RESEARCH ARTICLE

TITANIUM MINISCREWS UNDER CONTINUOUS LOADING IN A PIG JAW: A HISTOLOGICAL STUDY

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Summary

There is increasing interest in using titanium miniscrews for orthodontic anchorage. This experimental study aimed to evaluate the histological aspect of mandibular bone around miniscrews subjected to continuous forces in a pig. Three miniscrews were inserted on both sides of a pig mandible. Four weeks after implantation, a coil spring was fixed between two of the right miniscrews, while the left miniscrews remained unloaded. The pig was sacrificed nine weeks after implantation. Two right screws were lost during this period: one loaded screw and the unloaded one. Microradiographic and histological analysis revealed bone apposition in contact of about 1/3 of the unloaded screw surface, whereas the other 2/3 were surrounded by mesenchymal tissue. At distance of the implant, bone underwent important remodeling. No direct screw-bone contact was found around the loaded screw. Bone apposition and resorption were visible at distance of the screw, respectively on its traction and compression sides. In this preliminary study, osseointegration of the unloaded screw appeared more successful than that of the loaded ones.

Further studies should be conducted during a longer follow-up period in order to define the degree of minimal osseointegration associated with an efficient anchorage.

Key words: orthodontics, anchorage, mini-screw, implant, osseointegration

Introduction

Recent development of orthodontic treatments requiring minimal patient compliance, particularly in adults, has lead to the use of implants for anchorage (De Clerck et al. 2002). Orthodontic anchorage consists in the resistance of posterior teeth when anterior teeth are moved. Since a few years, implants have been used in a new application to suppress the dental reaction force in orthodontic treatments. Few studies have described peri-implant tissue adaptation to continuous or discontinuous orthodontic forces. Among them, few data definitely specified the range of loading generating osseointegration or peri-implant loss.

The aim of this experimental pilot study was to investigate by means of histological techniques the reactions of mandibular pig bone around orthodontically loaded mini-implants after a short healing time.

Materials and methods

A three-month-old pig was anesthetized with intramuscular injections of Rompun (Bayer, Germany), Zoletil (Zirbac, Belgium) and Anesketin (Eurovet, The Netherlands) for one and a half hour. Six titanium mini-implants (Surgi-Tec, Belgium) with an endosseous diameter of 1.6 mm and a length of 8, 10 or 12 mm (Fig. 1A) were placed in both hemimandibles. The implant surface was neither acid-etched nor sandblasted. The transmucosal part of the screw was covered by a 5-mm-long smooth cylinder, designed to allow the attachment of a coil spring without enhancing plaque retention (Fig. 1B).

Surgical procedure included muco-periosteal flap, pilot-drill with 1.6 mm bur under irrigation and manual insertion of the screws. In the left hemimandible, three screws were placed in the posterior edentulous ridge. On

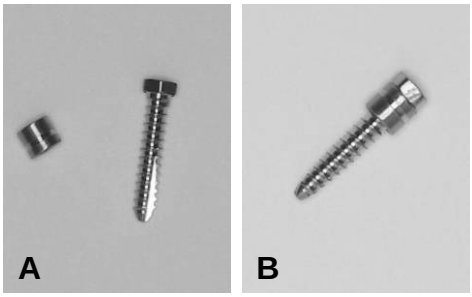

Fig. 1: 12-mm-long screw and fixation cylinder (A) - cylinder positioned on the transmucosal part of the screw (B).

