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Immediate loading of orthodontic mini-implants: a histomorphometric evaluation of tissue reaction

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SUMMARY Although immediate loading of orthodontic mini-implants can be clinically successful, a lack of histological data exists. The present investigation was performed to evaluate, in an animal model, tissue reaction to immediate loading. Fifty orthodontic titanium mini-implants were inserted in four adult male monkeys at four time intervals. Forty-two devices were loaded with 50 cN super-elastic coil springs immediately after insertion while eight were left unloaded and served as the controls. After euthanasia, the following histomorphometric parameters were evaluated: bone volume (BV/TV), bone-to-implant contact (BIC), mineralizing surface (MS/BS), and erosion surface (ES/BS). Statistical analysis was performed by means of non-parametric tests.

Four devices were removed because of loss of stability. A wide variation between animals was found for all parameters. BV/TV: slightly higher values were found in the unloaded sample. Although no particular trend was observed, at 3 months higher values were found in the lower jaw. BIC: a trend to a decrease between 1 week and 1 month followed by a significantly progressive increase was observed. Implants that showed some sections with as little as 3 per cent BIC successfully resisted loading. MS/BS: higher values were found in the lower jaw. MS/BS increased significantly between 1 week and 1 month, followed by a progressive decrease. ES/BS: there was a decrease between 1 week and 1 month, followed by a progressive re-increase.

BV/TV did not show any particular trend while BIC was a time-dependent factor. MS/BS and ES/BS demonstrated opposite trends during the healing period. Immediate loading with light forces did not negatively affect the bone healing pattern.

Introduction

Anchorage is a critical issue in orthodontics and, if inadequate, can be the most limiting factor of therapy, no matter which technique or philosophy the clinician follows. Especially when treating adults, the orthodontist faces the problem of lack of anchorage teeth or situations in which displacements in the reactive unit cannot be accepted. Furthermore, compliance may be difficult to obtain. Intraoral extra-dental anchorage has changed the limits of orthodontic therapy in such borderline cases and has developed exponentially in recent years.

Devices which do not use teeth as reactive units have the aim of avoiding unwanted tooth movement by loading a bone–metal interface and are defined as 'skeletal anchorage'. As a consequence of the first successful attempt to use surgical screws for protrusion of incisors (Creekmore and Eklund, 1983), the use of skeletal anchorage has been extensively reported (Gray *et al.*, 1983; Roberts *et al.*, 1989; Linder-Aronson *et al.*, 1990; Higuchi and Slack, 1991; Ödman *et al.*, 1994; Block and Hoffman, 1995; Wehrbein *et al.*, 1996; Saito *et al.*, 2000; Melsen and Lang, 2001). Both surface-treated and smooth implants can be used as anchorage. However, the latter types are more suitable for orthodontic purposes, since they can be used in various anatomical sites and can be removed at the end of therapy without surgical intervention. Among this second category, mini-implants are the most widely investigated and the most used in clinical practice (Kanomi, 1997; Costa *et al.*, 1998; Kyung *et al.*, 2003; Maino *et al.*, 2003; Carano *et al.*, 2004). Although immediate loading is suggested by most clinicians, histological research of the healing pattern around immediately loaded mini-implants is limited (Melsen and Costa, 2000; Buchter *et al.*, 2006; Freire *et al.*, 2007), while in the case of early or delayed loading, several studies have been performed (Ohmae *et al.*, 2001; Deguchi *et al.*, 2003; Kim *et al.*, 2005; Freire *et al.*, 2007). Moreover, studies on immediate loading have only investigated bone healing in the form of bone-to-implant contact (BIC) without considering other parameters, such as resorption and formation indices.

The aim of the present research was to perform a bone histomorphometric study of the healing pattern surrounding immediately loaded orthodontic mini-implants in an animal model.

Materials and methods

The research protocol was approved by the Danish Board for Animal Research.

Fifty small titanium mini-implants (Aarhus Mini-Implant®, Medicon, Tuttlingen, Germany; length 9.6 mm, diameter 2.0 mm) were inserted in four adult male *Macaca fascicularis* monkeys (mean body weight 8.2 kg). Each animal received six implants on the buccal side of each jaw, one in the canine area, one in the premolar area, and one in the molar area, bilaterally, a total of 24 implants inserted in the maxilla and 24 implants inserted in the mandible (Figure 1). In addition, one monkey had two mini-implants inserted in the palate.

The screw insertion procedure was as follows: once general anaesthesia had been induced with an intramuscular injection of Ketalar (ketamine, 50 mg/ml: ~1.5 ml) and the implant site had been selected, the pilot bur from the mini-implant kit, using low rotation speed and under constant irrigation with sterile saline, was used to prepare a transmucosal hole through the cortical bone to a depth of approximately 2 mm. The self-taping mini-implants were then screwed manually into the selected sites for the whole length at 45 degrees in an apical direction in an attempt to avoid contacting the dental roots.

