EARLY TISSUE HEALING AROUND UNLOADED ORTHODONTIC MINI-SCREWS. A STUDY IN THE BEAGLE DOG. PART I. SOFT TISSUES

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Camillo Morea
Department of Orthodontics, Dentistry Faculty, University of Sao Paulo, SP, Brazil.

Maoela Domingues
Department of Oral Pathology, Porto Alegre, RS, Brazil

Décio Santos Pinto Jr
Department of Oral Pathology, Dentistry Faculty, University of Sao Paulo, SP, Brazil.

Gladys Cristina Dominguez
Department of Orthodontics, Dentistry Faculty, University of Sao Paulo, SP, Brazil.

Paula Guerino
Department of Stomatology, Dentistry Faculty, University of Santa Maria, RS, Brazil.

Mariana Marquezan
Department of Stomatology, Dentistry Faculty, University of Santa Maria, RS, Brazil.

Vilmar Antônio Ferrazzo
Department of Stomatology, Dentistry Faculty, University of Santa Maria, RS, Brazil.

ABSTRACT: The knowledge of tissue healing around orthodontic mini-screws can provide useful information for their clinical use. Even though there are some similarities with c.p. Titanium prosthodontics implants some differences exist in terms of materials, surface texture and load. The purpose of this study was to investigate the early phase of soft tissue healing around unloaded orthodontic mini-implants. In five Beagle dogs were inserted 20 self-tapping mini-screws. The animals were euthanatized at the day 0, 2, 7, 15 and 30. The bony specimens containing the screws were dissected, fixed, embedded in acrylic resin before they were cut. A novel orienting tool was developed to orient the cut exactly on the long axe of the screw. The samples were stained with Stevenel's blue and observed under light microscopy. Soft tissue adaptation to the neck of the mini-screw was excellent since from the beginning, forming a barrier against bacterial and foreign body penetration into the deeper tissue layers. A 2mm long junctional epithelium was observed at day 7. A connective tissue of about 1mm with predominantly parallel fiber orientation was also observed. A biologic width could be described with the features of the one around osseointegrated implants.

No gap was observed between soft tissue and implant neck throughout the whole observation period. At day 30 the maturation of the soft tissue was complete and either epithelium or connective tissue was in intimate contact with the implant neck forming a valid barrier against bacterial and foreign body challenge.
KEYWORDS: Orthodontics; mini-implants; soft tissues healing; animal study.

INTRODUCTION

Kanomi {Kanomi, 1997 #2034} introduced mini-screws in contemporary orthodontics in 1997. They are used routinely to obtain absolute Anchorage control during tooth movements {Park, 2003 #2040},{Carano, 2004 #2022}. To allow for absolute anchorage control the mini-screws have to be rigidly anchored to the bone {Davies, 1998 #2024}. Several Authors {Schenk, 1998 #2041},{Abrahamsson, 2004 #2014} have described as osseointegration the process of bone integration around mini-screws observed at the end of the bone-healing period. Nowadays the osseointegration process has been defined {Albrektsson, 2008 #2018}, understood and described with great detail {Davies, 1998 #2024},{Schenk, 1998 #2041},{Davies, 2003 #2025} for the osseointegrated implants as well as the implant-soft tissue relationship with the neck of the screws, defining a specific implant-related biological width {Abrahamsson, 1996 #2016},{Buser, 1992 #2021}.

With the aim of better understanding the healing process around orthodontic unloaded mini-screws and to drive some indication for their clinical use we decided to study and analyze histologically the phenomena occurring between their insertion, at day 0, 2, 7, 15 and 30.

In this study soft tissues have been analyzed while hard tissues will be described separately.

MATERIALS E METHODS

Before reading the paragraph of Material and Methods the reader should be aware that this experiment has been designed for studying histologically the healing process around unloaded mini-screws and also to perform pull-out tests on the same type of screws varying the healing time with the aim of comparing histological data with pull-out values in a future paper. For this reason, some of the described procedures make sense only if the two joint experiments are considered. A total of 80 X 6mm self-tapping mini-screws were inserted (4 in each quadrant) and to have enough space for insertion and avoid root contact the extraction of the 4 premolars was necessary for each quadrant. In this way the total number of animal lives was reduced.

The following protocol has been approved by the Ethic Committee for the Animal Well-being of the Veterinary Faculty of the Federal University of Santa Maria in 02/10/2006 with protocol n. 53/2006, process n. 23081.014353/2006-33.

Five adult Beagle dogs aging between 16 e 22 months and weighing between 8 e 10 kg were used for this study. Asepsis during clinical procedure was maintained with Chlorhexidine 1% spray.

Before extracting the teeth and inserting the mini-screws prophylactic antibiotic regimen was instituted with Spyramicin 75,000 UI/kg e Metronidazole 12.5 mg/kg (Stomorgyl 10).

All surgical procedures were executed under general anesthesia and local analgesia was obtained with Lydocaine 2%.

To have enough space for screw insertion and to avoid root impact it was necessary the extraction of the four premolars of each dental arch (P1, P2, P3, P4). After extracting the teeth a silk suture was applied and antibiotics and analgesics were administered. A healing period of 18 week was waited before inserting the mini-screws.

Twenty sterile self-tapping mini-screws tomas® (Temporary Orthodontic Micro Anchorage System, Dentaurum, J.P. Winkelstroeter KG, Ispringen - Germany) were inserted, one for each quadrant in a randomly assigned position (P1, P2, P3 or P4). The mini-implants were inserted following the technique recommended from the manufacturer.