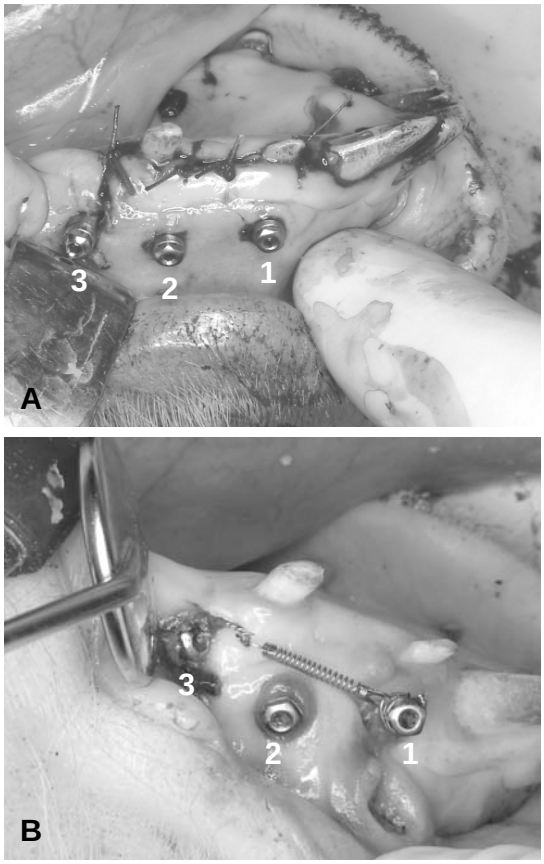

Fig. 2: Right hemi-mandible after implantation (A) - after loading (B). Screws 2 and 3 were lost at the end of experimentation. Screw 1 was the only loaded implant available for the microscopic analysis.

the right side, three mini-implants were placed in the anterior alveolar bone, between the incisor roots (Fig. 2A). Antibiotics (Suanovil, Merial, France) and anti-inflammatory drugs (Ketofen, Merial, France) were given for 5 days.

Four weeks after surgery, the pig was sedated with Rompun (Bayer, Germany) and Anesketin (Eurovet, The Netherlands) for 15 minutes. A nickel titanium coil spring (GAC, Japan) delivering a continuous force of 50 g was fixed between two of the three right implants (Fig. 2B), while the left, unloaded implants were considered controls.

Nine weeks after implantation, the pig was killed with an intracardiac injection of Nembutal (Abbott, Belgium). The experimental procedure was approved by the University Animal Care Committee.

The mandible was dissected to isolate the mini-screws. The implants and surrounding tissues were fixed in a 10% phosphate-buffered neutral formalin solution and stained *en bloc* with 1% basic fuchsine in a graded series of methanol. They were then embedded in methyl methacrylate without preliminary decalcification. After polymerization, serial 150-µm-thick sections were cut across the long axis of the screws with a circular diamond saw (Leitz, Germany). Each section was polished to a uniform thickness of 80µm with a rotating grinding machine (Planapol 2, Struers, Denmark).

Microradiographs were obtained by placing the sections on a fine grain emulsion (VRP-M film, Geola, Lithuania). The film was exposed to soft X radiations produced by a Machlett tube (Baltograph BF-50/20, Balteau, Belgium) at 14 kV and 15 mA. The exposure lasted 1 hour for a film-focus distance of 106 mm. After developing in D19 (Kodak, USA), the microradiographs were mounted like histological sections in order to observe them with an ordinary light microscope.

The sections were superficially stained with a 1% aqueous solution of methylene blue buffered with potassium biphthalate at pH 4.8. After mounting with Canada balsam (Brogniez et al. 2002), they were observed under ordinary light.

Results

Two right implants were lost during the 5 week loading period: one of the 2 loaded screws as well as the unloaded one (Fig. 2, screws 2 and 3). The date of loss could not be determined because of the absence of animal compliance.

Microradiographic analysis of the left hemimandible revealed bone apposition in contact of about one third of the unloaded screw surface (Fig. 3A). This new bone was

