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

Data de aceite: 01/12/2023

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: Understanding tissue healing around orthodontic mini-screws can provide useful information for their clinical use. Despite some similarities with 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 hard tissue healing around unloaded orthodontic mini-implants. Twenty self- tapping mini-screws were inserted in five Beagle dogs 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. The samples were stained with Stevenel's blue and Goldner and observed under light microscopy. Soon after insertion, primary stability was good for all mini-implants. In some implant areas, direct contact with the parent lamellar bone was observed. In other areas a gap due to the pilot bur drill was present and was initially filled by a coagulum. At day two the blood clot was transformed in granulation tissue and an amorphous protein matrix, containing osteoblasts, was noticed on the parent bone. Some lymphocytes and macrophages could be observed among the red blood cells and the small chips deriving from the bone preparation. At day 7 was evident the

osteoid deposition over the pristine lamellar bone. At day 15 woven bone could be seen within the osteoid matrix bridging from the parent bone towards the titanium surface. At day 30, beyond the woven bone, is visible parallel fiber bone deposited directly on the implant surface, indicating osseointegration of the screws.
KEYWORDS: Osseointegration; orthodontics; mini-implants; hard tissues healing; animal study.

INTRODUCTION

Introduced by Kanomi (Kanomi 1997) in 1997, Orthodontic mini-screws are routinely used in orthodontic treatments to obtain anchorage absolute control during teeth movements (Park, et al. 2003),(Carano, et al. 2004). To achieve absolute anchorage control is mandatory that the mini-screws have to be stably fixed to the bone (Davies 1998).

The healing processes happening into the bone after prosthetic implant insertion (Schenk & Buser 1998),(Abrahamsson, et al. 2004) have been described under the term osseointegration. This process has been defined (Albrektsson 2008), understood and describedwith great detail by several Authors (Davies 1998),(Schenk & Buser 1998),(Davies 2003) for the implants used in prosthodontic rehabilitations as well as the relationship between soft tissues and implant neck (Buser, et al. 1992), ((Berglundh & Lindhe 1996).

A difference in the quality of the osseointegration process has been described in the literature as depending from several factors. Among them we can find surface morphology (Davies 1998),(Di Carmine, et al. 2003), different surface coating materials (Gahlert, et al. 2007, Le Guehennec, et al. 2008) and other factors like surface energy (Schwarz, et al. 2007).

It is possible to speculate that exist some differences in the tissue healing process between a prosthetic implant and an orthodontic mini-screw due to the several differences existing amongst them. The construction material is grade 4 commercially pure (c.p.) titanium for the prosthetic implants and grade 5 titanium, an alloy of Titanium, Aluminum and Vanadium ($TiAl_4V_6$) for the orthodontic mini-screws. Both metals have a high degree of physical resistance and are widely used in medicine and dentistry to build the implants. The surface treatment type most recently used for the prosthetic implants is the SLA (Sandblasted-Large grit- Acid etched) or modSLA (modified SLA) because it has achieved the best results in terms of osseointegration (Buser 1999) when compared to other surface types as TPS (Titanium Plasma Sprayed) or turned or electropolished surfaces (Buser 1999). Orthodontic mini-screws are fabricated with turned or electropolished surface because they are meant to be easily removable after their time-limited clinical use. Prosthetic receive an axial load while orthodontic implants and mini- screws are loaded laterally. The time to wait until loading for the prosthetic implants and for the Orthosystem® (Straumann Holding AG, Waldenburg, Switzerland) is variable and it is related to the type of surface treatment and of bone where they are inserted varying between 4-8 weeks for the SLA/modSLA surfaces

and 4-6 months for other surfaces as TPS. For the orthodontic mini-screws the time to wait until loading has varied between immediate (Costa, et al. 1998),(Maino, et al. 2003) and 2-3 weeks (Kyung, et al. 2003).

Aim of this study was to study *"in vivo"* and describe the healing process around self-tapping orthodontic mini-implants during 30 days and to compare it to the prosthetic implants.

PREMISE

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.

MATERIALS E METHODS

Twenty sterile self-tapping mini-screws tomas® (Temporary Orthodontic Micro Anchorage System, Dentaurum, J.P. Winkelstroeter KG, Ispringen - Germany) were inserted in 5 adult Beagle dogs, 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).

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 topical Lydocaine 2%. Asepsis was maintained with Chlorhexidine 1% spray.

All mini-screws were meant to have a monocortical anchorage (Huja, et al. 2005) even though this was not always possible, especially in the maxillary arch due to the reduced thickness of theresidual ridge.

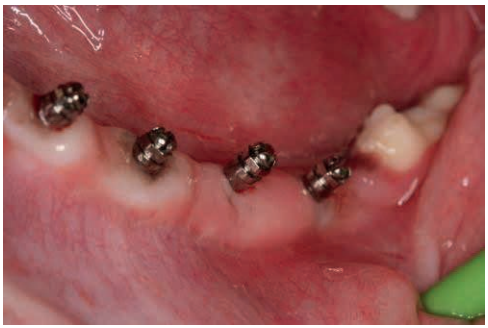

Fig. 1: Mini-screws inserted in the mandible.

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) 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: Specimen after cutting in the Exakt® machine.

A 50 μ (Donath 1988) thickness for each specimen was obtained with diamond disks. Stevenel's staining as described by Gotfredsen (Gotfredsen, et al. 2002) and Masson-

Goldner were made.

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) andstored for further analysis.

Detailed descriptions of the procedures and of the materials used for this experiment have beenpreviously described [REF: Morea et al, 2008 or 2009, if approved by the referee].

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%. Noinflammation or infection of the peri-implant area was observed during the healing phase.

HARD TISSUES ANALYSIS

A substantial difference was encountered during implant insertion either in the maxilla or in the mandible: the availability of cortical and marrow bone in contact with the mini-screw. The relative quantities of cortical and medullar bone change in the maxilla between anterior and posterior region. In maxillary anterior region the residual alveolar ridge (basal bone) is very thin and in the average measures approximately 3mm. In the case shown in Fig. 3A there was a bicortical perforation having the screw reached the nasal cavity of the dog. In maxillary posteriorregion corresponding to the fourth premolar area the cortical bone thickness is still very subtle but the trabecular bone availability is much higher surrounding completely the mini-screw (Fig. 3B).

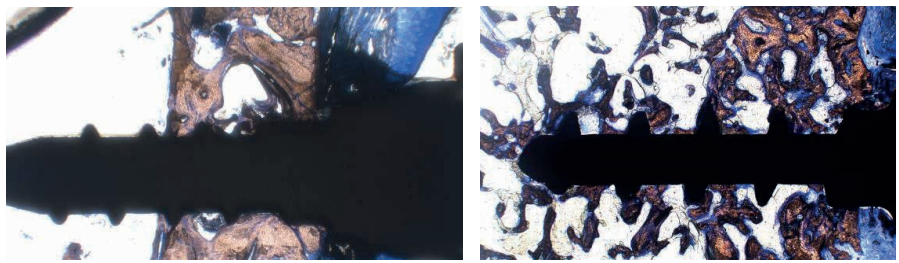

Fig. 3: A) Mini-screw inserted in the maxillary P2 region; B) Mini-screw inserted in the maxillaryP4 region.

In Fig. 4 is shown macroscopically the mini-screw that reached the nasal cavity of the dog. In a bicortical perforation (Fig.4B) approximately 40% of the body of the mini-screw is penetrated the nasal cavity pulling up the respiratory mucosa (Fig. 4A) and forming a coagulum between the bone and the soft tissues (Fig. 4C). Even though in some cases it

was observed some nose bleeding after the surgery, it was minimal and none of the inserted implants was lost till animal sacrifice.

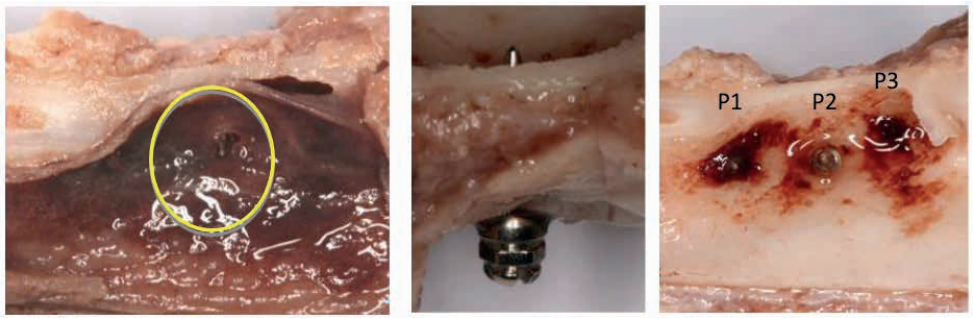

Fig. 4: Mini-implant inserted in P2 region: A) The nasal mucosa appears to be perforated by the point of the mini-screw; B) Longitudinal section of the alveolar ridge in the same area; C) Tips of mini-screws inserted bicortically penetrating the nasal cavity.