The surgical protocol was as follows (Figure 2): 12 screws were inserted 96 days before euthanasia (3 month group), 12 screws were inserted 70 days before euthanasia (2 month group), 14 screws were inserted 39 days before euthanasia (1 month group), and 12 screws were inserted 7 days before euthanasia (1 week group). Forty-two of the mini-implants were loaded immediately with 50cN super-elastic springs (Sentalloy® extra light closed coil springs, GAC International, Bohemia, New York, USA) tied either to other implants or to the teeth for the following observation periods: 3 months (n = 10), 2 months (n = 10), 1 month (n = 12), and 1 week (n = 10). Two mini-implants for each time group were left unloaded and served as the controls (n = 8). All monkeys underwent a weekly oral hygiene programme during which stability of the mini-implants was assessed. Tetracycline (20 mg/kg) and calcein (10 mg/kg) intravital labels were used as bone markers for dynamic histomorphometry and were administrated intravenously 7 and 3 days before euthanasia, respectively. At the end of the experiment, the monkeys were killed by means of a Mebumal overdose, and just before the heart stopped beating the veins were rinsed with saline solution followed by 70 per cent ethanol. Both jaws were then excised, the soft tissues removed, and cut into separate blocks of bone tissue around every single mini-implant. After 1 week fixation in 70 per cent ethanol, the specimens were sequentially dehydrated and embedded in methylmetacrylate.

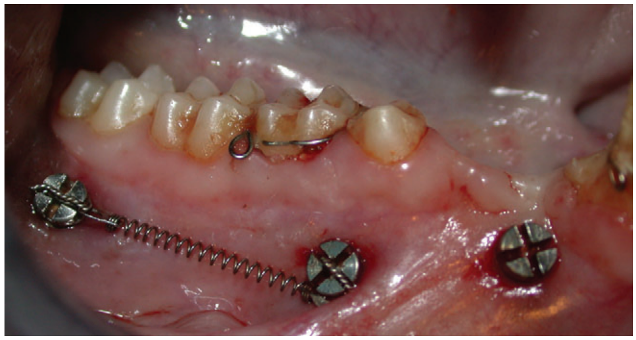

Figure 1 Mini-implants in the lower molar and premolar area loaded reciprocally after insertion. The unloaded control mini-implant is in the canine area.

Approximately 10 undecalcified bone sections, 70 μm thick, perpendicular to the long axis of the mini-implant were produced from each embedded specimen by means of a Leiden saw (KDG-95, Meprotech, Heerhugowaard, The Netherlands) and half were stained with toluidine blue. Three different layers (cutting levels) were selected in an apical-coronal plane (bottom, middle, and top; Figure 3). Histomorphometric analyses were performed by means of an Olympus BX51 microscope (Olympus Danmark A/S, Ballerup, Denmark) equipped with an integrating reticle (Carl Zeiss, GmbH, Oberkochen, Germany) at a magnification of ×100, both under visible and ultraviolet light. All measurements were carried out by the same operator (SM). The following parameters, expressed as a percentage, were analysed:

Bone volume. A custom-made grid, consisting of two concentric circles centred on the axis of the mini-implant and two perpendicular lines crossing in the circle centre, was used (Figure 4a). The inner circle had a diameter of 3.75 mm and, together with the lines, defined four regions. Two regions lay along the line of action of the force (one where the mini-implant was loaded 'towards' the bone and the other 'away' from the bone). The other two regions lay perpendicular to the line of action of the force (one apically and the other occlusally). Only the areas lying along the line of action of the force were used for histomorphometric evaluation of bone volume (BV/TV). The BV/TV was assessed by means of a point-counting method counting approximately 300–400 points within each region.

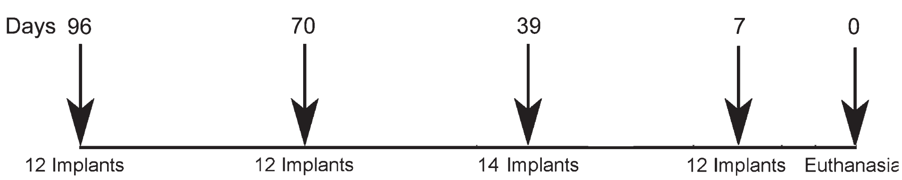

Figure 2 Timing of placement of the mini-implants during the experimental phase.

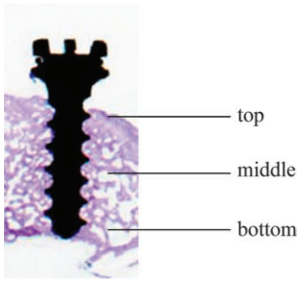

Figure 3 The three levels at which the histological sections were sampled.

BIC. To determine BIC, a different custom-made grid was used (Figure 4b), consisting of 32 equidistant concentric test lines converging to the mini-implant long axis. The parameter was assessed by means of an intersection counting method under conventional light.

Mineralizing surface. The same custom-made grid as used to determine BV/TV was used to assess the mineralizing surface (MS/BS). The parameter was assessed by means of an intersection counting method using ultraviolet light with a U-MWBV-2 filter (wavelength: 400–410) to identify the surfaces labelled with the bone markers (Figure 4c). An integrating reticule consisting of 10 parallel lines was used and rotated randomly when the microscopic field was changed.

