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REVIEW ARTICLE

A systematic review of methods for tissue analysis in animal studies on orthodontic mini-implants

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Abstract

Anchorage devices are increasingly used in orthodontics, and their clinical performance is directly dependent on the tissue response to these devices. This study aims to identify assessment parameters for evaluating tissue reactions around orthodontically loaded implants and to propose parameters to be included in a standardized method. Several electronic databases (PubMed, ScienceDirect, the Cochrane database) were explored for papers from January 1999 to December 2009. The preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement was used as a guideline for the methodology of systematic reviews. Twenty-five publications were selected from 123 potentially relevant abstracts. The selected studies mainly aimed to answer a clinical question and particularly the ability of immediate loading in orthodontics. Very few studies aimed to understand the healing mechanism around the devices leading to a lack of information on this topic. The most frequent combination of assessment methods was clinical evaluation, histology/histomorphometry and intravital bone labeling. Although the dog model is mainly used, pigs represent an interesting animal model, especially when studying devices in growing bone. Despite the extensive use of miniscrews in growing individuals, only few studies have included young subjects in their protocol. Moreover, in such studies, an oral hygiene program is absolutely necessary to avoid complications. Finite element analysis could improve the knowledge of the relationship between design and bone reaction; unfortunately, this elaborated method is complex and impossible to perform routinely. For standardization, the authors recommend to include specific criteria in study protocols when assessing tissue response to orthodontically loaded devices.

Key words: dental implant; methodology; miniscrew; orthodontics

Introduction

The use of dental implants and screws to provide intraoral anchorage for tooth movement is increasing in orthodontics. It avoids the need for extraoral sources of anchorage which are less comfortable, and it allows specific orthodontic movements. These devices, in particularly the miniscrews, are often inserted nearby teeth or between dental roots. The periodontal reaction in the peri-implant area needs to be evaluated, as tissue injury could lead to damage of the adjacent teeth. Moreover, considering the use of endosseous devices, the orthodontic procedure differs from the prosthetic one (angulations of the devices, removal at the end of the treatment, and lack of osseointegration). It leads to different tissue reactions and consequently to specific assessment methods of the peri-implant area. Concerning implants or screws devices, many systems are on the market, which differ in their characteristics (e.g., material or size) and clinical procedures. Studies are required to determine the safety and effectiveness of these systems. The aim of this systematic review was to identify the different assessment parameters and to suggest the most reliable parameters to be considered when studying tissue response to orthodontically loaded devices.

Material and methods

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) (1) has been used as a guideline throughout the manuscript.

A literature search was performed using several electronic databases (PubMed, ScienceDirect, the Cochrane database) on articles published from January 1999 to the end of December 2009 as this 10-year period corresponds to the major clinical use of these devices in orthodontics. To be included in this analysis, papers had to consist of animal studies (limits in the PubMed database, 'Animals') as these studies provide more information on tissue reactions compared with human or *ex vivo* studies. Language restrictions were not applied.

Furthermore, these studies had to meet the following criteria:

1. Orthodontically loaded metallic skeletal anchorage devices (implants or screws)
2. Evaluation of the periodontal reaction in the peri-implant area

The terms used in the search (in Title/Abstract) were as follows:

('Implant*' OR 'Screw*' OR 'Anchorage*' OR 'Plate*')
AND
('Bone' OR 'Periodon*')
AND
('Orthodon*')

Review articles and short communications were excluded. The titles and abstracts were screened independently by two reviewers. The full texts of all the abstracts in accordance with the inclusion criteria (by consensus) were collected and reviewed, and their reference citations screened for additional publications that might have been missed by the electronic search. Again, a consensus between the two readers was reached to determine which studies met the selection criteria.

The risk of bias of individual studies was assessed by a systematic analysis of papers regarding the use of a hygiene program or not, the number of animals, and the evaluation of gingival inflammation.