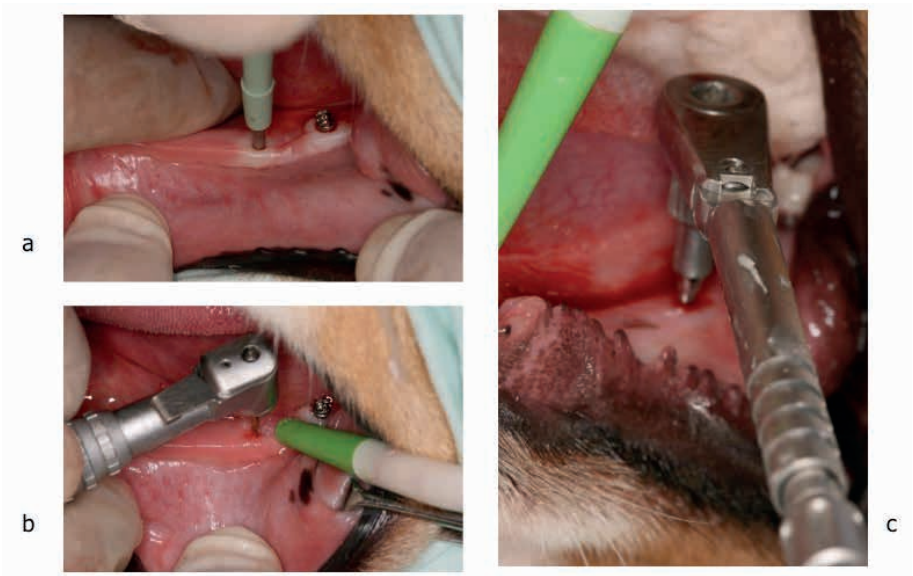

Fig. 1: a) Punching of the soft tissues; b) Pilot drill perforation; c) Insertion of the mini-implant with a torque ratchet.

To avoid implant fracture the mini-screws were inserted with a torque ratchet calibrated at 20 cN/cm^2 (ST tomas fracture limit: 28 cN/cm^2).

All mini-screws were meant to have a monocortical anchorage {Huja, 2005 #2032} even though this was not always possible, especially in the maxillary arch due to the reduced thickness of the residual ridge.

Post surgical medication (Tramadol HCl (Tramal) 1mg/kg/PO; Ketoprophen (Profenid) 1mg/kg (IM) and Spyramicin 75,000 UI/kg + Metronidazole 12.5 mg/kg (Stomorgyl 10 – 1 tablet 24/24h 10kg/PO) was given after implant surgery to reduce the pain and to prevent infections. Oral hygiene was maintained by 0.12% Chlorhexidine spray three times/day till animal sacrifice. To prevent implant loss due to occlusal forces the food was modified to a soft compound.

At day 0, 2, 7, 15 and 30 the animals were sacrificed under general anesthesia with 20ml KCl and soon after perfused with Zamboni's fixative.

Bony blocks containing the mini-implants were dissected and stored into fixative.

The specimen were dehydrated in a growing series of alcohol {Donath, 1988 #2028} and embedded in metacrylate for being cut along its longitudinal axes. To facilitate the aligning procedure in the cutting machine (Exakt®, Kulzer, Norderstedt, Germany) a custom made extension of the implant axes was realized (Fig.2).

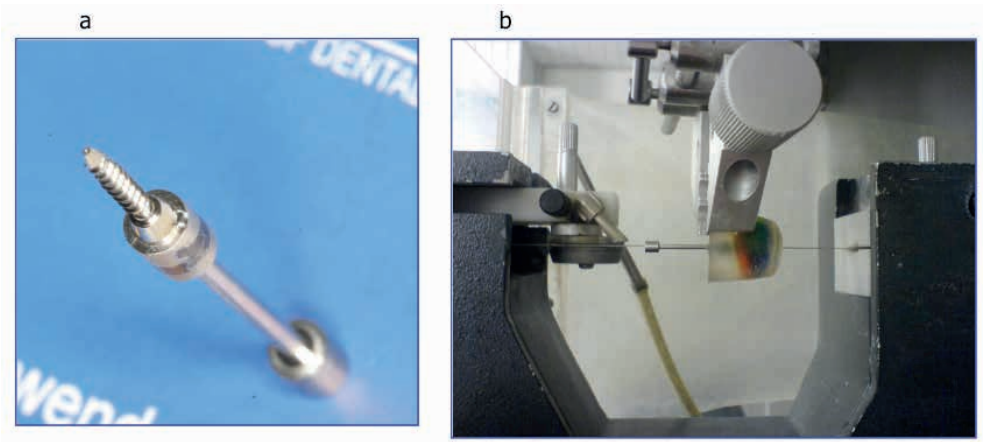

Fig. 2: a) Implant axes extension device; b) implant aligned in the cutting machine

A 50 μ thickness for each specimen was obtained with diamond disks {Donath, 1988 #2028} and the Stevenel's staining was made as described by Gotfredsen et al. {Gotfredsen, 2002 #2029}.

The specimens were examined under light microscope (AxioScope, Zeiss, Jena, Germay), and high-res digital images of the tissues were captured (AxioCam HRc, Zeiss, Jena, Germany) and stored for further analysis.

RESULTS

All five dogs showed good health during the experiment time and post surgical time was uneventful. All 80 (20 for the current study and 60 for pull-out test) inserted mini-screws were successfully maintained till the end of the experimental time giving a success rate of 100%. No inflammation or infection of the peri-implant area was observed during the healing

phase.

SOFT TISSUES ANALYSES

Soft tissues around orthodontic mini-screw neck, soon after its insertion, show the structure of a normal gingival tissue well adapted to the metallic structure and exhibits a light depression provoked by the conical shape of the neck on the cut orifice by whom the screw pierces the tissues and reaches the underlying bone. The surgical wound determined by the punch cut shows sharp clean edges that favor healing process.

It is possible to observe the normal composition of the gingival tissue: a thin keratinized epithelium with deep digitations overlaying a dense and organized connective tissue (Fig. 3).

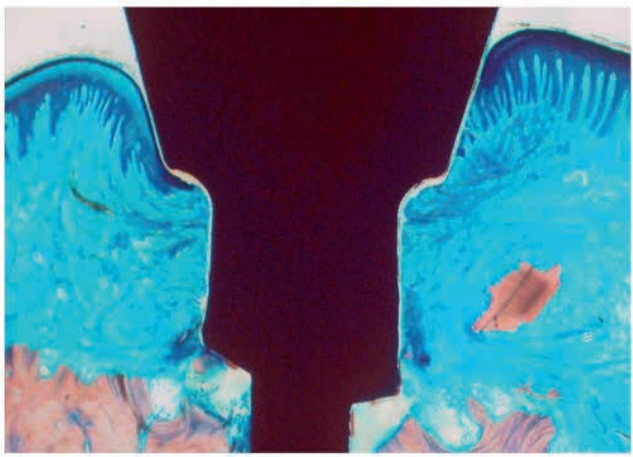

Fig. 3: Soft tissues aspect soon after implant insertion. It is visible a good adaptation of the epithelium and connective tissue at implant neck.