In the mandible the bone presents different characteristics (Fig. 5). The cortical bone has a much higher thickness than the maxillary one and the mini-screw is in contact with it for almost 2mm. The reminder of the screw is in contact with a trabecular bone of thickness and structure similar to the maxillary one. Are not detectable relevant differences among the areas P1 through P4.

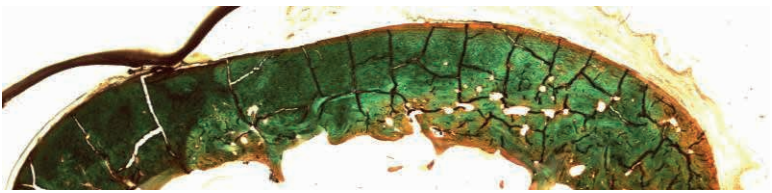

Fig. 5: Mini-screw inserted in Mandibular P3 area.

The bone tissue soon after mini-screw insertion is of mature, lamellar type (Fig. 6A e 6B) and shows the signs of the pilot perforation made by the bur and a space of 50-60μm is visible between the bone wall and the screw surface. This space is occupied by a blood clot.

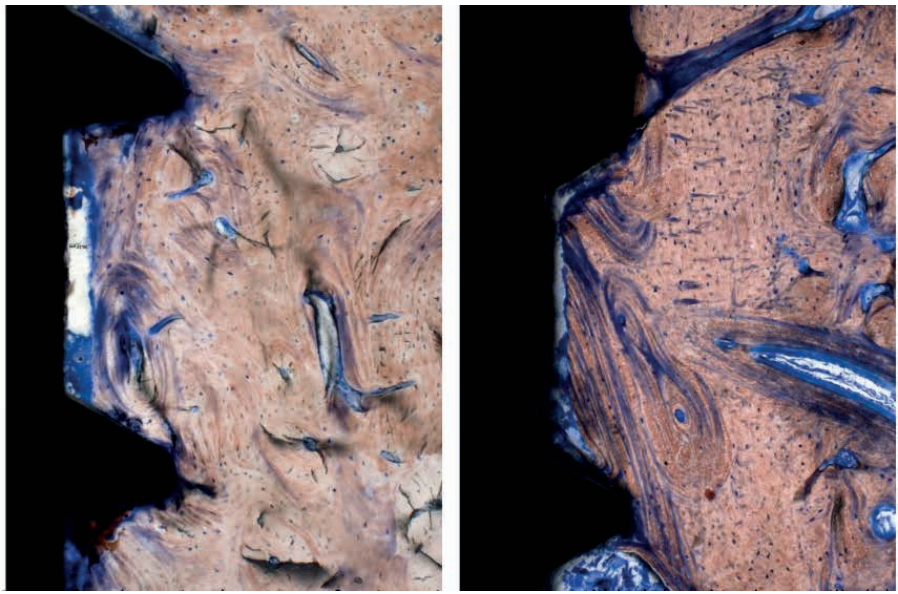

Fig. 6: Interface bone-implant soon after insertion in the mandible (A) and in the maxilla (B). A50-60 μm space is occupied by a blood clot.

An excellent adaptation between the threads of the screw and the bone can be observed since from the beginning (Fig. 6A and 6B) for the presence of a great amount of mature lamellar bone in contact with the metallic surface of the implant. At the pitch of the threads some micro- fractures due to implant insertion are visible (Fig. 7).

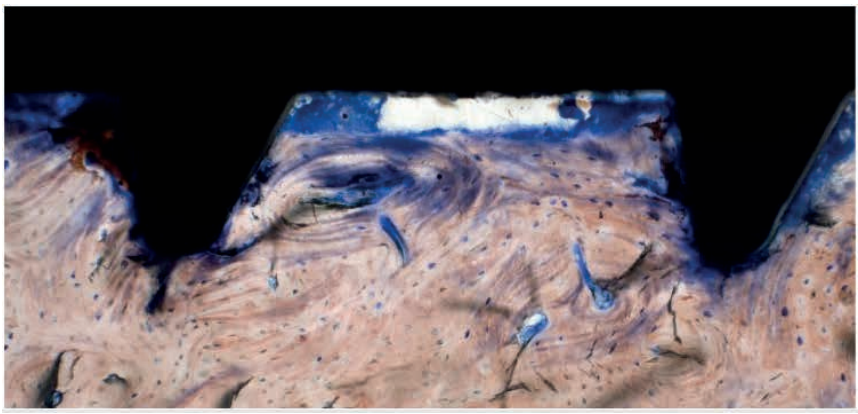

Fig. 7: Micro-fractures due to implant insertion are visible in the bone at the thread pitches.

In some areas, even though soon after the insertion of the implant exists an excellent and full adaptation of the bone to the implant surface (Fig. 8).

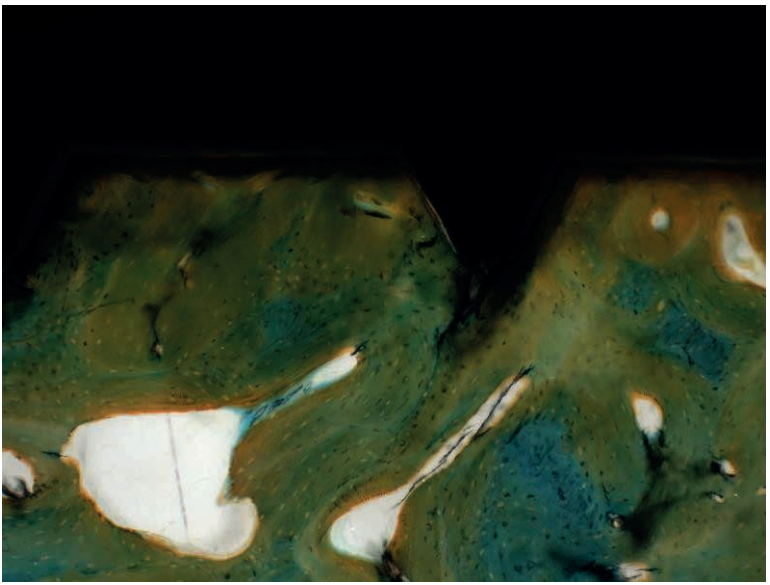

Fig. 8: Area of excellent adaptation between implant and bone soon after insertion.

Apically to the implant tip is visible, in the perforation made during the pilot drilling, some bone together with a blood clot derived from the cut of the blood vessels as a consequence of the preparation of the bone prior to implant insertion (Fig. 9).

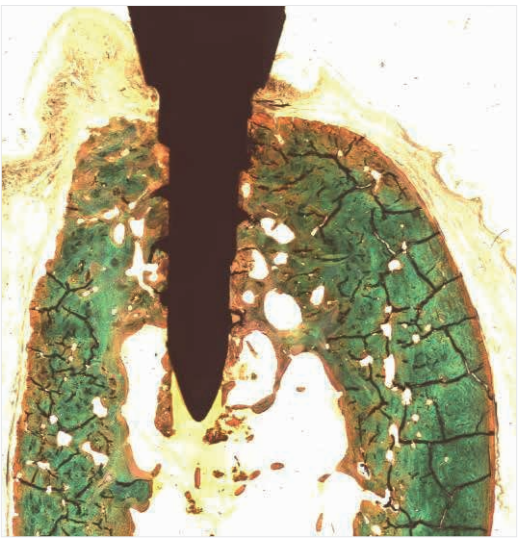

Fig. 9: Apically to the implant tip is visible a blood clot and some bone chips due to thepreparation of the bone.

In Fig. 10 is visible an implant inserted in the mandible of a dog sacrificed after 48 hours.In his case the screw was not completely inserted with the base of the neck in contact

with the cortical bone being the first thread and the space between the pitches confined within the soft tissues. The majority of the screw is in contact with the cortical and trabecular with the exception of the most coronal and most apical part of the implant tip. Also in this case the perforation exceeded the implant length leaving an empty space occupied by bone chips and a blood clot (Fig. 10).