mainly lamellar, but woven bone (WB) was also visible at a short distance of the implant and recognizable to its thin and highly mineralized trabeculae with randomly distributed osteocyte lacunae. Bone resorption was attested by the presence of clear-cut, scalloped lacunae in mineralized trabeculae (Fig. 3A, white arrows). Microscopic observation of the corresponding stained sections confirmed the microradiographic aspect. Bone in contact with the control screws was highly stained and separated from older bone by cement lines (Fig. 3B, c). Bone surface was covered with a preosseous layer (black arrows), lined by a row of osteoblasts. Beside the bone-implant contact areas, the screw was surrounded by a highly cellular and dense mesenchymal tissue (Fig. 3C, M). Further away from the screws, the bone trabeculae underwent remodelling, as certified by the presence of osteoclasts in How ship lacunae (white arrows). Microradiographic analysis demonstrated resorption of the root dentin (D) in front of the implant (Fig. 5A, arrows). In the stained sections, the resorption cavities contained osteoclast-like cells (Fig. 5B, arrows). Microradiographic analysis did not show any bone contact with the loaded screw (Fig. 4A) although small bone deposits on the screw surface were visible in the stained sections (Fig. 4B). The bone tissue around the implantation site was mainly constituted of were clearly aligned along the orthodontic woven bone (Fig. 4A, WB). Its trabeculae were clearly aligned along the orthodontic traction line (long arrow), whereas numerous resorption lacunae were obvious only on the compression side (thick arrows). Enlargement of these lacunae in the corresponding stained section showed the presence of osteoclasts (Fig. 4C, arrows). The space between the screw and the surrounding bone was filled with connective tissue constituted of numerous fibres and undifferentiated mesenchymal cells (Fig. 4B, M).

Necrosis was visible neither around the control screws nor around the loaded one.

One control screw was found to be placed near a dental root.

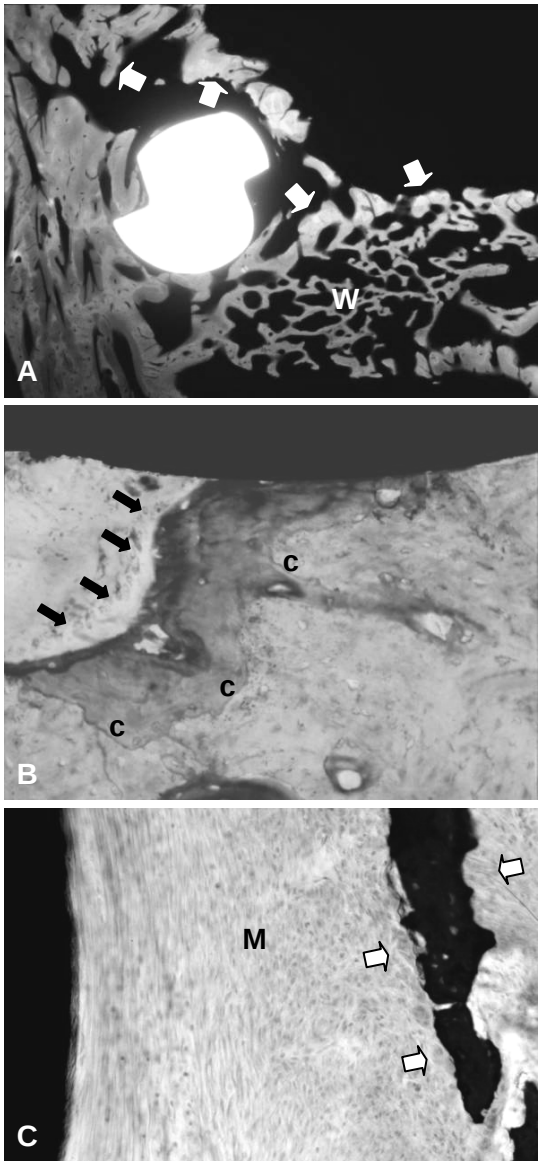

Fig. 3: Microscopic aspect of a control screw. (A) Microradiograph. WB: woven bone; white arrows: Howship lacunae. (B) Undecalcified stained section illustrating direct screw-bone contact. c: cement lines; black arrows: preosseous layer. (C) Undecalcified stained section. M : mesenchymal tissue; white arrows: Howship lacunae.

Discussion

Titanium implants constitute a structural and functional direct bone anchorage, as defined by Branemark (1969). Despite protocol divergences regarding animal species (Asikainen et al.1997, Linder-Aronson et al. 1990, Wehrbein & Diedrich 1993), implant dimensions (Roberts et al. 1984, Roberts et al. 1989, Ohmae et al. 2001, Melsen & Lang 2001) and localization (Turley et al. 1988), orthodontic force magnitude (Smalley et al.