The pressure exerted by the implant neck over the soft tissues determines the presence of a small depression of the tissue mimicking the gingival sulcus.

The epithelium of approximately 0.5 mm shows the characteristics of the keratinized mucosa with no signs of inflammation (Fig. 4).

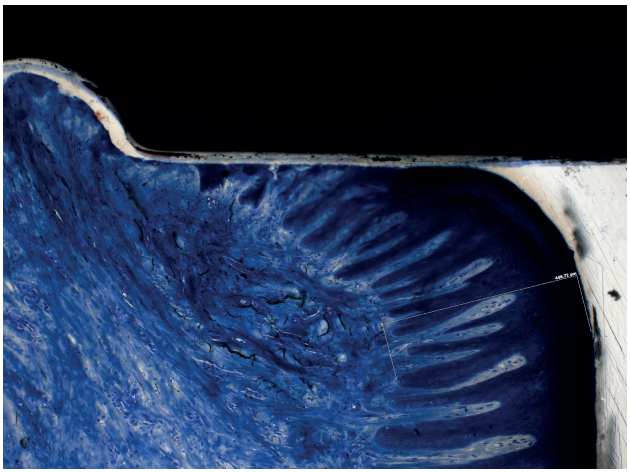

Fig. 4: Peri-implant epithelium soon after implant insertion.

The dense connective tissue (Fig. 5) shows the orientation of the collagen fibers of the bundles with a direction similar to the one observed near natural teeth. Both components of the soft tissue show a good adaptation to the implant neck providing an efficient seal against bacterial and foreign substances penetration to the underlying bone. Neither epithelial nor connective tissue adhesion to implant neck could be shown at this time.

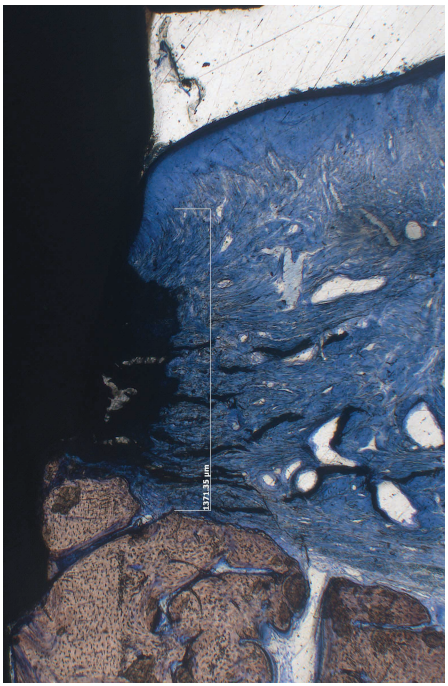

Fig. 5: Peri-implant connective tissue soon after implant insertion.

Two days after insertion (Fig. 6a and 6b) the epithelial morphology is not changed, showing an excellent adaptation to implant neck. An initial cellular down growth of about 1 mm, constituting the future junctional epithelium is visible at the interface with the metallic surface and ends before the beginning of the connective tissue layer (Fig. 4B). It cannot be seen a gingival sulcus like around normal teeth or dental implants.

Moderate signs of inflammation are visible in the context of the subepithelial conn

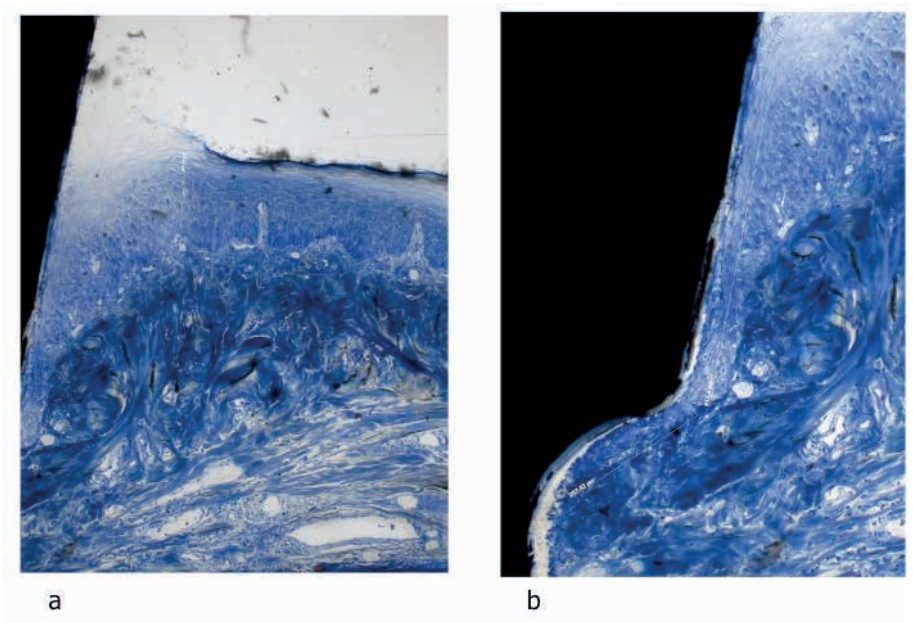

Fig. 6: a) Epithelium in contact with the neck of the implant 48 hours after insertion; b) Vertical down growth of the Junctional Epithelium

Connective tissue attachment (Fig. 7) is composed by dense tissue whose bundles are still disposed perpendicularly to the implant surface. Signs of a moderate inflammatory cell invasion can be remarked into the tissue. In the deeper layers the space between the bundles of collagen fibers seems to be increased as the caliper of the blood vessels. Subepithelial layers of the connective tissue appear to be less edematous and to keep the physiologic organization. The tissue in contact with the implant neck has an edematous aspect too and still cannot be demonstrated adhesion between the collagen fibers and the implant surface. The presence of various types of inflammatory cells testifies that there is an ongoing healing process.