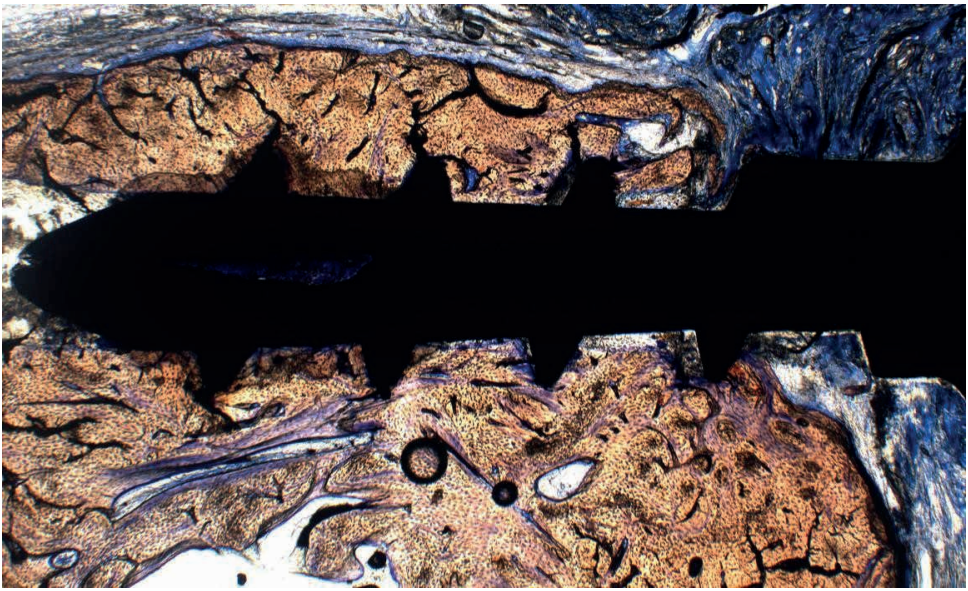

Fig. 10: Mini-mplant 48 hours from its insertion.

Analyzing he interface between the bone and the implant body (Figs. 11 e Fig. 12) after 48 hours from insertion we can observe the presence of inflammatory cells, mainly lymphocytesand macrophages together with red blood cells. On the bone surface is present an amorphous matrix containing osteoblasts. In the interface between the matrix and the bone can be noticed the reversion line. On the implant body is visible a thin layer of a proteic substance, amorphous and without cellular content. In this phase neo-osteogenesys is occurring together with the healing of the wound.

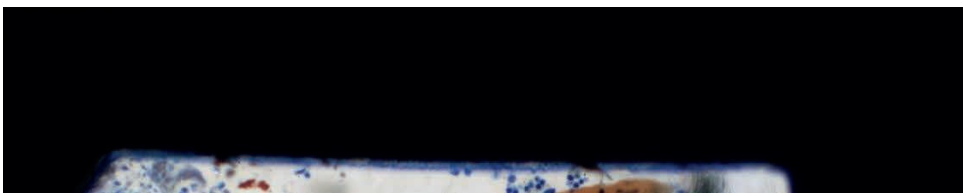

Fig. 11: Interface bone-implant 48 hours after insertion surgery.

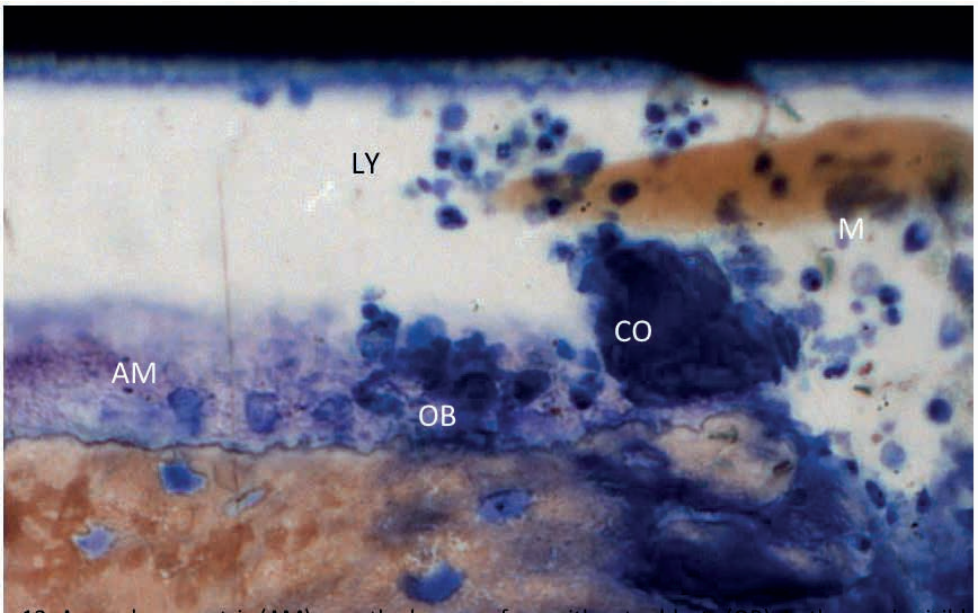

Fig. 12: Amorphous matrix (AM) over the bone surface with osteoblasts (OB). In the gap is visiblea blood clot (CO) with lynphocytes (LY) and macrophages (M).

On the areas of the implant body that do not have intimate contact with the bone (Fig. 13) adheres a fibrin net, especially in the pitches of the threads.

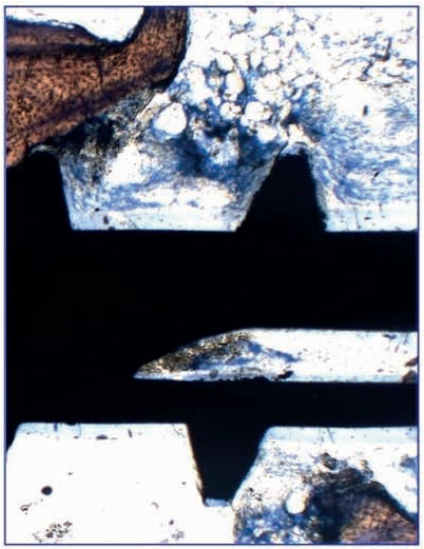

Fig. 13: Fibrin network adhered to the implant surface 48 hours after implantation.

In some other areas (Fig. 14) the matrix between the bone and the implant body shows a network pattern and contains the cells of the osteoblastic line thus exhibiting osteogenic

activity. Is still visible in this phase of the tissues the presence of numerous inflammatory cells involved in the healing process of the surgical wound.

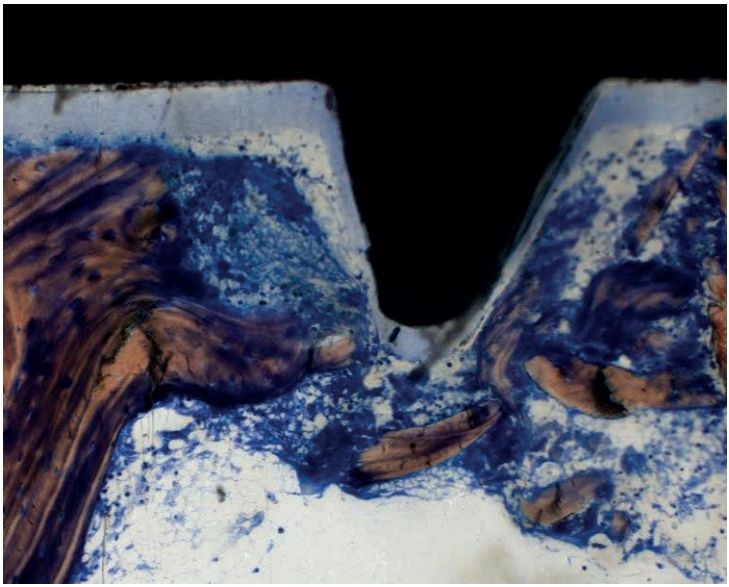

Fig. 14: Osteogenic activity area.

After one week from the insertion of the mini-screws into the bone is still visible the space left between the implant body and the screw during the drilling of the pilot perforation (Fig. 15).