Results

Study selection

A total of 123 abstracts were identified through the electronic database with the selected terms (Fig. 1). After screening, 86 publications were rejected based on the title and abstract because they did not meet the inclusion criteria. The full texts of the 37 remaining studies were read as follows: 11 of them also did not meet the inclusion criteria [tissue reaction at the bone–device interface not evaluated (2–5), no orthodontic loading (6–11), insertion of the devices in the caudal part of the mandible and not in the alveolar bone (12)], and one more used only one animal per studied parameter (13), leading to a low methodological quality. The citations of the 25 remaining publications were screened carefully for additional

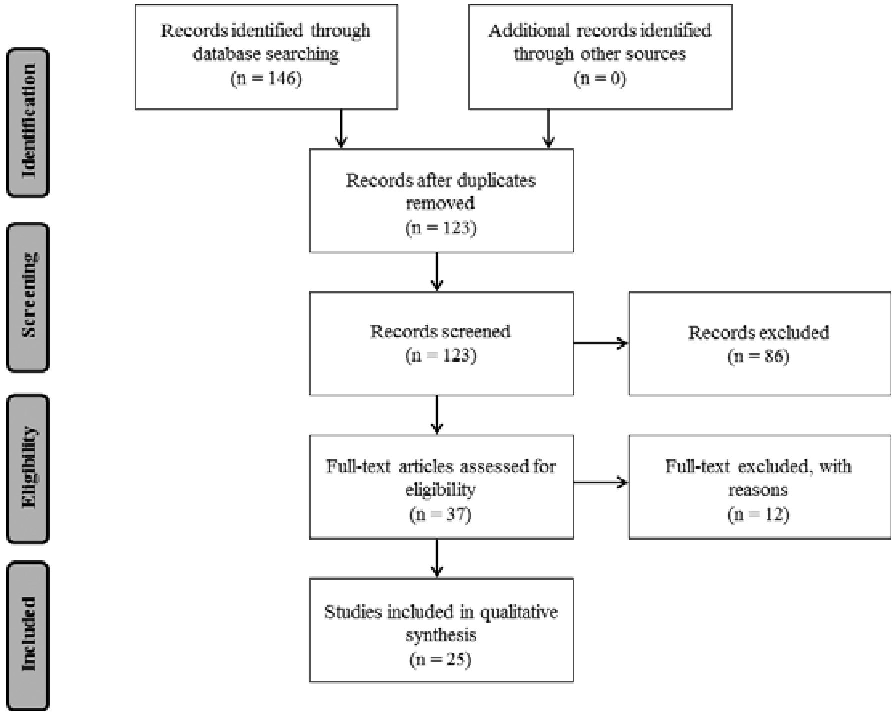

Fig. 1. Flow diagram.

studies that might have been missed by the electronic search (0 papers found). Finally, 25 papers remained to be included (14–38). Our analysis focused, for each study, on the scientific questioning, the protocol parameters, and the assessment methods and criteria of the tissue reaction in the peri-implant area.

Study characteristics

Study objectives

All the remaining papers studied the tissue integration of the selected devices. However, scientific objectives differed and can be classified into five groups, studying the influence of a part of the clinical procedure (group I), of the design characteristics of the device (group II), of the behavior of a specific system of devices (group III) or the behavior of devices for one specific clinical indication (group IV), and/or studying mechanism of bone adaptation to such devices (group V). The objectives are detailed in Table 1.

Table 1. **Study objectives (5 themes) of the 25 included papers**

Themes	Detailed objectives
Clinical procedure (group I)	Immediate-loading or delayed loading (17, 20, 21, 23, 24, 27, 28, 35)
	Drill procedure (18, 19, 26)
	Insertion with root contact (25)
Devices characteristics (group II)	Metal and alloys (31*, 32*, 33*)
	Devices length (24)
	Devices surface characteristics (14, 31*, 32*, 33*)
Specific system of devices (group III)	Newly designed devices (38)
	Length reduced-devices (15, 36, 37)
Specific clinical indication (group IV)	Molar intrusion (22, 30)
Alveolar adaptation (group V)	Unloaded devices (29, 34)
	Loaded devices (16, 29, 34)

*Same study.