Erosion surface. The parameter was assessed similar to MS/BS by means of an intersection counting method using conventional light with polarization filters to identify resorption lacunae with or without osteoclasts.

Error of the method

Measurements were repeated on 10 per cent of the sections by the same operator after a 1 month interval. The graticule which had been removed after the first measurement, was placed again on the randomly selected sections and the same measurements were performed.

Statistical analysis

Since the data did not show a normal distribution, non-parametric tests were performed. The differences between jaws were analysed by means of a Mann–Whitney *U*-test considering the different cutting levels separately. The overall differences between loading times were analysed by means of a Kruskal–Wallis test. Once a difference was detected, loading times were compared with each other by means of a Mann–Whitney *U*-test. For the BV/TV, MS/BS, and erosion surface (ES/BS), the side (towards or away) was also considered in relation to the direction of the load. Comparison between sides was performed within the same animal by means of a Wilcoxon test for paired data, in order to minimize between-animal variations. When there was no significant difference, the mean values of both were used, as in the case of BV/TV, BIC, and MS/BS. For ES/BS, the paired tests showed significant differences, and therefore the values of the towards and away side were not combined and were analysed at each cutting level. Data analysis was performed using the

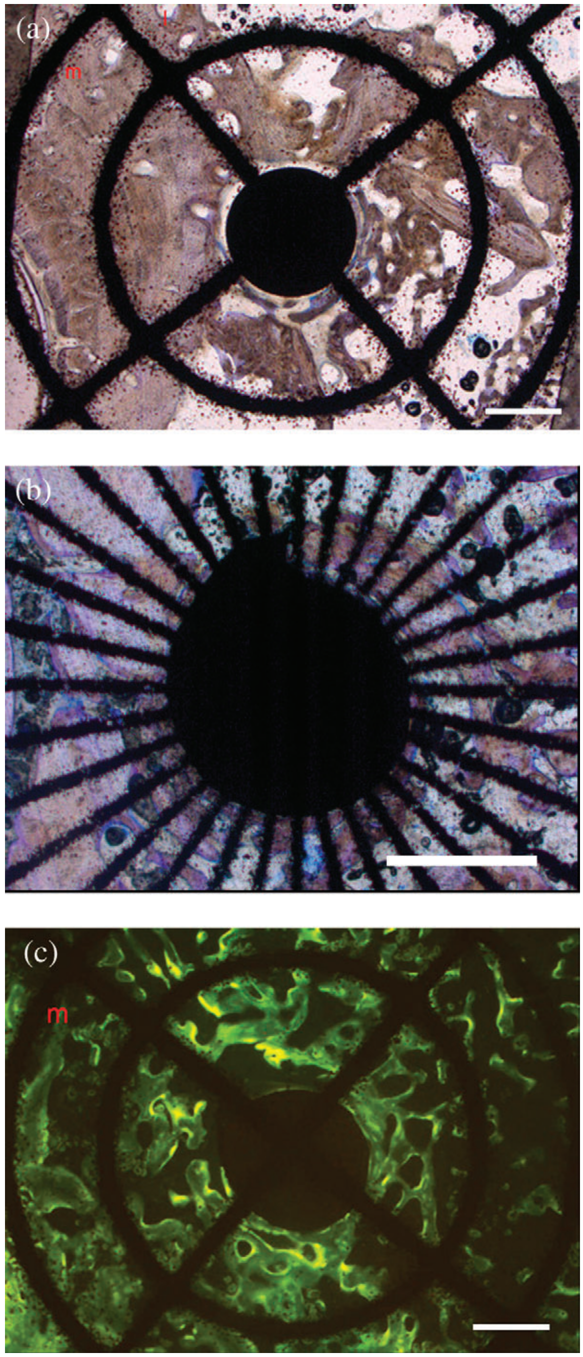

Figure 4 Histological section used to assess bone volume and erosion surface (a), bone-to-implant contact (b), and mineralizing surface (c). Bar = 1 mm.

Statistical Package for Social Sciences 11.0 (SPSS Inc., Chicago, Illinois, USA). A significance level of 5 per cent was used.

Results

During the entire experimental period, the four monkeys maintained good physical health and their body weight did not show any significant variations. Four mini-implants were found to be mobile and had to be removed. Two devices, a loaded and an unloaded one, which had been inserted at the 1 month observation, were removed from the same animal, while the other two mobile loaded mini-implants, inserted at the 1 week observation, were removed from two different animals. Two super-elastic springs were found to be broken in the same animal at the 1 month and 1 week observations and were immediately replaced. A low to medium degree of inflammation was always present in all animals and some of the coil springs were found to be impinging the alveolar soft tissues, without affecting the load on the mini-implants.