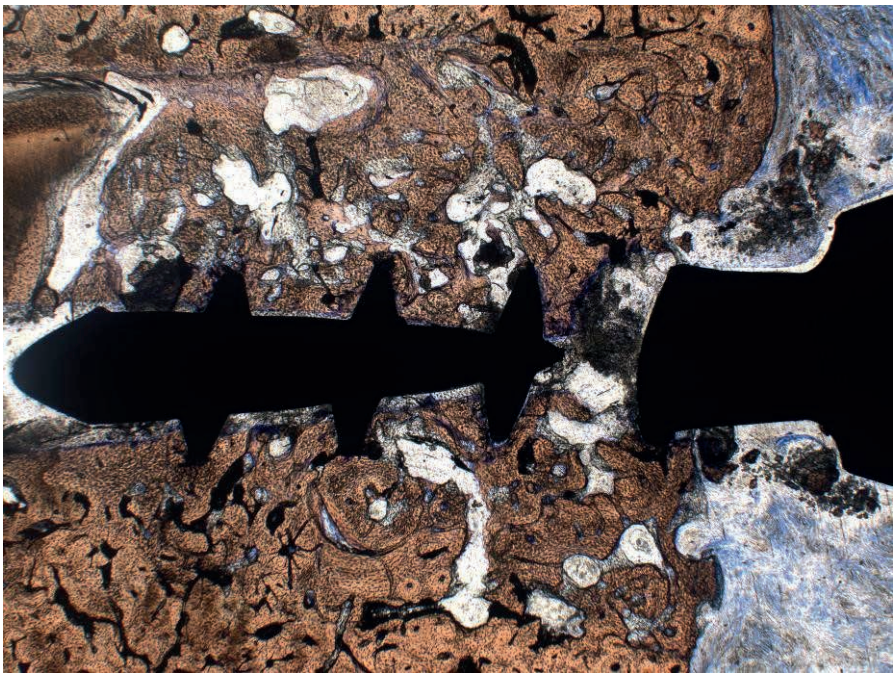

Fig. 15: Mini-screw after 1 week from its insertion.

Examining the interface between the implant body and the bone is possible to see the evolution of the healing process (Fig. 16).

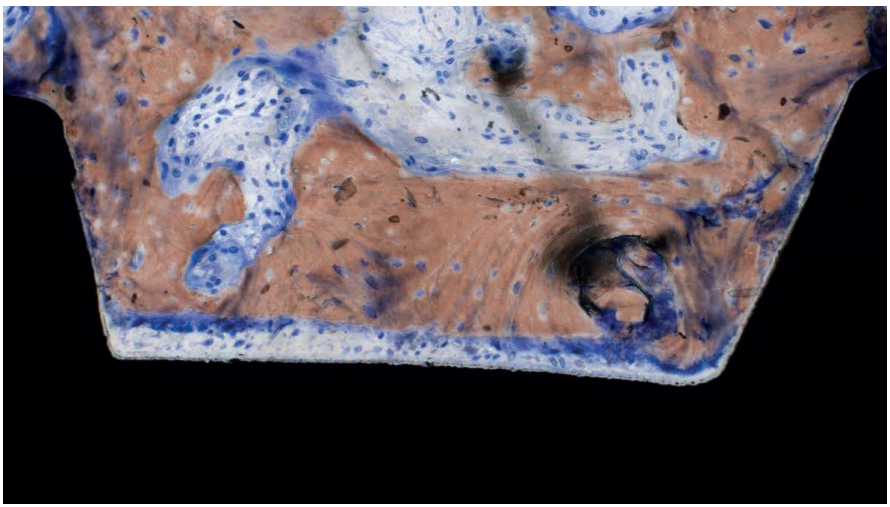

Fig. 16: Interface bone-implant after 7 days from implantation.

The gap is now filled by an amorphous matrix rich in osteoblasts coming from the neighboring marrow spaces rich of those cells.

It is interesting to notice that the bone in contact with the thread pitches as well as in

the other areas in direct contact with the screw since from the beginning of the implantation the lamellae are intact and do not show any sign of remodeling due to the absence of osteoclastic activity (Fig. 15).

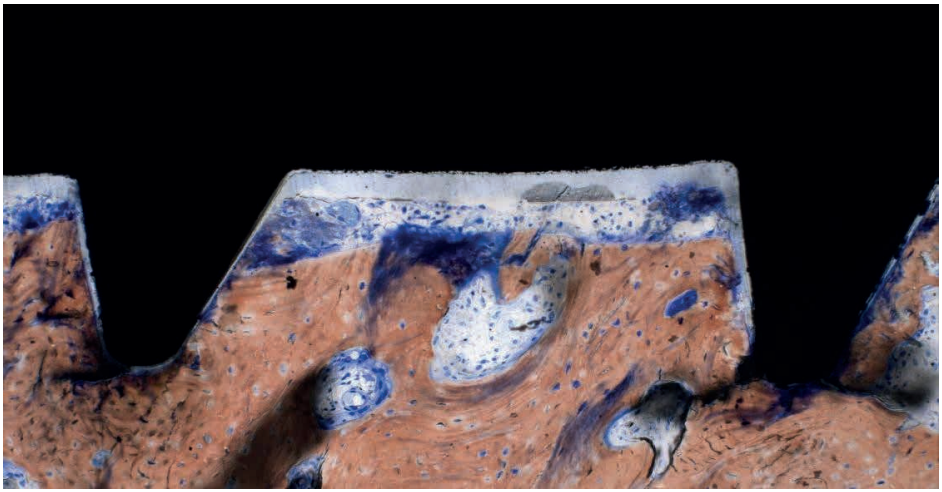

Fig. 17: Threads in contact with the lamellar bone 7 day after implant insertion.

Fifteen days after implantation, in the areas where initially there was a gap between theimplant body and the bone surface, there is not yet a bone fill (Fig. 18). In the tip of the screw area (Fig. 19) where at the beginning there was an empty space due to the pilot drill action and the tip was initially covered by a coagulum and then from a fibrin layer, is now visible a protein matrix containing newly formed bone (woven bone) which is going to form new trabecular primary bone with a central vessel.

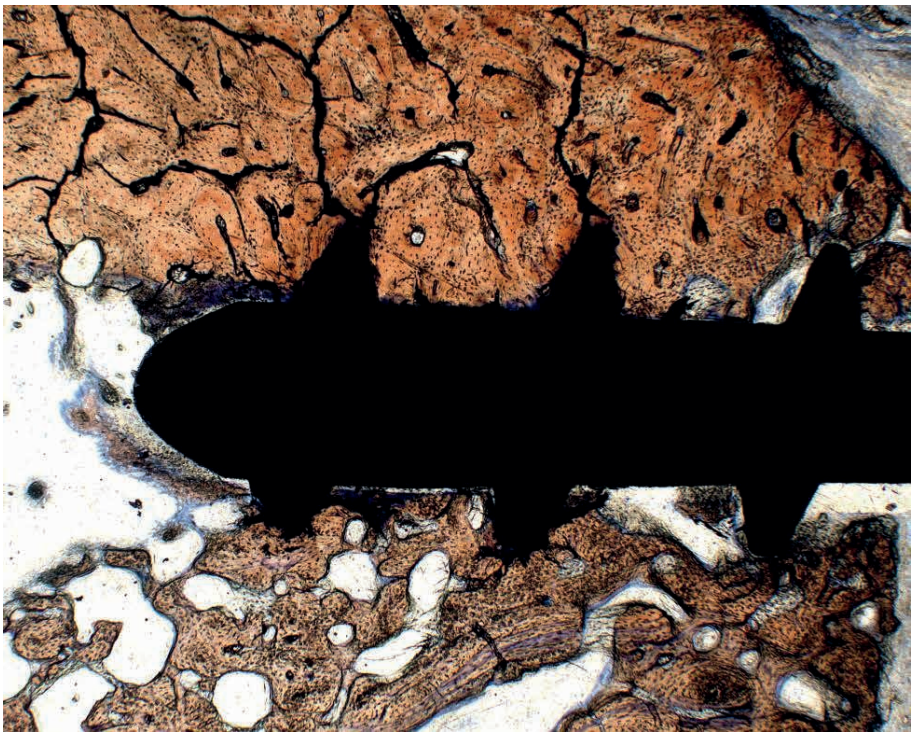

Fig. 18: Mini-implant 15 days after insertion.

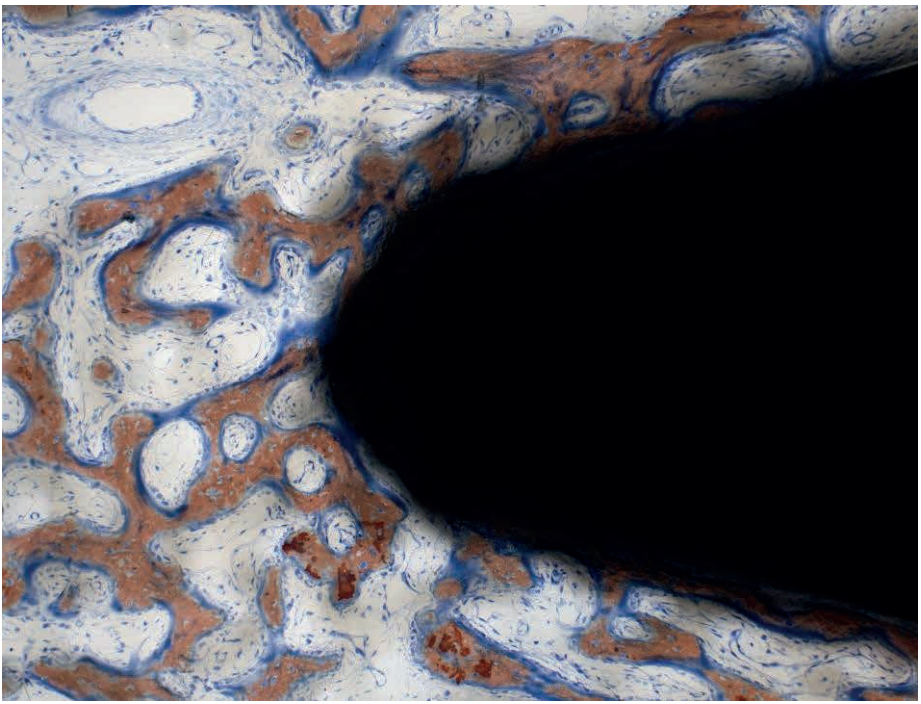

Fig. 19: Neo-osteogenesys (woven bone) around implant tip 15 days after insertion.