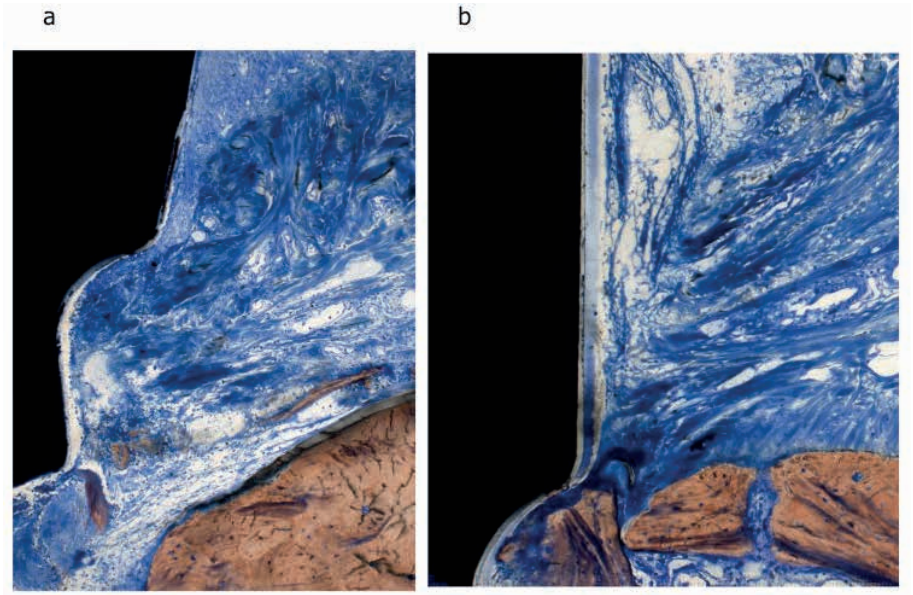

Fig. 7: Connective tissue aspect after 48 hours from insertion a) Diffused edema of the deeper layers; b) Peri-implant edema.

At day 7 after mini-screw insertion (Fig. 8a), the epithelium is still subtle, showing normal aspect of the digitations and of the cell layers (Fig. 8b). It' s well adapted to the implant neck and a small gingival sulcus is present (Fig. 8a). The cells that form the sulcular epithelium have a more clear aspect compared to the mature cells of the oral epithelium revealing an ongoing differentiation process. A proliferative activity is clearly visible as the cells proliferate in apical direction as to form the future junctional epithelium (Fig. 8b, SE).

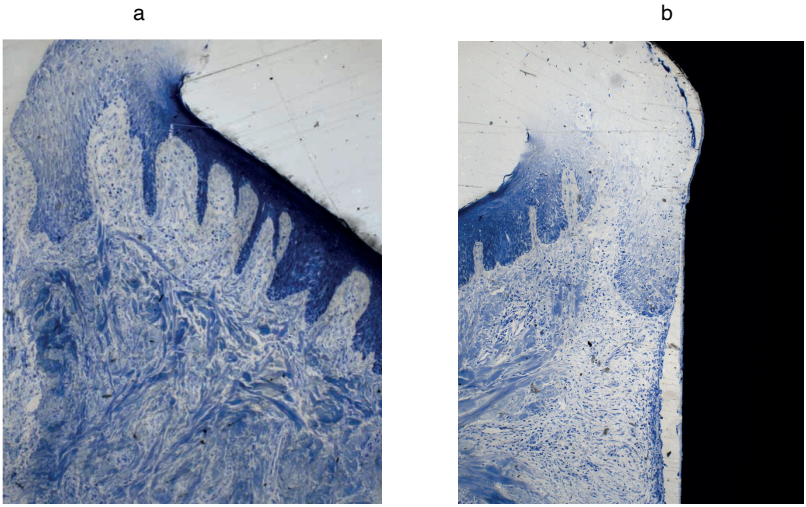

Fig. 8: a) Soft tissues after 7 days from mini-screw insertion; b) Sulcular epithelium and forming junctional epithelium (JE)

The collagen of the supracrestal connective tissue appears now to be normal with bundles oriented still perpendicularly to the implant surface. A moderate inflammatory infiltrate is still present as normal reaction to bacterial challenge but without clinic significance. There is an excellent adaptation to the implant neck. Some bony remnants derived from the surgical preparation of the bone are present in the tissue context (Fig. 9). The edema observed in the first stages of the tissue healing is not any more visible being the collagen bundles closer one to the other and the global aspect of the tissue is more compact. The blood vessels show in this phase normal caliper (Fig. 8b and Fig. 9).

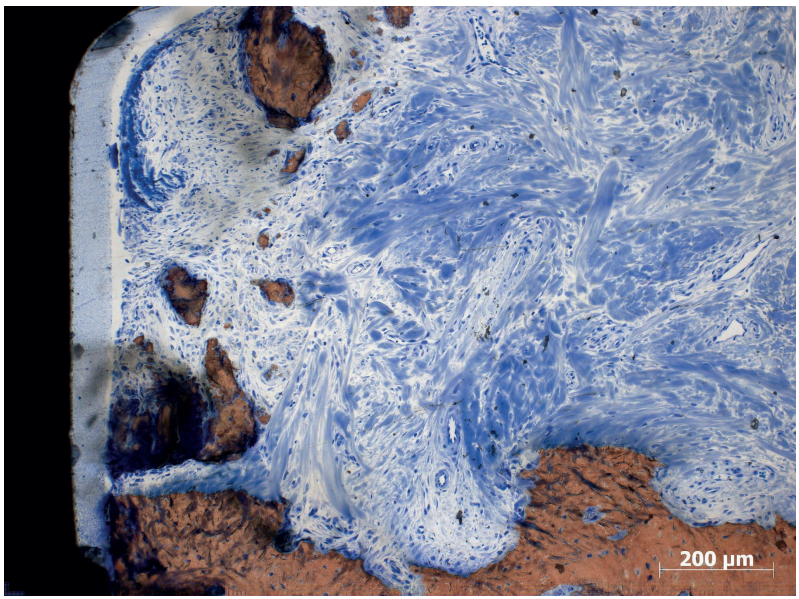

Fig. 9: Connective tissue aspect after 7 days from the surgery.