Calcein labelling was found to be very weak under microscopic examination and, therefore, only tetracycline labelling was taken into consideration in the analysis of MS/BS. Not all the histological sections could be analysed: in some sections the mini-implant was found in close proximity to the dental root surface or fractured away from the surrounding tissues during the sectioning phase. All the data related to the histomorphometric analysis showed a wide variation between animals (Figure 5a–c), thus giving elevated values of the standard deviation and range. The coefficients of variation to evaluate the error of the method were found to be 2.2 per cent for BV/TV, 6.9 per cent for BIC, 5.2 per cent for MS/BS, and 3.6 per cent for ES/BS. Analysis of the single parameters revealed the following:

BV/TV. The mean values (±SD) of the BV/TV percentage at the four time intervals for the loaded group and the corresponding values for the unloaded controls are reported in Table 1. The mean values in the unloaded group were slightly higher compared with the loaded group but, due to the relatively small sample of unloaded controls, no statistical tests were performed. Table 2 shows the mean values of the loaded mini-implants for the four observation periods. Statistical analysis revealed no significant differences between the upper and lower jaws, except for the bottom level at 3 months where the BV/TV of the lower jaw was significantly higher compared with the upper jaw. No difference was found between the four time intervals for both jaws or between the three different cutting levels. When all levels were tested together, no significant difference was found between the upper and lower jaw or at the four different time intervals.

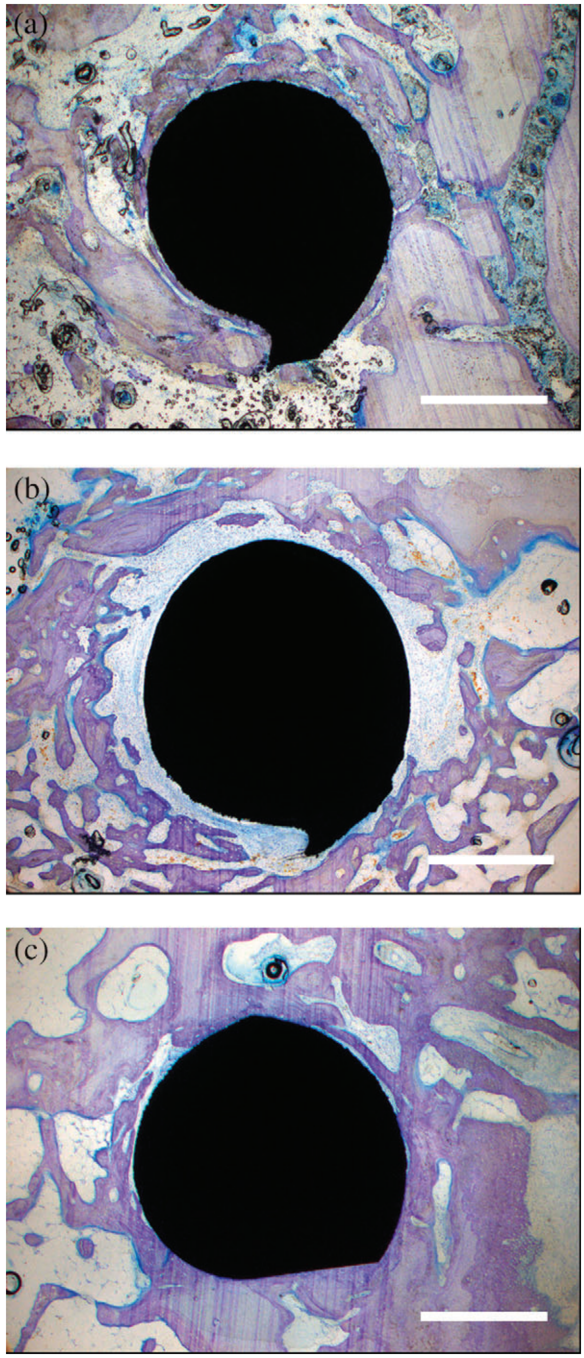

Figure 5 Histological section taken at the top level to assess bone-to-implant contact (BIC): (a) and (b) are taken from different animals at the 1 month observation, while (b) and (c) are taken from the same animal at the 1 and 3 months observations, respectively. Note the inter-animal variability at the same time interval and the time-dependent increase in BIC in the same animal. Bar = 1 mm.

BIC. The mean values (±SD) of the BIC percentage at the four time intervals for the loaded group and the corresponding values for the unloaded controls are reported in Table 1. Although the difference in sample size between the loaded and unloaded groups did not allow for statistical testing,

Table 1 Histomorphometric parameters of the loaded and the unloaded mini-implants.