Protocol parameters

Anchorage devices. Miniscrews were studied in 16 articles (17–30, 35, 38); two of which are screws of miniplates. Dental implants are used in nine publications (14–16, 31–34, 36, 37). For the miniscrews, the number of devices ranged from 10 (38) to 160 (21), the diameters from 1 (23, 30) to 2.5 mm (24), and the lengths from 4 (30) to 14 mm (38). Eight studies used Ti6Al4V devices

(17–21, 24, 28, 35) and 8 used pure titanium (22, 23, 25–27, 29, 30, 38) (Table 2). Concerning the implants, the number of devices ranged from 8 (14, 15) to 30 (31–33); with diameter ranged from 3.3 (15, 16) to 4.1 mm (14, 31–33) and the lengths from 4 (37) to 10 mm (14). In all the publications, Ti devices were placed except in 3 (31–33) (parts of the same study) where both Ti and Ti6Al4V are used (Table 2).

Regarding miniscrew implant devices, the most frequently observed objective was the assessment of the ability of immediate loading in orthodontics (group I). All other groups were investigated too, but in only one or two studies per group. For the one using dental implant devices, only group II (14, 31–33), III (15, 36, 37), and V (16, 34) were investigated (Table 1).

Devices were retrieved before periodontal reactions could be evaluated in three studies (24, 30, 35), using miniscrew implants.

Animal model

Two animal models were listed (Table 3): dogs were used in most of experimental studies (14, 15, 17–26, 28, 30–38) as well as monkeys (16, 27, 29). Related to the studies' objectives, monkeys were used in studies working on alveolar adaptation to implants or screws (group V). In all other studies, dogs were used. The number of animals ranged from 2 (18, 26) to 10 (21) for studies including miniscrews and from 2 (36) to 5 (14, 31–33) for studies including dental implants. Adult animals were always selected except in two articles that used growing animals (23, 35). No correlation was

Table 2. **Characteristics of the endosseous devices in the 25 selected papers**

Study	Implant material	Number of devices	Length × diameter (mm)	Manufacturer
Cha et al. (17)	Ti6Al4V	48	7 × 1.8	ORLUS; Ortholution
Chen et al. (20)	Ti6Al4V	60	7 × 1.2–1.3	Absoanchor; Dentos, Daegu, Korea
Kang et al. (25)	Ti	48	8.5 × 1.8	C-implant; C implant, Seoul, Korea
Luzi et al. (27)	Ti	50	9.6 × 2	Aarhus MiniImplant; Medicon, Tuttlingen, Germany
Ma et al. (28),	Ti6Al4V	24	*	Absoanchor; Dentos, Daegu, Korea
Wehrbein et al. (37),	Ti	16	4 × *	Orthosystem®; Straumann, Basel, Germany
Cornelis et al. (21)	Ti6Al4V	160	5 × 2.3	SurgiTec, Bruges, Belgium
Chen et al. (19)	Ti6Al4V	24	7 × 1.3	Absoanchor, Dentos, Daegu, Korea
Chen et al. (18)	Ti6Al4V	56	7 × 1.3	Absoanchor; Dentos, Daegu, Korea
Borbely et al. (15)	Ti	8	6 × 3.3	Orthosystem®; Straumann, Basel, Germany
Vande Vannet et al. (35)	Ti6Al4V	20	6 × 1.7	Leibinger-Stryker, GmbH and Co, Freiburg, Germany
Wu et al. (38)	Ti	10	12–14 × 1.15	Westlake Biomaterial (authors design), Hangzhou, China
Freire et al. (24)	Ti6Al4V	78	6–10 × 2.5	Bicon, Boston, MA, USA
Cattaneo et al. (16)	Ti	16	7 × 3.3	Exacta; Biaggini Medical Devices, La Spezia, Italy
Pilliar et al. (33)	Ti/Ti6Al4V	30	5 × 4.1	Innova Corporation (custom-made implants), Toronto, ON, Canada
Kim et al. (26)	Ti	32	* × 1.6	Osas; Epoch Medical, Seoul, Korea
Oyonarte et al. (32)	Ti/Ti6Al4V	30	5 × 4.1	Innova Corporation (custom-made implants), Toronto, Canada
Oyonarte et al. (31)	Ti/Ti6Al4V	30	5 × 4.1	Innova Corporation (custom-made implants), Toronto, Canada
Aldikaçti et al. (14)	Ti	8	10 × 4.1	Institute Straumann AG, Waldenburg, Switzerland
Deguchi et al. (23)	Ti	96	5 × 1	Stryker Leibinger, Kalamazoo, MI, USA
Ohmae et al. (30)	Ti (99.5%)	36	4 × 1	Sankin Industrial Company, Tokyo, Japan
Melsen and Lang (29)	Ti	12	6 × 2.2	Institute Straumann AG (specially designed), Waldenburg, Switzerland
Daimaruya et al. (22)	Ti	48	5–7 × 2	Leibinger Co, Freiburg, Germany
Saito et al. (34)	Ti	16	7 × 3.75	Bränemark
Wehrbein et al. (36)	Ti	10	6 × 4	Bonefit; Institue Straumann, Waldenburg, Switzerland