In the areas where the amorphous matrix with the osteoblastic cells was laid at this stage of the healing is visible primary bone within a protein matrix. Numerous osteoblasts are present at the around the mineralized bone revealing an intense osteogenic activity (Fig. 21).

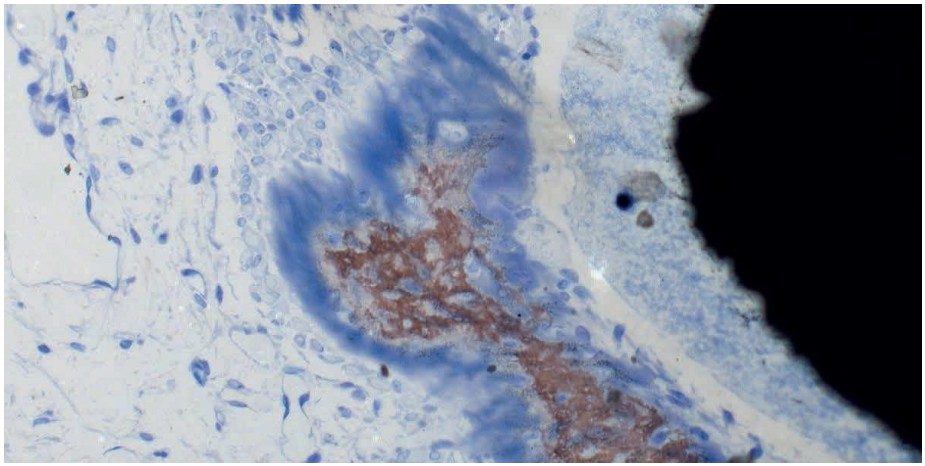

Fig. 21: Neo-osteogenesys around implant tip 15 days after insertio

One more example of the ongoing osteogenesys is visible in picture 22 where an osteoblasticlayer depositing bone matrix covers the pre-existing lamellar bone, surrounded by a large number of blood vessels for the nutrients transportation in the reparation area.

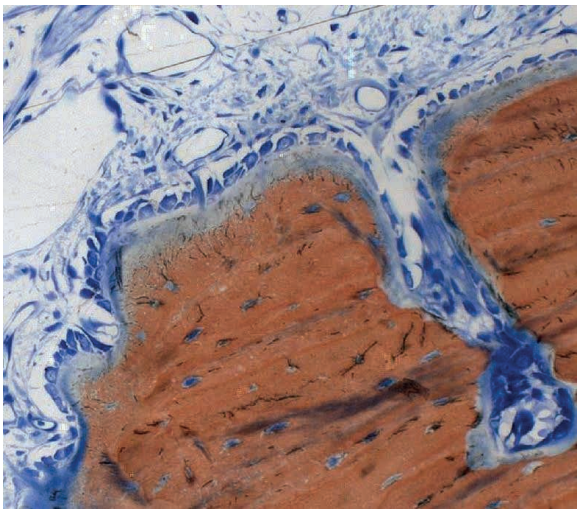

Fig. 22: Neo-osteogenesys area

In Fig. 23 can be seen the transformation of the granulation tissue into bone for the great number of cells present into the matrix and blood vessels of medium and small caliper together with the newly formed bone joining the pre-existing lamellar bone.

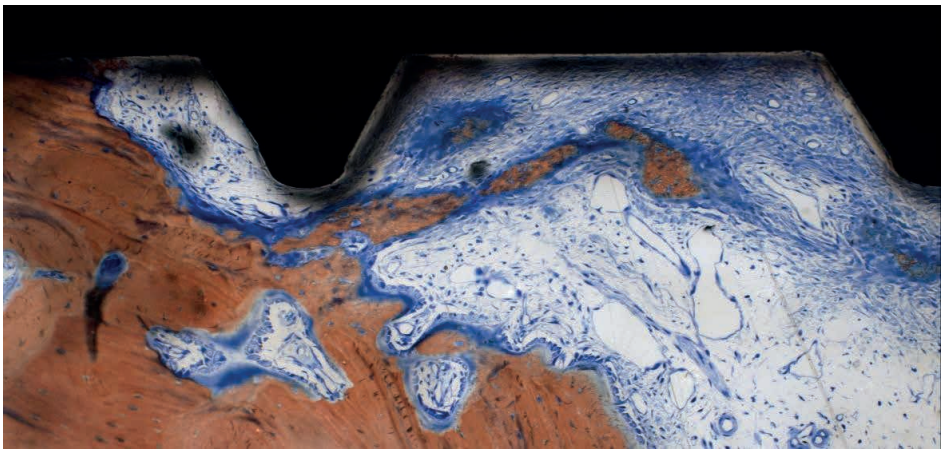

Fig. 23: Newly formed bone area occurring in the interface between bone and implant after 15days of healing.

In some other areas pristine lamellar bone can be seen in direct contact with the implant body without any sign of remodeling (Fig. 24).

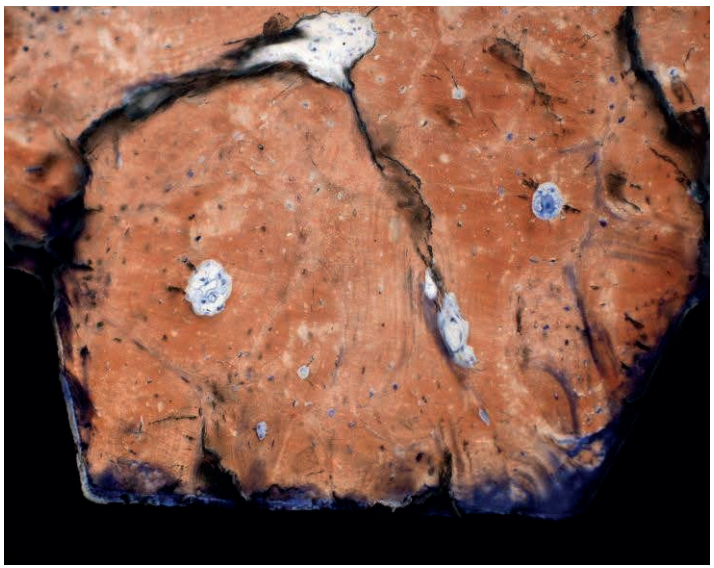

Fig. 24: Lamellar secondary bone with Havers channels in contact with the implant body inserted15 day before.

After 30 days from implant insertion direct bone deposition on implant surface is visible (Fig. 25), woven bone, primary osteons and lamellar bone are in contact with the pristine bone. In the newly formed bone areas can be seen osteoblasts layers depositing new matrix to be mineralized.