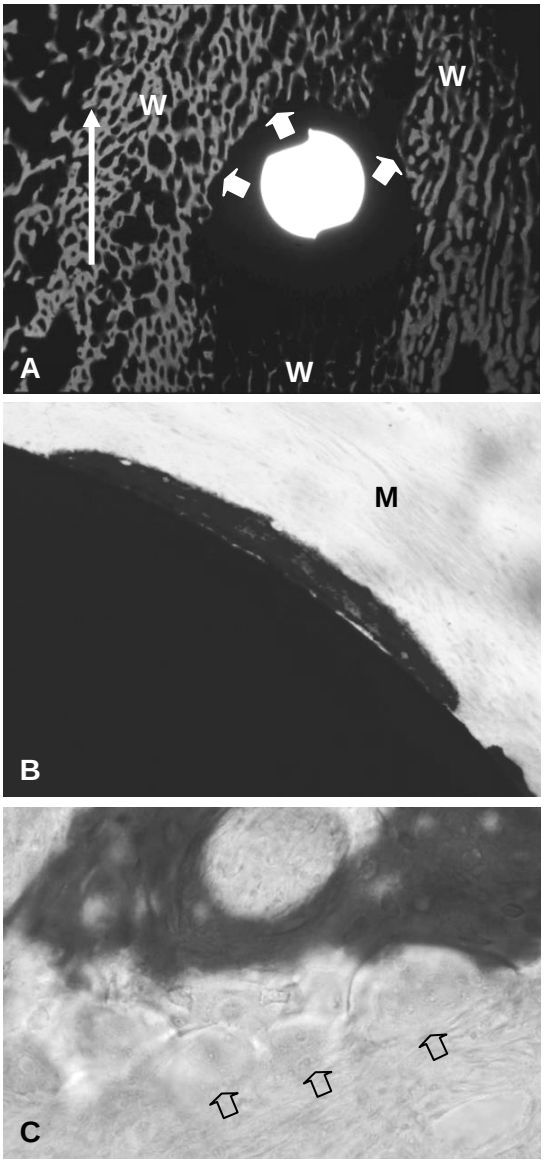

Fig. 4: Microscopic aspect of a loaded screw. (A) Microradiograph. Long white arrow: direction of orthodontic traction; thick white arrows: Howship lacunae. (B) Undecalcified stained section illustrating a small bone deposit on the implant surface. M: mesenchymal tissue. (C) Undecalcified stained section showing osteoclasts (arrows) in Howship lacunae.

1988, De Pauw et al. 1999) and direction (Southard et al. 1995) as well as healing period (Majzoub et al. 1999, Saito et al. 2000) and loading time (Wehrbein et al. 1998, Akin-Nergiz et al. 1998), all the studies reported clinical stability and histological osseointegration after orthodontic loading. Clinical stability was described as the resistance of osseointegrated implants to displacement forces in all directions and maintenance of their initial position without

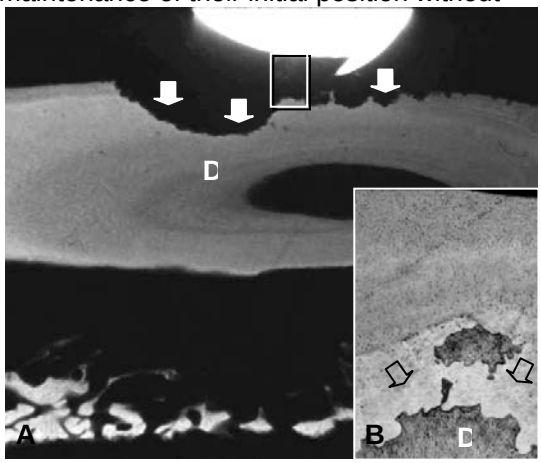

Fig. 5: (A) Microradiograph of a section through the left hemimandible. D: dentin; white arrows: resorption lacunae in front of the screw**.** (B) Enlargement of the framed area in A, observed in the stained section. Arrows: osteoclast-like cells.

loss of marginal bone support. However, the loss of 2 screws in the present pilot study was predictable from the 3 to 45% failure rate noted by some authors (Turley et al. 1988, Roberts et al. 1989, Deguchi et al. 2003).