		No. of implants	Loading time							
			1 week		1 month		2 months		3 months	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
BV/TV	Loaded	39	41.01	24.37	45.87	19.32	42.64	23.92	52.99	25.91
	Unloaded	7	56.39	13.92	46.51	24.69	48.58	18.39	64.65	23.97
BIC	Loaded	39	32.53	25.91	23.33	26.38	40.64	27.79	52.87	26.79
	Unloaded	7	15.70	11.17	22.83	7.47	46.88	40.86	42.98	23.00
MS/BS	Loaded	39	40.16	17.83	60.75	24.70	56.09	15.11	44.43	17.92
	Unloaded	7	57.31	11.68	32.08	8.50	62.44	23.36	44.61	15.60
ES/BS	Loaded	39	45.91	24.39	27.13	19.49	35.30	13.94	36.78	13.57
	Unloaded	7	50.19	20.58	40.52	13.04	38.81	15.47	55.43	17.82

Mean values and SD values are expressed in percentages.
SD, standard deviation; BV/TV, bone volume; BIC, bone-to-implant contact; MS/BS, mineralizing surface; ES/BS, erosion surface.

higher values were found at 1 week and 3 months in the loaded sample compared with the controls. Table 2 shows the mean values of the loaded mini-implants for the four observation periods. No significant differences were observed between the upper and lower jaws or at the three cutting levels, although BIC at the top level was low compared with the other two cutting levels. When tested at the four observation periods, BIC showed a trend to decrease between 1 week and 1 month, and to increase again at 3 months, with statistically significant differences between 1 month/3 months and 1 week/3 months at the middle and bottom cutting levels, while no difference was found at the top level (Figure 6a).

MS/BS. The mean values (±SD) of the MS/BS at the four time intervals for the loaded group and the corresponding values for the unloaded controls are reported in Table 1. The unloaded sample, as opposed to the loaded implants, showed decreased mineralization activity after 1 month, which reached the level of the loaded mini-implants at 2 and 3 months.

Table 2 reports the mean values of the loaded group for the four observation periods. In general, higher levels of MS/BS were found in the lower jaw at all four observation intervals compared with the upper jaw. No difference was found between the different cutting levels for paired data analysis. When the difference between loading times was tested, statistical significance was found with a higher MS/BS at 1 month compared with 1 week, 2 months compared with 1 week, 1 month compared with 3 months, and 2 months compared with 3 months (Figure 6b).

ES/BS. The mean values (±SD) of the ES/BS at the four time intervals for the loaded group and the corresponding values for the unloaded controls are reported in Table 1 and the mean values of the loaded group for the four observation periods in Table 2. Since a significant difference was found between the mesial and the distal side of the mini-implants, the data were additionally separated according to the direction of the load (towards or away).

No significant differences were found when the results were analysed at the different observation intervals or for the upper and lower jaws separately (Figure 6c). When tested at the three cutting levels, significantly higher values for ES/BS were found at the bottom level compared with the top level on the side of the implants away from the orthodontic load.

Discussion

Although immediate loading of mini-implants has been suggested in clinical case reports (Kanomi, 1997; Costa *et al.*, 1998; Kyung *et al.*, 2003; Maino *et al.*, 2003; Carano *et al.*, 2004), little supporting histological data have been published. In this study, the aim was to investigate, in an animal model, the bone healing pattern to immediate loading with light forces (50 cN super-elastic springs) of orthodontic mini-implants. The choice of monkeys in the experiment was due to the similarity to humans both with respect to morphology (Garetto *et al.*, 1995) and to bone remodelling. On the other hand, the use of these animals had a disadvantage in that they are never uniform in physiological traits, which can cause wide inter-animal variation in the data (Gotfredsen *et al.*, 1991; Pilon *et al.*, 1996) as confirmed in the present study (Figure 5). These wide variations were observed both for the loaded and unloaded mini-implants (Table 1). Apart from inter-animal variations, external factors, such as oral hygiene, also contributed to the differences, as confirmed by the constant low to medium degree of inflammation present on the intraoral soft tissues of the four animals, despite routine oral hygiene sessions. The four mini-implants that came loose and had to be removed were found in the two animals with the greatest soft tissue inflammation levels. Three of these implants were loaded while one was an unloaded control, suggesting that individual resistance to inflammation

Table 2 Histomorphometric parameters of the loaded mini-implants in the upper and lower jaws, at the three different cutting levels and at the four time intervals.