In a lateral area to the implant neck extending approximately 300-400 μm (Fig. 9), a mesenquimal type connective tissue is present, rich in cells and with a very small number of blood vessels, remembering the initial stage of a scar tissue formation. The outer portion of this area is formed by a more mature type of tissue with collagen fiber with random direction. In this area the vascular system is also reduced and no edema or inflammation is visible.

Bony chips deriving from the surgical act and the outer part of the crest show signs of osteoclastic resorption (Fig. 9).

After 15 days from he insertion the epithelium shows a more mature aspect when compared to the previous two weeks, especially with concern to the sulcular and junctional portion (Fig. 10).

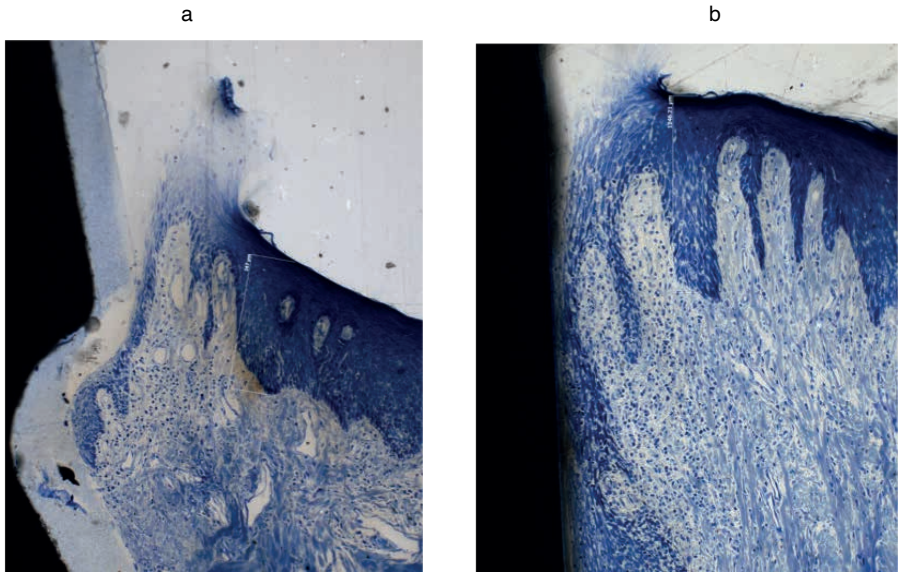

Fig. 10: Epithelium after 15 days from implant insertion: a) Juncional Epithelium (JE) and underlying Connective Tissue (CT); b) Sulcular Epithelium (SE).

A small gingiva sulcus is visible around implant neck. The epithelial cells of the sulcus show a more intense stain, which indicates a more mature cellular development stage. Below these cells a 2mm thick junctional epithelium is present (Fig. 10).

In subepithelial connective tissue a small inflammatory infiltrate is visible which is a normal finding in the presence of bacterial plaque challenge. Both epithelium and junctional epithelium appear to be away from the implant surface but this is due to a technique artifact.

Connective tissue layer in this maturation stage appear to more mature showing a well-organized tissue and functionally oriented architecture. There is no evidence of an inflammatory infiltrate and the tissue appears to be healthy (Fig. 11).

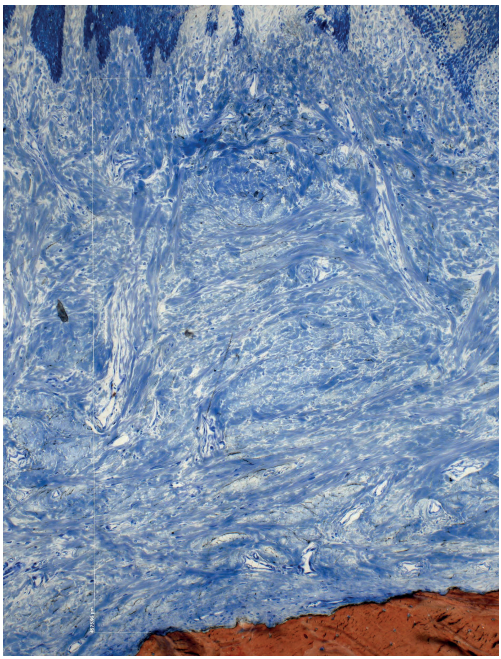

Fig. 11: Connective tissue after 15 days from implant insertion.

The collagen fibers bundles of the subepithelial layer show a direction parallel to the implant surface (Fig. 10) while the direction of the deeper layers, close to the bony surface, show a more perpendicular direction (Fig. 11).

The connective tissue close to the metallic surface (Fig. 12) shows a more pronounced maturity of the tissue in relation to he day 7: the connective tissue that was mainly composed by a mesenquimal type now contains less round cells and more fibroblast-like ones, with their nuclei oriented parallel to the implant wall and a collagen fibers group of periostal origin that inserts perpendicularly in a less mature tissue.