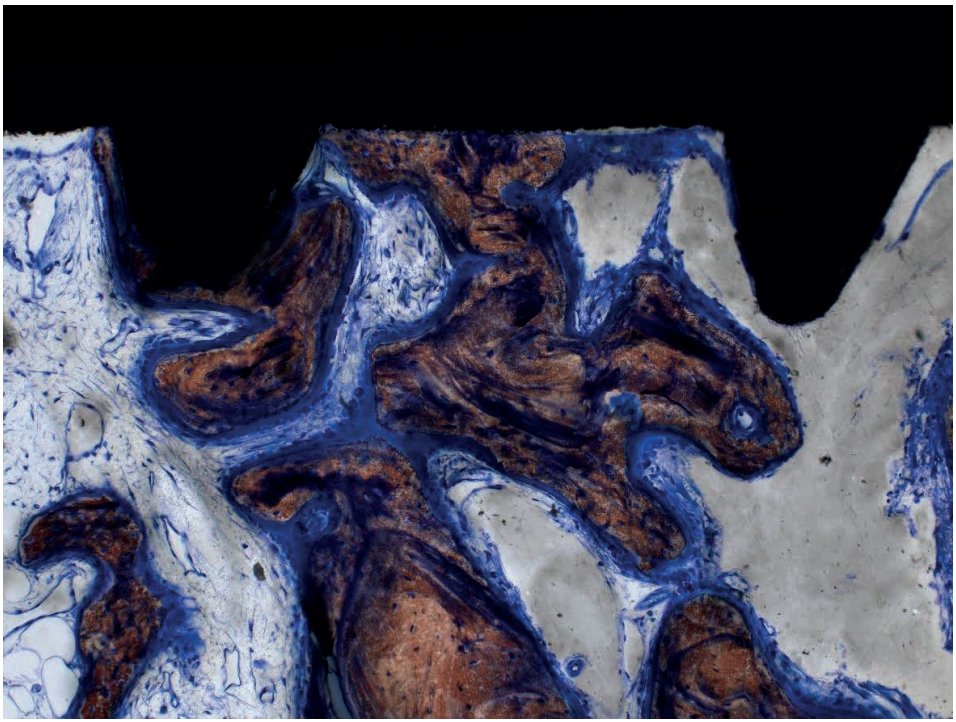

Fig. 25: Osteogenic area after 30 of healing.

Laterally to the areas of woven bone genesis (Fig. 26) where the osteoblasts (OB) keep on laying down the bone matrix can be observed a connective tissue characterized by a collagen fibers matrix with reticular pattern containing a great number of fibroblast-like fuse-shaped cells together with a large number of blood vessels remembering the immature connective tissue (CT).

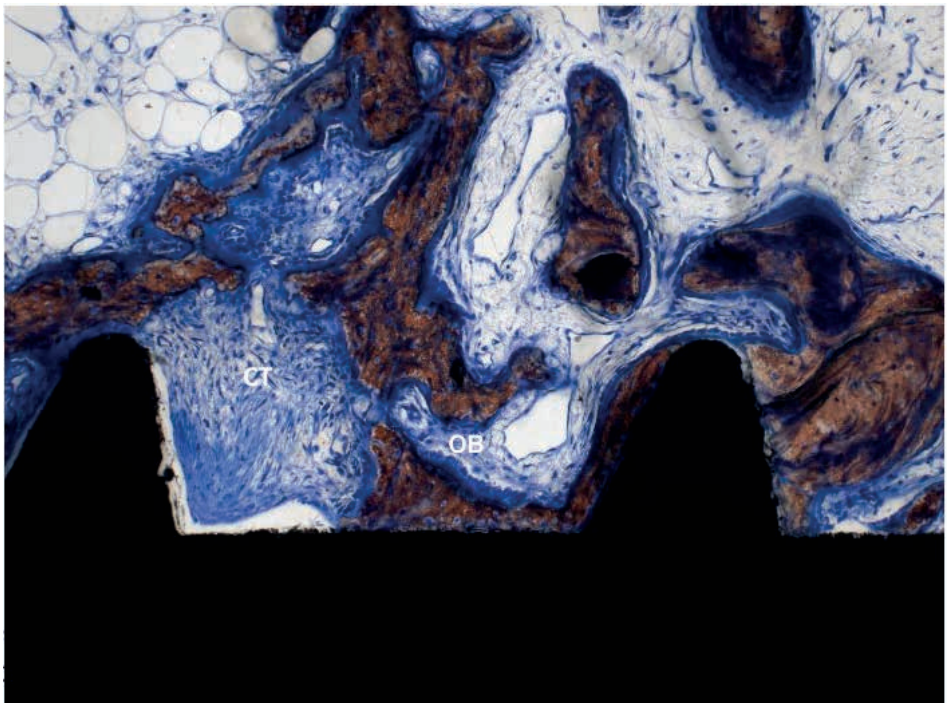

Fig. 26: Immature connective tissue area (CT) between newly formed bone and implant body.

Osteoblasts (OB) forming bone directly on the implant surface.

DISCUSSION

The use of orthodontic mini-implants is become in the last decade of paramount importance forthe absolute control of anchorage in orthodontics making possible and much more easy to obtain some dental movements like intrusion (Ohmae, et al. 2001) that was very difficult to be achieved prior to mini-screw insertion. Nowadays with aid of mini-implant it is possible to control very precisely and in the three dimensions of the space the movement of one or more teeth at the same time like it happens in the anterior area (Kanomi 1997). Distalization (Horiuchi, et al. 2008) and uprighting (Park, et al. 2002) are other very useful movements that allow for the correction of anterior crowding, space management and tooth proper positioning before fixed or removable prosthodontics without affecting the position of the teeth that do not need to be moved (Vigorito 2004).

Even though frequently used in the daily routine there are some controversial issues and opened questions about the correct use of the mini-screws. Very little amount of histological evidence is available in the orthodontic literature (Deguchi, et al. 2003) with respect to the healing of Titanium grade 5 mini-screws with smooth surface more with the intent of setting theideal timing for loading. At the present time no data are available for the description of the earlystages of healing around unloaded orthodontic mini-screws.

A common believe and hope of many users is that the Temporary Anchorage Devices (Costa, etal. 2005) (TADs) do not osseointegrate. This is due to the reason that the concept of osseointegration has been described as a very solid and resistant relationship between the implant and the bone hopefully lasting for a very long period of time (Buser 1999). This concept is in contrast with the need of removing easily (Kanomi 1997) (Costa, Raffainl & Melsen 1998) (Ohmae, Saito, Morohashi, Seki, Qu, Kanomi, Yamasaki, Okano, Yamada & Shibasaki 2001) the mini-implant after its clinical use and led to the idea that orthodontic mini-screws do not osseointegrate because they can be removed easily even after 2 years or more of permanence into the bone and even in the case of Titanium grade 5 SLA surface (Kim, et al. 2005). Through the discussion of the data of this experiment the reader will have the possibility to evaluate thebone healing of around unloaded, electropolished, orthodontic titanium grade 5 mini-screws assoon as inserted, after 2, 7, 15 and 30 days.

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, Berglundh & Lindhe 2002),(Deguchi, Takano-Yamamoto, Kanomi, Hartsfield, Roberts & Garetto 2003) but in disagreement with the data presented from Huja (Huja, et al. 2006) with self-drilling mini-screws and did not do tooth extractions scoring losses forapproximately 20%.

HARD TISSUES EVALUATION

There is a great difference between an implant inserted in the maxilla and one inserted into the mandible: the amount of cortical and trabecular bone contact with the screw. He relative amounts of cortical an trabecular bone change in the maxilla and in the mandible depending if we consider the anterior or posterior region (Huja, Rao, Struckhoff, Beck & Litsky 2006). In the maxillary anterior region of the dog the residual ridge, after tooth extraction (basal bone), is very thin measuring nearly 3mm in coronal-apical direction and it is very easy to make perforations that end into the nasal cavity when inserting even the shortest mini-screw. In the maxillary posterior region corresponding to the fourth premolar (P4), the thickness of the cortical bone is still very thin but the amount of trabecular bone is much higher and this makes a complete monocortical insertion of the screw possible. The studies where the difference between anterior and posterior region of the maxilla and mandible through pull-out tests confirm the finding of our study (Huja, Litsky, Beck, Johnson & Larsen 2005),(Huja, Rao, Struckhoff, Beck & Litsky 2006),(Kim, Ahn & Chang 2005).

The differences existing between the anterior and posterior region of the jaws has to taken into high consideration when the screw type is chosen: in the areas where a minor density and bone to implant contact is expected it is better to choose screws that offer a mayor primary stability which are the self-drilling ones (Deguchi, Takano-Yamamoto,

Kanomi, Hartsfield, Roberts & Garetto 2003), (Huja, Rao, Struckhoff, Beck & Litsky 2006), (Kim, Ahn & Chang 2005), (Wu, et al. 2008). Only in the areas where the cortical bone is ticker can be used self-tapping screws because enough primary stability is achieved and the prognosis of the mini-screw survival is more favorable due to the minor insertion pressure (Schenk & Buser 1998) and also the post- surgical pressure discomfort related from the patient disappears.

In our observations the mandible has a much higher thickness of cortical bone than in the maxilla and the screw body is in contact with it for about 2mm. Remarkable differences between the different premolars areas were not observed.