				Loading time											
				1 week			1 month			2 months			3 months		
			*	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
BV/TV	Upper	Top		6	62.11	15.79	5	53.86	15.83	6	46.76	24.69	6	49.12	24.84
		Middle		6	35.73	17.15	5	46.89	21.36	6	38.23	23.57	6	52.06	21.18
		Bottom		6	28.64	9.32	5	44.78	24.50	6	33.04	17.15	6	37.53	23.25
	Lower	Top		4	43.45	37.48	5	51.23	20.60	4	44.66	28.63	3	60.19	26.02
		Middle		4	40.96	21.89	5	46.79	15.91	4	61.48	22.22	3	63.68	28.33
		Bottom		4	44.83	19.19	5	41.23	13.18	4	50.50	18.95	3	74.32	25.97
BIC	Upper	Top		6	42.50	16.91	5	13.28	19.16	6	27.50	27.19	6	46.10	35.58
		Middle		6	20.00	16.92	5	19.53	25.31	6	53.73	22.67	6	53.75	13.87
		Bottom		6	22.40	23.59	5	34.38	37.76	6	46.70	23.83	6	64.24	34.39
	Lower	Top		4	31.25	21.87	5	20.84	33.42	4	28.13	29.81	3	25.01	13.26
		Middle		4	33.34	52.32	5	18.75	25.13	4	41.41	29.90	3	43.76	4.42
		Bottom		4	39.59	26.94	5	41.41	28.11	4	59.38	43.75	3	77.08	9.55
MS/BS	Upper	Top		6	28.34	17.54	5	56.08	31.03	6	54.85	11.36	6	54.26	15.37
		Middle		6	40.94	15.98	5	59.87	30.21	6	53.79	14.63	6	44.40	17.37
		Bottom		6	42.56	18.13	5	53.75	32.61	6	51.85	11.16	6	25.17	19.05
	Lower	Top		4	47.29	6.80	5	70.65	15.58	4	64.76	18.58	3	57.43	2.20
		Middle		4	33.99	3.64	5	73.91	11.41	4	64.86	9.02	3	49.78	7.90
		Bottom		4	50.17	14.14	5	60.97	18.81	4	49.48	18.63	3	47.86	6.32
ES/BS	Upper	Top	t	5	60.00	23.97	4	47.69	23.80	5	31.52	19.06	4	32.52	1.15
			a	5	39.60	24.68	4	28.94	24.51	5	35.03	11.47	4	37.41	30.49
		Middle	t	5	52.72	31.52	4	36.69	21.10	5	29.93	13.72	4	45.88	22.97
			a	5	47.02	43.14	4	32.12	18.90	5	17.90	2.11	4	40.92	19.19
		Bottom	t	5	45.95	37.42	4	33.57	16.63	5	35.54	17.16	4	35.86	3.44
			a	5	33.06	30.17	4	32.25	29.47	5	41.20	22.10	4	39.48	23.65
	Lower	Top	t	4	39.46	0.51	5	18.77	13.72	4	41.54	14.53	3	33.75	6.67
			a	4	53.39	20.77	5	14.00	11.29	4	29.98	4.55	3	41.21	18.99
		Middle	t	4	52.61	7.57	5	20.40	16.21	4	32.91	5.51	3	18.96	2.31
			a	4	50.60	20.32	5	22.59	19.27	4	41.15	8.49	3	35.56	14.37
		Bottom	t	4	26.80	13.78	5	31.39	29.19	4	34.24	15.11	3	36.70	18.81
			a	4	49.92	30.63	5	33.76	34.02	4	41.12	15.36	3	45.40	7.95

Mean values and SD values are expressed in percentage.
SD, standard deviation; BV/TV, bone volume; BIC, bone-to-implant contact; MS/BS, mineralizing surface; ES/BS, erosion surface.
*t, towards the orthodontic load; a, away from the orthodontic load.

might have been the main cause of failure. Other authors who used beagle dogs reported failures either exclusively in the unloaded group (Deguchi *et al.*, 2003) or in the loaded group (Freire *et al.*, 2007) when loaded with a 250 g force. Histological analysis revealed that some mini-implants had a close proximity to the dental roots, not allowing for measurement of BIC. The accidental impingement of mini-implants in the dental root and periodontium is a possible complication, especially in the inter-radicular areas. However, histological studies have demonstrated repair of the periodontal tissues in cases of accidental contact of dental roots (Roberts *et al.*, 1989; Asscherickx *et al.*, 2005).

BV/TV. Slightly higher values were found at all four time intervals in the unloaded sample compared with the loaded sample (Table 1). The data did not show any particular trend, although at 3 months higher BV/TV values were found in the lower compared with the upper jaw, as expected due to the higher density of mandibular bone (Table 2). One explanation could be that 3 months corresponds approximately to one remodelling cycle in monkeys (Garetto *et al.*, 1995), i.e. the healing pattern in the peri-implant area could have taken place entirely within the 3 months interval. If this was the case, no detrimental effects would be generated by orthodontic loading, confirming that the force applied to the implants induces physiological bone adaptation (Roberts *et al.*, 1984). Akin-Nergiz *et al.* (1998) found that the alveolar bone surrounding osseointegrated implants loaded by continuous forces was more compact on the pressure than on the tension side. This finding cannot be supported by the results of the present study, where the BV/TV percentage values at the pressure and tension sides showed no significant differences.

BIC. Comparison between the loaded and unloaded samples showed higher percentage BIC values for the loaded group at the 1 week and 3 month intervals, although the differences