According to Roberts (1993), histological osseointegration consists in transformation of the surrounding bone into woven bone and lamellar bone, distinguishable by matrix organization, mineral content and cellules. These features, visible around both control and loaded screws (Figs. 3-4), correspond to the adaptation of bone healing process to the presence of a biocompatible implant.

Some studies, using conventional implants (Turley et al. 1988; Wehrbein et al. 1997; Akin-Nergiz et al.1998; Majzoub et al. 1999) as well as mini-implants (Melsen & Lang 2001; Ohmae et al. 2001), reported an initial increase in bone remodelling which was more pronounced under loading than without it. This was evidenced under fluorescent light by increasing spacing between consecutive marker lines, as compared to control implants (Ohmae et al., 2001). Despite the absence of such data in our study, the respective microradiographic aspects of control (Fig. 3A) and loaded screws (Fig. 4A) speak for the same difference in bone remodelling intensity, which clearly gave rise to a negative balance at the end of the experiment.

In the relatively long-term histological studies (Roberts et al. 1984, Roberts et al. 1989, Linder-Aronson et al. 1990, Asikainen et al. 1997, Akin-Nergiz et al. 1998, Hürzeler et al. 1998, Wehrbein et al. 1997, Wehrbein et al. 1998, Wehrbein & Diedrich. 1993, Majzoub et al. 1999, De Pauw et al. 1999, Saito et al. 2000, Aldikacti et al. 2004), the first bone remodeling stage was followed by a real osseointegration stage, revealed by the presence of viable bone tissue in close contact of the implants, without any connective tissue interposition or inflammatory reaction. Integration indices up to 75,5% were obtained with orthodontically loaded conventional implants (Wehrbein et al. 1998) whereas lower values were observed around mini-implants: 25% (Ohmae et al. 2001) to 40-45% (Melsen & Lang 2001). Nevertheless, these poor osseointegration indices were associated with clinical stability and successful orthodontic resistance (Ohmae et al. 2001, Deguchi et al. 2003), while allowing implant removal after orthodontic treatment. So complete bone encapsulation is not to be considered a histological criterion of clinical success, as the only object is to maintain durable stability towards continuous orthodontic load. Absence of any significant bone-screw contact after 5 weeks loading (Fig. 4) could result from the immediate, and hopefully temporary, increase in bone remodelling around a loaded implant. It can be positively interpreted regarding the results of Ohmae et al. (2001), who attributed the poor osseointegration scores of their stable implants to the small size of the screws, the shorter healing time as well as the different locations compared to conventional implants.
As the loss of one of the 2 loaded screws could not be dated, the loading efficiency on the remaining implant was questionable. However, the bipolarization of the bone cell activities around the remaining loaded screw indirectly attested that the orthodontic traction had been exerted at least during a significant time. The woven bone trabeculae formed along the orthodontic force axis (Fig. 4A), whereas osteoclastic resorption occurred on the compression side of the screw, evoked the distraction osteogenesis mechanism classically used to elongate long bones or mandible (Ilizarov, 1990). Only few authors (Wehrbein et al. 1997; De Pauw et al. 1999) noted slight and contradictory asymmetries in bone cells activities. In our material, this distribution appeared consistent with the biomechanical rules of bone metabolism and could correspond to the first integration stage of a loaded implant.
Finally, the present microscopic study showed that implants could provoke root resorption even without direct contact with dental cement (Fig. 5). Roberts et al. (1989) also described root resorption in the microradiographic study of an implant located close to the periodontal ligament. Therefore, a careful pre-implantation evaluation should define a device location at distance of the roots.

Conclusion

In the present short-term pilot study, the mini-implants were moderately osseointegrated with no bone-implant contact around the loaded screw. Increased bone remodelling was observed around the implants, particularly the loaded one. Long-term qualitative and quantitative observations have to be carried out in order to distinguish a transitory remodelling reaction towards loading from the permanent bone adaptation to functional implants. Therefore, further experimental studies should try to define the minimal osseointegration percentage correlated to stability, as well as the appropriate healing period before loading. This information will be decisive in the elaboration of a standard protocol for the use of orthodontic mini-implants.

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