In this study we tested only self-tapping mini-implants inserted in the four quadrants in the premolar area (P1-P4). The total number of mini-implants inserted was 80 and the clinical success score was 100% because no implant was lost for any reason during the observation period. Huja (Huja, Rao, Struckhoff, Beck & Litsky 2006) testing in dogs the mechanical behavior of self drilling mini-screws scored 81,4% clinical success (80/102 screws). Deguchi (Deguchi, Takano-Yamamoto, Kanomi, Hartsfield, Roberts & Garetto 2003) in an animal study in dogs scored 97% clinical success after inserting mini-screws following a pilot drill. The unsuccessful implants were the mandibular ones and the Author relates that the most probable failure cause is the root proximity. In a recent study (Morea, et al. 2007) in 27 consecutive Class II patients treated with four extractions, only self-tapping screws were inserted to retract the anterior

teeth and the most frequent cause of failure was root proximity (3,94%), allowing a clinical success rate of approximately 92% (70/76 screws).

In our study it was observed between the lamellar mature bone and the implant body, soon after its insertion, the consequence of the action of the bur used to make the pilot drill: a 50-60 μm small gap (Schenk & Buser 1998). In this space a coagulum occupies homogeneously the available space (Davies 1998),(Abrahamsson, Berglundh, Linder, Lang & Lindhe 2004),(Schwarz, Ferrari, Herten, Mihatovic, Wieland, Sager & Becker 2007),(Berglundh, et al. 2003).

Is present from the beginning an excellent adaptation between the threads and the parent lamellar bone (Schenk & Buser 1998, Roberts, et al. 1987), (Schwarz, et al. 2007), (Schwarz, Ferrari, Herten, Mihatovic, Wieland, Sager & Becker 2007). Microfractures of the lamellar bone (Roberts, Turley, Brezniak & Fielder 1987) are visible in correspondence of the pitch of the threads (Schenk & Buser 1998). These are due to the insertion pressure generated from implant insertion into the pilot hole (Schenk & Buser 1998), (Schwarz, Herten, Sager, Wieland, Dard & Becker 2007),(Schwarz, Ferrari, Herten, Mihatovic, Wieland, Sager & Becker 2007).

In the implant tip area is visible into the trabecular bone the deepest part of the pilot hole occupied by some bone chips deriving from the action of the bur together with a blood clot deriving from the cut of the blood vessels during the preparation of the bone to receive

the implant.

In the case of an implant inserted into the mandibular bone, after 48 hours from the surgery, we can notice that the major part of the screw is in contact ether with the cortical or the trabecular bone achieving a high percentage of bone to implant contact (Deguchi, Takano-Yamamoto, Kanomi, Hartsfield, Roberts & Garetto 2003) with the exception of the implant tip where the pilot drill exceeded the implant length and left an empty space.

Analyzing the interface between bone and implant after 48 hours from its insertion inflammatory cells of the lymphocyte type are detectable together with macrophages and erythrocytes. On the bone surface there is an amorphous matrix containing osteoblasts. In this particular phase together with the healing processes new bone is being formed.

On the implant body, where there is no contact with the bone, there is a fibrin network adhered especially on the threads area. In other areas, the matrix deposited on the implant body takes a network shape and contains cells belonging to the osteoblastic line revealing osteogenic activity. In this phase are still visible some inflammatory cells that have the main function of cleaning the wound from the remnants originated during the surgery (Abrahamsson, Berglundh, Linder, Lang & Lindhe 2004).

After one week from insertion of the mini-screws into the bone is still present a gap created from the bur during the pilot drill. At the interface between the bone and the implant body there has been an evolution of the healing process of the bone. The space between the

bone and the titanium surface is totally occupied by an amorphous matrix (Berglundh, Abrahamsson, Lang & Lindhe 2003), rich in osteoblasts originated from the neighboring marrow spaces (Abrahamsson, Berglundh, Linder, Lang & Lindhe 2004) where these cells are very numerous.

In some studies on osseointegrated implants with SLA or modSLA surface, differently from our observations, already during the first week of healing can be seen woven bone (Roberts, Turley, Brezniak & Fielder 1987) either in contact with the parent lamellar bone bridging towards the implant surface or direct bone formation on the titanium surface (Berglundh, Abrahamsson, Lang & Lindhe 2003).

It is interesting to notice that around the implant threads as in the other areas where there was intimate direct contact between the implant and the bone since from the beginning of the insertion the lamellar bone seems to be intact without any evident osteoclastic remodeling activity as described from some Authors (Schwarz, Herten, Sager, Wieland, Dard & Becker 2007), (Berglundh, Abrahamsson, Lang & Lindhe 2003) around prosthetic implants.

After 15 days from insertion is still not evident the direct bone deposition on implant surface, differently from what observed on SLA surfaces where in this phase this phenomenon is already visible (Abrahamsson, Berglundh, Linder, Lang & Lindhe 2004), (Schwarz, Herten, Sager, Wieland, Dard & Becker 2007), (Berglundh, Abrahamsson, Lang & Lindhe 2003), (Bornstein, et al. 2008). In the region of the screw tip where after the insertion it was observed a space occupied before by a coagulum, followed from a fibrin

network bonded to the titanium surface, now there is a tissue composed by an amorphous proteic matrix (osteoid) containing newly- formed bone (woven bone). The maturation of this tissue leads successively to the formation of primary osteons (Schwarz, Ferrari, Herten, Mihatovic, Wieland, Sager & Becker 2007), in the space previously carved by the bur.

In the areas where the amorphous matrix containing osteoblasts had been deposited is now clearly visible parallel fiber bone (primary bone) (Schwarz, Herten, Sager, Wieland, Dard & Becker 2007) within a protein matrix. The presence of primary bone characterizes the maturation of the woven bone previously formed in the osteogenic areas (Schwarz, Herten, Sager, Wieland, Dard & Becker 2007),(Schwarz, Ferrari, Herten, Mihatovic, Wieland, Sager & Becker 2007). Numerous osteoblasts are present around the newly formed bone revealing that an intense osteogenic activity is still ongoing (Bornstein, Valderrama, Jones, Wilson, Seibl & Cochran 2008).

The new osteogenesis areas area characterized by the presence of a layer of osteoblasts depositing bone matrix over the parent lamellar bone together with abundant blood vessels (Schwarz, Herten, Sager, Wieland, Dard & Becker 2007) for the transport of the nutrients.

In the areas where the lamellar parent bone is in contact with the implant surface could not be seen a remodeling activity as observed from several Authors (Schwarz, Herten, Sager, Wieland, Dard & Becker 2007),(Berglundh, Abrahamsson, Lang & Lindhe 2003), being the bone perfectly adapted to the screw.

After 30 days of healing can be found areas where there is a direct deposition of bone over the implant surface (Schenk & Buser 1998) beyond woven bone, primary osteons and lamellar bone (Schwarz, Ferrari, Herten, Mihatovic, Wieland, Sager & Becker 2007),(Bornstein, Valderrama, Jones, Wilson, Seibl & Cochran 2008) in contact with the pristine lamellar bone. In the same area, over the newly-formed bone, osteoblasts layers deposit new matrix to be mineralized confirming that the osteogenesis process is still ongoing and the implant body will be surrounded by more bone (Abrahamsson, Berglundh, Linder, Lang & Lindhe 2004)·(Schwarz, Ferrari, Herten, Mihatovic, Wieland, Sager & Becker 2007),(Wu, Deng, Wang, Zhao & Wang 2008) if the healing process is not disturbed from inflammatory or infectious processes. Deguchi (Deguchi, Takano-Yamamoto, Kanomi, Hartsfield, Roberts & Garetto 2003) evaluating self- drilling mini-screws found that the bone to implant contact (BIC) decreased from the third to the twelfth week after implant insertion but this data are different from our finding and from the previously cited studies where the BIC was always increasing during the healing period.

In contiguity with new-bone genesis areas where the osteoblasts keep on depositing bone matrix around the woven bone there is connective tissue characterized by a thin collagen fiber network containing elongated fibroblast-like cells together with a large number of blood vessels remembering immature connective tissue (Bornstein, Valderrama, Jones, Wilson, Seibl & Cochran 2008).

CONCLUSIONS

The hard tissues healing process around orthodontic self-tapping mini-screws can besummarized as follows:

- Soon after implant insertion there is immediate contact between the implants threadsand the bone. Due to pilot drill exists also a 50-60 μm gap between the implant body and the bone surface occupied by a blood clot.
- Since day 2 from insertion start the coagulum transformation processes that will lead tothe formation of a connective tissue containing a large number of cells and blood vessels. It can be noticed also the deposition of an amorphous matrix over the pristine secondary lamellar bone.
- At day 7 osteogenic processes are already evident. These processes increase and are more evident at day 15 where woven bone formation occurs along implant surface. Inthis phase there is not yet direct bone deposition on implant surface.
- At day 15 after implantation woven bone formation starts and within primary bone canbe seen primary osteons containing osteocytes and blood vessels (Haversian system).
- After 30 days of healing there is primary lamellar bone in contact with the woven bonebridging towards the secondary pre existing bone. In this phase of healing direct deposition of the bone occurs directly on implant surface characterizing osseointegration of the mini-implants.

