Original Article

Histomorphometric Study of Tissue Reaction to Mini-screws with Composite Coating Implanted in the Maxilla of Rabbits

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Abstract

Background and Aim: Stability of mini-screws in bone depends on mechanical factors in the primary phase and biological factors after osseointegration. mini-screws can transfer thermal shocks in the oral cavity to bone marrow. It has been shown that impaired osseointegration and inflammation can lead to failure of mini-screws. The aim of this study was to compare the tissue response to implanted mini screws with and without composite coating of the head.

Materials and Methods: In this animal study, mini-screws with 1.4mm diameter and 6mm length were implanted in the maxilla of 12 male rabbits. One mini-screw was implanted in each of the right and left quadrants of the maxilla. The right side mini-screws received composite coating on their head. The left side mini screws did not receive coating. In the next three months, cold (1°C) and warm (40°C) water were injected into their mouth 10 times daily. After three months, the rabbits were sacrificed, and paraffinized tissue blocks were prepared. The tissue reaction was examined under light microscopy. The paired t-test and Wilcoxon signed rank test were used for statistical analysis.

Results: The mean rate of osseointegration of mini-screws with and without composite coatings was 32.1±5.3 and 20.4±3.43, respectively. The rate of osseointegration was significantly higher around mini-screws with composite coating than mini-screws without coating ($P<0.001$). In both groups, the newly formed bone was woven and no inflammatory reaction was noted.

Conclusion: The rate of osseointegration of mini-screws with composite coatingof the head was higher than that of mini-screws without it.

Key Words: Bone Screws, Osseointegration, Thermal Conductivity

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Introduction

Anchorage has been a major challenge for orthodontists since the introduction of edgewise system by Edward H Angle [1]. In the past, head gears and elastics were used to reinforce anchorage; mini-screws were later introduced and gained significant popularity since they could be placed in different areas of the jaws with low cost via a simple surgical procedure [2,3]. Anchorage reinforcement in retraction, distalization and intrusion movements and stability against orthodontic forces are among the main advantages of mini-screws [4-6]. Stability of mini-screws depends on mechanical factors in primary phases and biological factors after osseointegration [7]. Factors such as tissue status in which, mini-screws are placed, bacterial contamination and penetration of epithelium into the bone surface can result in formation of fibrous tissue instead of bone and lead to treatment failure [8]. The implant-bone interface determines the success or failure of implant [9]. The stability of mini-screws can be assessed by evaluation of the screw-bone contact area. This can be doneby analyzing the amount of newly formed bone through microscopical examinations. Histomorphometric assessments are performed for qualitative analysis of this topic [8]. Several studies have shown that diameter and length of mini-screws and their loading time affect their stability [3,10-13]. Moreover, it has been stated that the heat generated during site preparation and placement of mini-screws is correlated with bone necrosis [14]. Despite the previous studies on the effect of physical and mechanical factors on mini-screws, biological studies on these implants are scarce. Oral cavity is subjected to thermal changes on a daily basis. Since mini-screws are made of metal, they transferheat to the bone. This can compromise implant osseointegration. The effect of thermal shocks on mini-screws in the oral environment has not been previously evaluated. This study aimed to assess and compare the tissue reaction to composite-coated mini-screws and those without coating.

Materials and Methods

This animal study was conducted on 12 white New Zealand rabbits weighing 2kg. Surgical procedures and placement of mini-screws were all done by the same operator. General anesthesia was induced by 10mg/kg intramuscular injection of ketamine into the thigh muscle [15] and mini-screws with 1.4mm diameter and 6mm length were placed in the lateral part of the anterior region of their maxilla. One mini-implant was placed in the right and another one in the left side of the maxilla ofeach rabbit. Mini-screws were placedin the maxilla at 90°angleusing a screw-driver. In the right side, the mini-screw head, which was out of the mucosa was completely covered with composite resin (Z250, 3M ESPE, St. Paul, MN, USA) with 2mm thickness and light-cured. mini-screws in the left side remained uncoated. The rabbits were kept for three months at room temperature (25°C) and received 300g concentrated nutritional regimen including fishbone powder, soy bean and wheat grain (Behparvar, Tehran, Iran) and 1cc of multivitamin per 1liter of water. To transfer thermal shocks, cold (1°C) and hot water (40°C) were injected into the oral cavity of rabbits by a lavage syringe 10 times a day. After three months, two rabbits died and the remaining 10 were sacrificed by overdose of sodium thiopental administered intravenously (10mg/kg).

By separating the soft tissue, the anterior segment of the maxilla was cut out and placed in 10% formalin. After decalcification of bone in formic acid and methanol, mini-screws were extracted. After tissue processing of decalcified tissues, paraffin blocks were prepared. Histological sections with 5-micron thickness were made of each block and stained with hematoxylin-eosin for histomorphometric assessment [16], which was done using a light microscope (Eclipse E 600, Nikon, Tokyo, Japan). For each sample, the bone-implant contact (BIC) area was calculated [7]. Type of newly formed bone was determined as lamellar (mature) or woven (immature). Presence of acute or chronic inflammation was also assessed [17].

The rate of osseointegration in the two groups was compared using paired t-test and Wilcoxon signed rank test. $P \leq 0.05$ was considered statistically significant. The data were analyzed using SPSS version 20 (SPSS Inc., IL, USA).