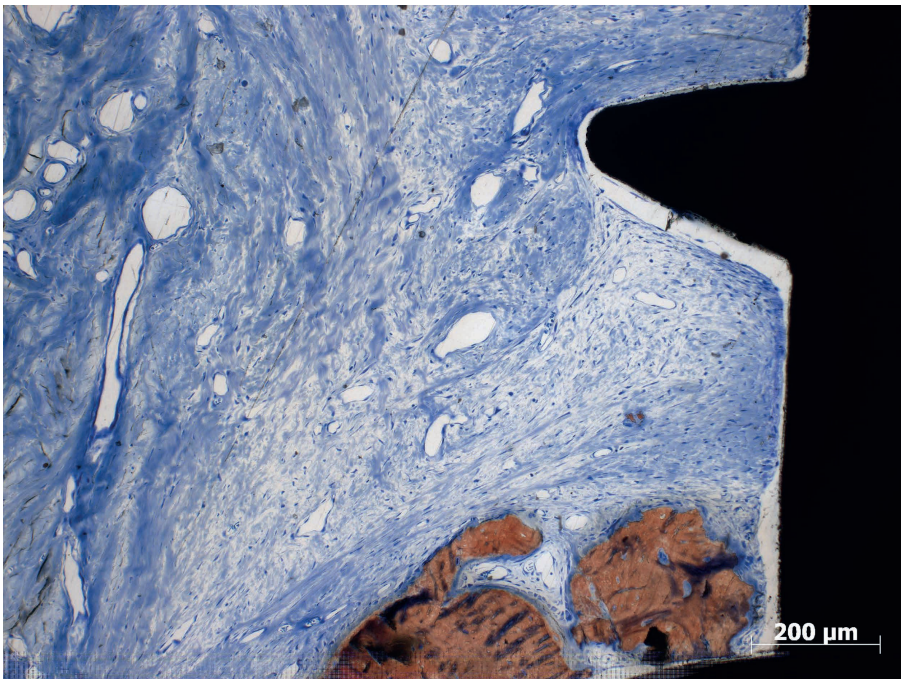

Fig. 12: Peri-implant collagen tissue after 15 days from implant insertion: close to the implant surface collagen fibers are oriented parallel to the metallic surface. Close to the bone surface periostal fibers inserts perpendicularly in a less mature connective tissue.

Vascular system appears to be moderately dilated showing more widened vessels where a metabolic activity is more intense, answering to a functional need (Fig. 12).

Active osteoclastic remodeling is present in the more superficial part of the bone crest and on the bone chips near to the implant surface (Fig. 12).

The aspect of the epithelium after 30 days from implant insertion is shown in Fig. 13.

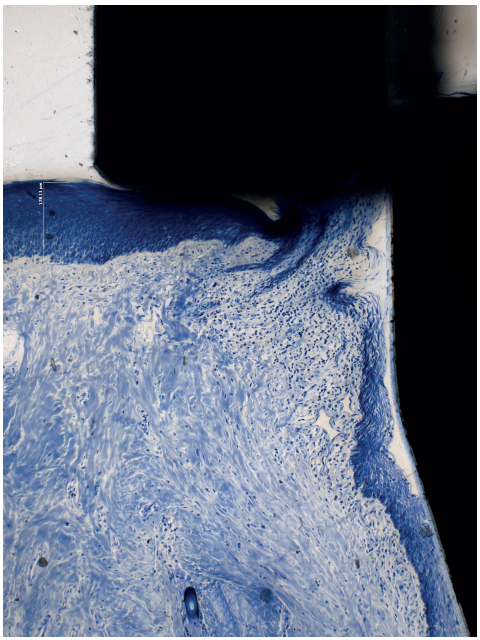

Fig. 13: Epithelium after 30 days from implant insertion.

In this healing phase of the epithelium shows the features of a mature tissue that is physiologically oriented to be a barrier against bacterial and foreign body invasion coming from the oral cavity. A subtle, keratinized tissue, adhering to the implant surface by means of a junctional epithelium measuring about 2mm, forms it. A small inflammatory infiltrate without clinical significance is present in the underlying connective tissues where the oral turns into the sulcular epithelium and continues more apically with the junctional epithelium.

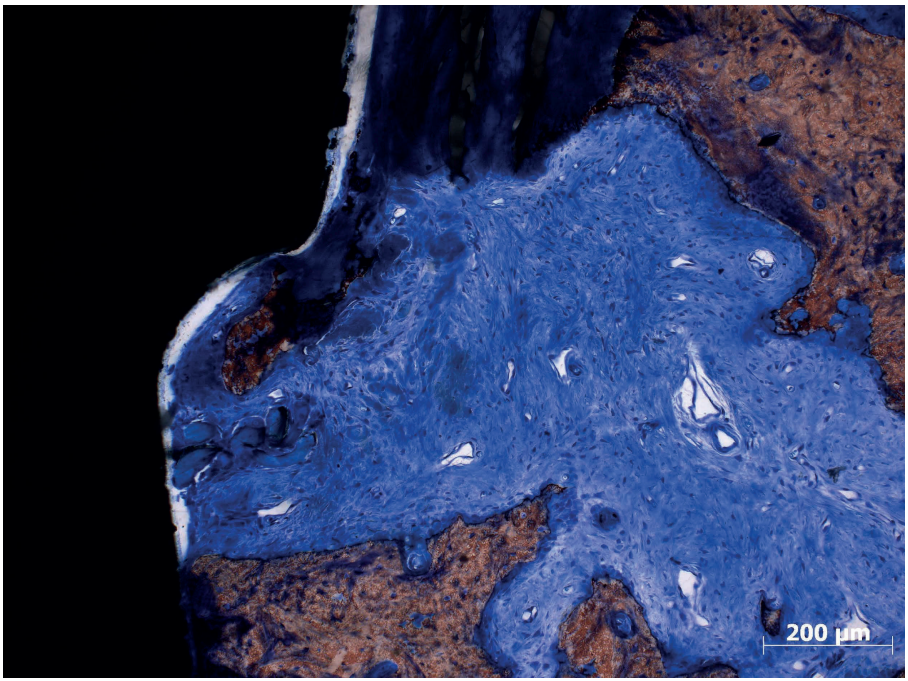

Fig. 14:Connective tissue 30 days after implants insertion.

The underlying connective tissue (Fig. 14) shows also the characteristics of tissue maturity compatibles with one month of healing. The compact subepithelial bundles of collagen fibers show a parallel direction to the implant surface (Fig. 13) and the vascular system is not dilated and the edema is totally disappeared.

In the deeper layers the connective tissue appears to be adhered to the implant surface and its dense collagen fiber bundles show a direction parallel to the implant surface. The mesenquimal-type tissue has been totally substituted by a more mature one. The part of the tissue in closer contact to the implant surface has a reduced vascular system and appears to be scar-like, especially when compared to the previous healing phases. Small vessels are visible in areas where the demand for metabolic activity is higher for remodeling and resorption of the bone chips deriving from the insertion of the screws.

DISCUSSION

The use of orthodontic mini-implants is very frequent when absolute orthodontic anchorage is needed. Some orthodontic movements like intrusion {Ohmae, 2001 #2038}, very difficult to be achieved prior to the introduction of these devices, now can be executed with greater amounts and controlled precisely and three dimensionally and can involve groups of teeth at one time like during anterior teeth intrusion {Kanomi, 1997 #2034}. Other teeth movements of paramount importance during orthodontic treatment that often require

absolute anchorage control are distalization {Horiuchi, 2008 #2031} and uprighting {Park, 2002 #2039}, allowing for severe anterior crowding or kipping solution without jeopardizing the position the other teeth already correctly located {Vigorito, 2004 #2044}.