AKNOWLEDGMENTS

To the FAPESP (Foundation for Support of Research of the State of São Paulo) for supporting and financing this project (grants N. 2007/50572-0 and N. 07/50522-2).

To Dentaurum for supporting the project and donating the materials and financing histology costs.

To Drs. B. Böhm and A. Bernstein from University of Halle (Germany) for the histology processing.

REFERENCES

Kanomi, R., (1997) Mini-implant for orthodontic anchorage. *Journal of Clinical Orthodontics* **31**: 763-767.

Park, Y.C., Lee, S.Y., Kim, D.H. & Jee, S.H., (2003) Intrusion of posterior teeth using mini-screw implants. *American Journal of Orthodontics and Dentofacial Orthopedics* **123**: 690-694.

Carano, A., Velo, S., Incorvati, C. & Poggio, P., (2004) Clinical applications of the mini-screw-anchorage-system (m.A.S.) in the maxillary alveolar bone. *Progress in Orthodontics* **5**: 212-235.

Davies, J.E., (1998) Mechanisms of endosseous integration. *International Journal of Prosthodontics* **11**: 391-401.

Schenk, R.K. & Buser, D., (1998) Osseointegration: A reality. *Periodontology 2000* **17**: 22-35.

Abrahamsson, I., Berglundh, T., Linder, E., Lang, N.P. & Lindhe, J., (2004) Early bone formation adjacent to rough and turned endosseous implant surfaces. An experimental study in the dog. *Clinical Oral Implants Research* **15**: 381-392.

Albrektsson, T., (2008) Hard tissue implant interface. *Australian Dental Journal* **53 Suppl 1**: S34- 38.

Davies, J.E., (2003) Understanding peri-implant endosseous healing. *Journal of Dental Education* **67**: 932-949.

Buser, D., Weber, H.P., Donath, K., Fiorellini, J.P., Paquette, D.W. & Williams, R.C., (1992) Soft tissue reactions to non-submerged unloaded titanium implants in beagle dogs. *Journal of Periodontology* **63**: 225-235.

Berglundh, T. & Lindhe, J., (1996) Dimension of the periimplant mucosa. Biological width revisited. *Journal of Clinical Periodontology* **23**: 971-973.

Di Carmine, M., Toto, P., Feliciani, C., Scarano, A., Tulli, A., Strocchi, R. & Piattelli, A., (2003) Spreading of epithelial cells on machined and sandblasted titanium surfaces: An in vitro study. *Journal of Periodontology* **74**: 289-295.

Gahlert, M., Gudehus, T., Eichhorn, S., Steinhauser, E., Kniha, H. & Erhardt, W., (2007) Biomechanical and histomorphometric comparison between zirconia implants with varying surface textures and a titanium implant in the maxilla of miniature pigs. *Clin Oral Implants Res* **18**: 662-668.

Le Guehennec, L., Goyenvalle, E., Lopez-Heredia, M.A., Weiss, P., Amouriq, Y. & Layrolle, P., (2008) Histomorphometric analysis of the osseointegration of four different implant surfaces in the femoral epiphyses of rabbits. *Clinical Oral Implants Research* **19**: 1103-1110.

Schwarz, F., Ferrari, D., Herten, M., Mihatovic, I., Wieland, M., Sager, M. & Becker, J., (2007) Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: An immunohistochemical study in dogs. *Journal of Periodontology* **78**: 2171-2184.

Buser, D. (1999) Effect of various titanium surface configurations on osseointegration and clinical implant stability In: N. P. Lang, ed. *Proceedings of the 3rd European Workshop on Periodontology.*, 88-101. Ittingen: Quintessenz Publishing.

Costa, A., Raffainl, M. & Melsen, B., (1998) Miniscrews as orthodontic anchorage: A preliminary report. *International Journal of Adult Orthodontics and Orthognathic Surgery* **13**: 201-209.

Maino, B.G., Bednar, J., Pagin, P. & Mura, P., (2003) The spider screw for skeletal anchorage. *Journal of Clinical Orthodontics* **37**: 90-97.

Kyung, H.M., Park, H.S., Bae, S.M., Sung, J.H. & Kim, I.B., (2003) Development of orthodontic micro-implants for intraoral anchorage. *Journal of Clinical Orthodontics* **37**: 321-328; quiz 314.

Huja, S.S., Litsky, A.S., Beck, F.M., Johnson, K.A. & Larsen, P.E., (2005) Pull-out strength of monocortical screws placed in the maxillae and mandibles of dogs. *American Journal of Orthodontics and Dentofacial Orthopedics* **127**: 307-313.

Donath, K., (1988) Die trenn-dünnschliff-technik zur herstellung histologischer präparate von nicht schneidbaren geweben und materialien. - apparate- und methodenbeschreibung. *Der Präparator* **34**: 197-206.

Gotfredsen, K., Berglundh, T. & Lindhe, J., (2002) Bone reactions at implants subjected to experimental peri-implantitis and static load. A study in the dog. *Journal of Clinical Periodontology* **29**: 144-151.

Ohmae, M., Saito, S., Morohashi, T., Seki, K., Qu, H., Kanomi, R., Yamasaki, K.I., Okano, T., Yamada, S. & Shibasaki, Y., (2001) A clinical and histological evaluation of titanium mini-implants as anchors for orthodontic intrusion in the beagle dog. *American Journal of Orthodontics and Dentofacial Orthopedics* **119**: 489-497.

Horiuchi, Y., Horiuchi, M. & Soma, K., (2008) Treatment of severe class ii division 1 deep overbite malocclusion without extractions in an adult. *American Journal of Orthodontics and Dentofacial Orthopedics* **133**: S121-129.

Park, H.S., Kyung, H.M. & Sung, J.H., (2002) A simple method of molar uprighting with micro- implant anchorage. *Journal of Clinical Orthodontics* **36**: 592-596.

Vigorito, J.W., (2004) Ortodontia clínica: Diagnóstico e terapêutica. 1, Sao Paulo, Ed. Santa Madonna.

Deguchi, T., Takano-Yamamoto, T., Kanomi, R., Hartsfield, J.K., Jr., Roberts, W.E. & Garetto, L.P., (2003) The use of small titanium screws for orthodontic anchorage. *Journal of Dental Research* **82**: 377-381.

Costa, A., Pasta, G. & Bergamaschi, G., (2005) Intraoral hard and soft tissues depths for temporary anchorage devices. *Seminars in Orthodontics* **11**: 10-15.

Kim, J.W., Ahn, S.J. & Chang, Y.I., (2005) Histomorphometric and mechanical analyses of the drill-free screw as orthodontic anchorage. *American Journal of Orthodontics and Dentofacial Orthopedics* **128**: 190-194.

Huja, S.S., Rao, J., Struckhoff, J.A., Beck, F.M. & Litsky, A.S., (2006) Biomechanical and histomorphometric analyses of monocortical screws at placement and 6 weeks postinsertion. *Journal of Oral Implantology* **32**: 110-116.

Wu, X., Deng, F., Wang, Z., Zhao, Z. & Wang, J., (2008) Biomechanical and histomorphometric analyses of the osseointegration of microscrews with different surgical techniques in beagle dogs. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics* **106**: 644-650.

Morea, C., Dominguez, G.C., Tortamano, A. & Vigorito, J.W., (2007) Frequency and cause of failure of mini-screws for orthodontic absolute anchorage. *European Journal of Orthodontics* **29**: e27-e28.

Berglundh, T., Abrahamsson, I., Lang, N.P. & Lindhe, J., (2003) De novo alveolar bone formation adjacent to endosseous implants. *Clinical Oral Implants Research* **14**: 251-262.

Roberts, E., Turley, P.K., Brezniak, N. & Fielder, P.J., (1987) Bone physiology and metabolism. *Journal of Californian Dental Association* **15**: 54-61.

Schwarz, F., Herten, M., Sager, M., Wieland, M., Dard, M. & Becker, J., (2007) Histological and immunohistochemical analysis of initial and early subepithelial connective tissue attachment at chemically modified and conventional sla titanium implants. A pilot study in dogs. *Clinical Oral Investigations* **11**: 245-255.

Bornstein, M.M., Valderrama, P., Jones, A.A., Wilson, T.G., Seibl, R. & Cochran, D.L., (2008) Bone apposition around two different sandblasted and acid-etched titanium implant surfaces: A histomorphometric study in canine mandibles. *Clinical Oral Implants Research* **19**: 233-241.