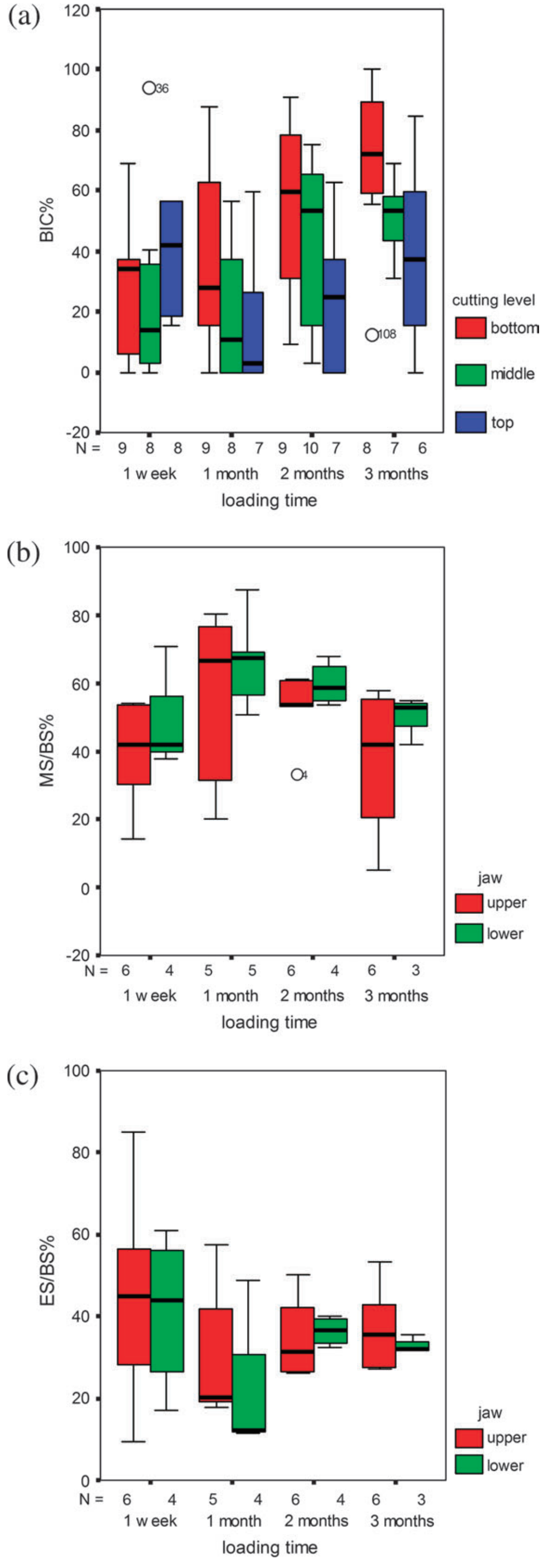

Figure 6 Bone-to-implant contact (BIC) at the different time intervals and at the different cutting levels (a), mineralizing surface (MS/BS) at the different time intervals in the upper and lower jaws (b), and erosion surface (ES/BS) at the different time intervals in the upper and lower jaws (c).

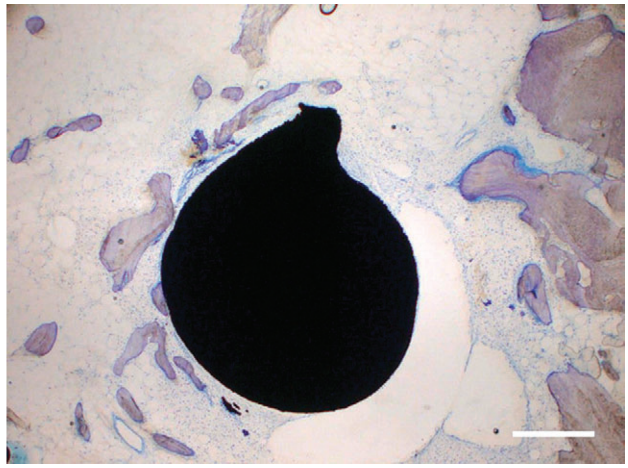

Figure 7 Histological section showing a very low bone-to-implant contact (3%). Bar = 1 mm.

were small (Table 1). This finding is in agreement with the results of Ohmae *et al.* (2001), Deguchi *et al.* (2003), and Freire *et al.* (2007) who found no significant differences between the force applied and the unloaded control implants in beagle dogs. Analysis of the data from the loaded sample revealed, as a general trend, higher BIC values at the bottom level when separated into the three cutting levels (Table 2). This finding was unexpected, since the top cutting level should correspond to the cortical bone area. On the other hand, the inflammatory reaction of the soft tissues around the head of the mini-implant could be the cause of this finding. When analysed at the four time intervals, there was a trend to a decrease in BIC values between the 1 week and 1 month observation periods, and a significantly progressive re-increase following the 1 month observation (Figure 6a). Melsen and Costa (2000) found BIC values to increase monthly and stated that osseointegration is a time-dependent factor, independent of the type of bone and magnitude of orthodontic force applied. A time-dependent increase of BIC was also found with immediate loading by Buchter *et al.* (2006) although the devices were not inserted in alveolar bone. The present results are in agreement with this statement except for the first month time interval. Aldikacti *et al.* (2004) stated that the healing period might be a factor that increases BIC value. However, those authors were referring to surface-treated implants that have osseointegration as a goal. Deguchi *et al.* (2003) observed that mini-implants with as little as 5 per cent bone contact at the bone–implant interface successfully resisted orthodontic force. Those results were obtained after as little as a 3 week healing period in dogs. In the current study, the mini-implants were loaded immediately with 50cN super-elastic springs and BIC values of the loaded sample ranged between 3 and 100 per cent. It is interesting to note that mini-implants with as little as 3 per cent BIC in one of the sections (Figure 7) successfully resisted the orthodontic load in the experimental phase, meaning that full BIC is not necessary for clinical success. No difference was found between the towards and away loading direction sides, in agreement with the results of Aldikacti *et al.* (2004). This latter finding again supports the fact that supplemental orthodontic loading does not play an important role in the healing pattern of the peri-implant bone.