Some histological data {Deguchi, 2003 #2026} have been published in the literature with respect to the healing of Grade 5 titanium orthodontic mini-screws with machined surface more with the aim of having histometric data related to search of an ideal parameter for loading. No one study was found to investigate early tissue healing around orthodontic mini-screws.

One other point to be elucidated is what kind of seal exists around orthodontic mini-screws as after their activation various plaque-retentive devices are used in the proximity of the soft tissues having the potential to induce a peri-implantitis.

The five Beagle dogs used in this experiment were healthy during the whole experimental period and all inserted mini-screws were maintained without signs of mobility or inflammation, registering 100% of success. These data are consistent with the findings of various Authors {Gotfredsen, 2002 #2029},{Deguchi, 2003 #2026} but in disagreement with the data presented from Huja{Huja, 2006 #2033} with self-drilling mini-screws and did not do tooth extractions scoring losses for approximately 20%.

Soon after their insertion the soft tissues around mini-implant neck show the normal composition of the gingival or mucosal tissue, precisely cut from a punch to allow for the insertion of the screw and are well adapted to the metallic surface allowing the immediate establishment of a tissue seal against the penetration of bacterial and foreign substances to the underlying layers. This observation disagrees with Schwarz et al. {Schwarz, 2007 #2042} studying the initial healing around SLA® implants where a 300 μm gap was encountered between the implant and the sutured flap. The penetration of bacterial and/or irritating substances in the surgical wound could lead to an infection and to the subsequent implant loss. For this reason is critical the post-surgical wound control with 0.12% or 0.2% Chlorhexidine {Heitz, 2004 #2030} rinsing.

The surgical technique for the insertion of the tomas® mini-screws does not need the elevation of a mucoperiostal flap like for osseointegrated implants but requires a 2mm punching of the gingival tissue before making the perforations in the bone. This procedure allows for an optimal adaptation of the soft tissues around the 2.75 mm implant neck and determines a better healing process of the soft tissue having been precisely cut by a scalpel and constitutes immediately an efficient barrier against infections. In this particular phase of the healing process has not been observes any union between the tissues and implant neck as in agreement with Schwarz et al. {Schwarz, 2007 #2042}.

Peri-implant tissues are composed by a keratinized epithelium with deep digitations entering into the subjacent connective tissue. The epithelium shows a light depression on implant side mimicking a small gingival sulcus. The connective tissue is dense with braided bundles with orientation similar to the one encountered around natural teeth as described

by Schwarz et al. {Schwarz, 2007 #2042} after one day of healing.

The inflammatory cell infiltrate encountered in the connective tissue, more localized under sulcular epithelium is normally present also around natural teeth in health conditions.

Vascular peri-implant system around mini-screws does not show any alteration because has not passed time enough for the vasodilatation to occur.

According with Schwarz et al. {Schwarz, 2007 #2042} there was no blood clot between the implant and the surrounding soft tissues.

After two days from implant insertion the epithelium is still subtle and shows normal morphology. The adaptation to the implant neck is very good. A peri-implant sulcus as the gingival sulcus around natural teeth is not visible. The epithelium shows a down growth of about 1mm in apical direction that is going to form the junctional epithelium. Moderate inflammatory signs are present in the subepithelial connective tissue.

The connective tissue shows a light inflammatory infiltrate together with the dilation of the vascular system corresponding macroscopically to the swelling and redness present at surgical sites. The spaces between the bundles appear to be increased as the caliper of the vessels, especially in the deeper layers while the more superficial bundles keep their more dense and organized aspect as described from Schwarz et al. {Schwarz, 2007 #2042}, {Schwarz, 2007 #2042}. Is not jet visible any union between the connective tissue and the implant surface {Schwarz, 2007 #2042}, {Schwarz, 2007 #2042}.

Even though some implants were not completely inserted, so that there was some small gap between the base of the neck and the cortical bone, the soft tissues were well adapted to the metallic structures keeping on maintaining the barrier against the bacterial or foreign substance penetration into the surgical wound.

After 7 days from implants insertion the epithelium is well adapted to the implants neck and does not show any morphologic abnormality. Where the oral turns into the sulcular epithelium is not consistently present a sulcus. This feature facilitates the cleaning procedure around the head of the orthodontic mini-screws because plaque accumulation is more difficult. A cleaning procedure in case of relatively deep sulcus would not be an easy task for the patient especially in those cases where the insertion place is in a posterior area.

The events sequence described by Abrahamson {Abrahamsson, 2002 #2017} during connective tissue healing around implants goes through the following stages: (a) formation of a blood clot followed by its transformation in granulation tissue at implant side; (b) migration of epithelial cells on connective tissue surface; (c) protein adsorption followed by cell migration on implant surface. In our specimens we could also see proliferation of non-keratinized epithelial cells, deriving from the oral epithelium, proliferating between the connective tissue and implant surface.

As observed by Schwarz et al. {Schwarz, 2007 #2042} either at day 4 or at day 7 the junctional epithelium was separated from the implant wall by a gap while connective tissue adhered to the implant surface already after one week. In our samples we could not see a

junctional epithelium after 7 days because the cells that composed the sulcular epithelium were less stained of the oral epithelium cells and showed a discrete proliferative activity but still they were not looking like mature cells of the junctional epithelium.

As found by Schwarz et al. {Schwarz, 2007 #2042} the bundles close to the implant wall are less dense and show collagen fibers and fibroblasts directed perpendicularly to the implant wall for the modSLA implants and parallel direction in the SLA. In our samples the outer layer of the connective tissue is denser with bundles oriented in random directions and contains an inflammatory infiltrate without clinical significance. The inner layer, closer to the implant wall, is less dense than the outer layer, remembering the mesenquimal aspect, rich in cells, and is well adapted to the implant wall but still does not show maturation enough to tell the orientation of the collagen fibers. There was no gap between the tissue and implant wall reinforcing the tissue barrier formed by the epithelium since the first moments from the surgical act.

