of the above studies have shown that the enhanced epithelial growth/adhesion, while the second influenced connective tissue attachment to the tita-

nium substrate. Koidou *et al.*[41] reported the use of peptide coating (lamin 332 and ameloblastin-derived peptides) on titanium surface. The immunofluorescence assay showed that the surface treatment improved the human keratinocytes proliferation for up to 48 hours. Furthermore, the epithelial adhesion was statistically higher (P<.01) on the test surface compared to the control.

In this preliminary study we investigate the formation of chemical bonds between the keratinized oral epithelium, the transmucosal path of the abutment and the dental adhesive. The aim of the present analysis was to test whether enamel-dentinal adhesive systems could be used in implant prosthesis to improve the biological seal. The FT-IR analysis showed that the enamel-dentin adhesive has established chemical bonds both on titanium and on the keratinized mucosa, involving different types of chemical interactions.

Limitations of the study

The main limitation of this study is the limited sample size. To best of our knowledge, there is a lack of studies on this topic.[42] Resin monomers have shown a certain degree of cytotoxicity.[43] Elias *et al.*[44] reported that the degree of toxicity was related to presence of residual hydrophobic monomers, such as acidic methacrylates, in the resin adhesive eluates. In the future further in-vitro studies will be required to test the safety of the procedure and the most suitable protocol.

Conclusions

The results of this in vitro study are encouraging, hoping in the future a comparative study with other adhesives, with the aim of looking for molecules that express a greater capacity for interaction with the different chemical groups exposed by both titanium and keratinized mucosa.

Before moving on to any in vivo trials, more research is needed. Further studies will be needed in the future to evaluate the degree of toxicity of the adhesive substances used against keratinocytes of the oral mucosa. In addition, characterization tests should be performed in the future, such as tensile tests, to measure the extent of these chemical bonds, and their resistance to prolonged stresses over time.

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