Results

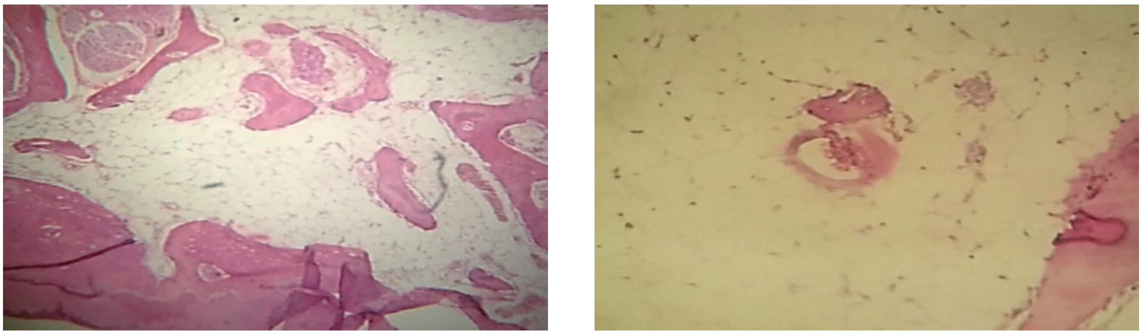
The mean rate of osseointegration was 32.1±5.3% around mini-screws with composite coating and 20.4±3.43% around mini-screws without coating. The rate of osseointegration around mini-screws with composite coating was significantly higher than that around mini-screws without coating ($P<0.001$). The percentage of BIC is presented in Table 1.

In both groups, type of newly formed bone was immature (woven) and no degree of inflammation was seen. Figure 1 compares osseointegration around mini-screws with and without coating.

Discussion

The results of this study showed that rate of osseointegration was significantly higher around mini-screws with composite coating compared to those without coating.

Anchorage is a major challenge in orthodontic treatment. To date, teeth and extra-oral devices have been used to provide anchorage. Use of mini-screws for anchorage in orthodontics was first described by Roberts et al, in 1984 [18].



(A) (B)

Figure 1. Comparison of osseointegration around mini-screws with and without composite coating: (a) the highest rate of osseointegration was noted around mini-screws with coating. No inflammation was seen (X100 magnification, hematoxylin-eosin staining); (b) osseointegration was insignificant around mini-screws without coating, no inflammation was seen (X100 magnification, hematoxylin-eosin staining).

Table 1. Osseointegration around mini-screws with and without composite coating (%)

Mini-screws / Osseo integration	1	2	3	4	5	6	7	8	9	10
With coating	24	28	29	35	41	32	26	37	35	34
Without coating	15	18	19	22	26	20	16	23	23	22

Evidence shows that method of placement, type of surgery, loading time, location of placement in the jaw, shape and surface area of mini-screws affect the rate of anchorage [7-9,13,14,19,20]. Stability of mini-screws depends on mechanical factors in the primary phase and biological factors after osseointegration [7].

Thermal shocks are among the most important biological factors affecting the outcome of treatment. Limited studies are available on the association of thermal shocks and bone response (BIC) around mini-screws.

Nagamatsu et al. [14] showed that 0.7°C heat caused bone necrosis around mini-screws. Our findings were in line with theirs; however, they examined the tibia of rabbits and thermal conduction was temporary and heat was transferred to bone only at the time of surgery. Whereas, in the current study, thermal shockswere transferred to jawbone (which is the target site for placement of mini-screws for orthodontic purposes) during three monthsin a longitudinal design. The current results more precisely indicated the effect of thermal shocks on mini-screws in the oral cavity**.**

Isler et al. [21] showed that 4°C water (compared to 25°C) was associated with greater activity of osteoblasts and greater dynamic activity of bone marrowat the location of placement of mini-screws. Their findings were in line with ours since both studies showed that thermal alterations can biologically affect bone response. The difference between the two studies was in that our study directly measured the effect of thermal shocks in the oral environment on osseointegration rate. Although both studies were in vivo, the study by Isler et al, had a cross-sectional design while our study was longitudinal and evaluated the effect of thermal shocks in the oral cavity on jawbone during three months.

Heat can cause bone necrosis at the time of implant placement in bone. Excessive heat results in infiltration of inflammatory cells around the mini-screws and subsequent treatment failure [14]. It has been shown that 47°C temperature for one minute can cause bone necrosis at the site of implant placement [22,23]. According to Eriksson and Alberktsson, 44-47°C temperature is the maximum thermal threshold for bone remodeling and in temperatures beyond this threshold bone loses its remodeling capacity [23]. High temperature impairs blood circulation and inactivates enzymes related to osteogenesis such as alkaline phosphatase [24,25]. Thus, heat generated at the site of implant placement can significantly affect bone vitality. Therefore, care must be taken to prevent over-heating during implant placement.

Another important issue in this study was that thermal shockswere applied to jawbone in a longitudinal pattern during three months. To the best of authors' knowledge, this study was the first to evaluate the role of heat on mini-screws in the oral cavity. In the current study, the effect of 1 and 40°C temperature was evaluated because most of the consumed foods and beverages are within this thermal range. Thus, findings of this study can desirably reflect tissue changes that occur in the clinical setting in response to oral thermal changes. Composite was used to cover the head of mini-screws in this study (for insulation). Further studies are required on the efficacy of other materials for this purpose.

Conclusion

Rate of osseointegration was significantly higher around mini-screws with composite coating compared to those without coating.

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