MS/BS. Since only the tetracycline labelled surfaces were considered, the MS/BS values may underestimate the 'real' amount of bone mineralization that took place. The lack of visualization of the calcein labels was due to the short time interval between labels to be clearly detected over thick sections. The analysis of the data from the loaded sample revealed, in general, higher values of MS/BS in the lower jaw when compared with the upper jaw, a finding that is consistent with the higher mandibular bone density. No significant differences were found at the three different cutting levels. When analysed at the four time intervals the data showed a particular trend. MS/BS seemed to increase significantly between the 1 week and 1 month observation intervals and then decrease gradually during the following 2 and 3 month intervals (Figure 6b). This finding is in agreement with that of Deguchi *et al.* (2003), where the MS/BS of their loaded sample was found to be greater at the 6 week observation period when compared both with the 3 and 12 week observation periods, in both jaws of dogs. Furthermore, those authors found intense formation of woven bone around the implants at the 3 week observation period, together with increased values of MS/BS, reflecting the elevation in the overall rate of bone activity. Although dogs and monkeys have been shown to have slightly different durations of remodelling (Garetto *et al.*, 1995), the present data show that following the initial bone healing reaction, characterized by intense formation of immature woven bone, MS/BS levels reached a maximum and then gradually decreased again until completion of the healing process. Compared with the loaded mini-implants, the unloaded samples showed decreased mineralization activity after 1 month, reaching the values of the loaded implants at 2 and 3 months. This could be due to the mechanical load that induces the repair to occur earlier as a regional acceleratory phenomenon (Frost, 1994). This is not consistent with the findings of Deguchi *et al.* (2003) who reported MS/BS of unloaded mini-implants in beagle dogs to have the highest values at 3 weeks, significantly higher when compared with the values of the loaded devices. Their values gradually decreased at the 6 and 12 week observations.

ES/BS. Analysis of the data from the loaded samples revealed that both the direction of the load and the cutting levels had a significant influence on the results. The difference was found to be on the away side, with higher ES/BS values at the bottom level compared with the top level. This could be a result of the strain distribution in the trabecular bone (Cattaneo *et al.*, 2007). Taking into consideration that the application of the load is at the level of the head of the screw, although stable and fixed, the orthodontic force would tend to tip the head towards the orthodontic load (Dalstra *et al.*, 2004). Due to the high density of the cortical bone, no major deformation took place at the top level, whereas the largest deformation, triggering cellular reactions, occurred in the less dense trabecular bone. The distribution of ES/BS at the four time intervals showed a trend opposite to that for MS/BS. There was a decrease between the 1 week and 1 month observation and a progressive re-increase following the 1 month observation (Figure 6c). The unloaded sample showed, although a large variation was observed, a tendency towards larger values for ES/BS, especially after 1 month, compared with the loaded implants with less time-dependent fluctuation. Again, in a load history perspective, mechanical loading might after 1 month inhibit bone resorption that occurs around unloaded implants, thus drifting the bone balance towards positive values. The lack of data in the literature on bone resorption indices in the peri-implant area dealing with orthodontic mini-implants did not allow for any comparison of the present data.

If related to the MS/BS, it could be assumed that the bone healing reaction is at a certain point characterized by a maximum level of mineralization which coincides with the minimum level of erosion, approximately at 5–6 weeks after insertion and immediate loading. The loaded sample maintained MS/BS equal to or larger than ES/BS until the end of the observation period, while the unloaded mini-implants showed a larger fluctuation in the ratio between resorption and formation, reaching a peak of large formation after 2 months. This fluctuation could be ascribed to the lack of a constant load delivered by the springs.

Conclusions

BV/TV around loaded mini-implants did not show any particular trend within the 3 month healing period, similar to unloaded implants.

The healing pattern around the immediately loaded orthodontic mini-implants showed a trend to a decrease in BIC after 1 month followed by a progressive re-increase over time, while BIC in the unloaded controls was much lower at 1 week and progressively increased, but without reaching the same level as the loaded sample.

MS/BS and ES/BS showed opposite trends during the healing period. The first parameter increased between 1 week and 1 month and progressively decreased throughout the following two observation intervals, while the latter decreased between 1 week and 1 month and progressively increased at 2 and 3 months. As compared with the unloaded samples, mechanical loading seems to induce an earlier increase in bone formation and a decrease in bone resorption, thus confirming the positive role of mechanical loading in increasing bone activity.

As to the timing of loading, it can be concluded that immediate loading of orthodontic mini-implants with light forces does not negatively affect the bone healing pattern.

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