Some bone fragments deriving from the surgical act are visible close to the cortical bone and show active osteoclastic remodeling. These data cannot be discussed with other papers in the literature as for the osseointegrated implants a mucoperiostal flap is elevated during the surgery and any eventual bone fragment is washed away from the saline used for rinsing after each phase during implant insertion. In the case of mini-screws a mucoperiostal flap is not elevated and only a small tissue roll is removed prior to pilot drilling and even though a saline is used for cooling the bur and rinsing the surgical area, its use is not very much effective in a such a small area. The edema observed in the first stages of healing disappeared as can be told from the vascular system that is not anymore dilated and in the outer area the collagen fiber bundles show again a more dense aspect with well interwoven amongst them. Vascular lumens appear to be numerous and dilated only in the inner part of the peri-implant connective tissue where an intense proliferative activity is ongoing.

The epithelium after 15 days of healing is already completely healed. The oral epithelium turns into the sulcular one making a small sulcus covered by a non-keratinized epithelium that more apically ends with a well developed junctional epithelium adhering to the implant surface, differently from what has been described from Schwarz et al. {Schwarz, 2007 #2042},{Schwarz, 2007 #2042} where, in this healing phase there was still a space between the implant neck and the epithelium. The intensity of the stain absorbed by the cell demonstrates a different degree of maturity from day 7 where the more clear aspect of the cells was related to a minor maturity degree. The junctional epithelium length is approximately 2mm according to the findings of Abrahamsson et al. {Abrahamsson, 2002 #2017},{Abrahamsson, 1996 #2016},{Abrahamsson, 1999 #2015},{Abrahamsson, 1996 #2016}. In the subepithelial connective tissue is present a light inflammatory infiltrate answering to the irritating elements present in the peri-implant sulcus. In any phase of the experiment were not observed clinical signs of inflammation of the peri-implant tissues, confirming the lack of clinical significance of the inflammatory cells present into the

subepithelial tissue.

Connective tissue, in this healing phase, shows already an advanced maturation degree with a functionally well-organized structure. No inflammatory infiltrate can be seen and the tissue. Collagen fiber bundles, in the subepithelial layer, show a direction parallel to the implant neck surface while in the deeper part, close to the cortical bone, the pattern is perpendicular. These findings are similar to Schwarz et al. {Schwarz, 2007 #2042}, {Schwarz, 2007 #2042} for the day 14 of healing around SLA or modSLA osseointegrated implants where the tissues are in contact with the implant surface and connective tissue is partially mature and is perpendicularly inserted.

The connective tissue closer to the implant wall shows a more pronounced tissue maturation as in the findings of Berglundh & Lindhe {Abrahamsson, 1996 #2016} related to day 7 of healing while the tissue that was previously only mesenquimal now has less round cells and more fibroblasts with nuclei predominantly parallel oriented towards the implant and a group of collagen fibers inserted perpendicularly in the less mature tissue.

Vascular system appears to be moderately dilated in response to metabolic activity for new collagen formation.

Active osteoclastic remodeling is visible on the cortical bone near to the implant and on the bone chips eventually present in the surgical area.

The aspect of the epithelium after 30 days from the surgery is a mature tissue and functionally oriented to isolate the area from bacterial or foreign body invasion in agreement with the observations of Buser et al. {Buser, 1992 #2021} around unloaded implants in dogs. A thin keratinized tissue that adheres to implant surface by means of a junctional epithelium constitutes the epithelium. The length of the junctional epithelium is about 2mm as observed by several Authors{Abrahamsson, 1996 #2016},{Abrahamsson, 2002 #2017},{Abrahamsson, 1996 #2016},{Abrahamsson, 1999 #2015}. A small inflammatory infiltrate is present especially in the area where the oral epithelium turns into the sulcular one and also under the junctional epithelium.

The underlying connective tissue shows also maturity features. The compact subepithelial bundles of collagen fibers follow a pattern parallel to the implant neck. The vascular system is not dilated because the edema is not any more present in this area. These findings are similar to the ones described from several Authors after 3 {Buser, 1992 #2021}, 6 {Abrahamsson, 2002 #2017},{Abrahamsson, 1996 #2016} and 12 months {Abrahamsson, 1999 #2015} testifying that the healing process of the soft tissue is already completed. Moon et al. {Abrahamsson, 1999 #2015} describe in the supracrestal connective tissue context 200 μm band that extends from the implant wall in which two portions can be distinguished: the first, called “central zone or zone A” 40 mμ thick, is characterized by the a reduced number of blood vessels and for the abundance of fibroblasts between collagen fibers; the second called “lateral zone or zone B” 160 μm thick is characterized by the presence of less fibroblasts but more collagen fibers and blood vessels.

In the deeper layers the connective tissue is adhering to the metallic surface of the implant and its collagen fibers, characterized by dense bundles, physiologically oriented to protect the bone, show a pattern parallel to the neck of the implant as in the findings of Moon et al. {Abrahamsson, 1999 #2015}. The portion of the tissue in closer contact to the implant surface has a reduced vascular system and a scar-like aspect in comparison to the findings of the day 15. Small vessels are always more visible in remodeling areas where bone chips, remnants of the surgery, can be found.

CONCLUSIONS

The healing process of soft tissues around unloaded self-tapping orthodontic mini-screws can be described as follows:

- Both epithelium and connective tissue are well adapted to implant neck soon after the completion of the insertion surgery.
- Soft tissue healing is already visible at day 2 from insertion with the apical proliferation of the cells that will form the junctional epithelium.
- The inflammatory infiltrate seen in the examined area is minimal and compatible with the physiologic healing process happening in the peri-implant area.
- Connective tissue adheres to the neck of the implant already during the first week after implant insertion and its collagen fibers show a pattern parallel with the implant surface.
- Epithelial down growth to the cortical bone was not observed in any of the examined specimen.
- A peri-implant biologic width similar to the one described for the osseointegrated implants, formed by a 2mm junctional epithelium and a 1mm connective tissue layer could be described.
- The barrier function against the bacterial and foreign substance penetration was effective since from the early moments after implant insertion.

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