*Not specified.

Table 3. **Animal models used in the 25 selected papers.**

Animal model	Study	Number of animals	Age of animals
Dog			
Beagle dog	Cha et al. (17)	8	Adults
	Kang et al. (25)	3	Adults
	Ma et al. (28)	4	Adults
	Cornelis et al. (21)	10	Adults
	Vande Vannet et al. (35)	5**	Growing
	Wu et al. (38)	5	Adults
	Freire et al. (24)	6	Adults
	Pilliar et al. (33)*	5	Adults
	Kim et al. (26)	2	Adults
	Oyonarte et al. (32)*	5	Adults
	Oyonarte et al. (31)*	5	Adults
	Ohmae et al. (30)	3	Adults
	Daimaruya et al. (22)	6	Adults
	Saito et al. (34)	4	Adults
Foxhound dog	Wehrbein et al. (37)	4	Adults
	Wehrbein et al. (36)	2	Adults
German shepard	Borbely et al. (15)	4	Adults
Mongrel dog	Chen et al. (20)	4	Adults
	Chen et al. (19)	3	Adults
	Chen et al. (18)	2	Adults
Turkish sheepdog	Aldikaçti et al. (14)	5	Adults
Dog (unspecified)	Deguchi et al. (23)	8	Growing
Monkey			
Macaca fascicularis	Luzi et al. (27)	4	Adults
	Cattaneo et al. (16)	4	Adults
	Melsen and Lang (29)	6	Adults

*Same study.
**Same mother

observed between a specific objective and the use of young animals, and with the number of animals used.

Hygiene program

From the 25 studies, 13 included an oral hygiene program (14, 16–18, 21, 22, 27, 29, 31, 32, 34, 37, 38) for plaque control; from these 13 studies, only two carried out rinsing with antiseptic agent (14, 18), and the other applied teeth and appliance brushing with chlorhexidine; its concentration varied from 0.2% (14, 18, 23) to 2% (29, 38), and three papers have not provided information about it (17, 21, 37). In one study, toothpaste was combined to chlorhexidine (34), and in another one, a subgingival ultrasonic scaling was performed (31, 32), both under anesthesia. The frequency of oral hygiene application ranged from once daily (14, 17) to once weekly (27, 38).

Tissue response criteria and assessment methods

According to the study objectives, the assessment methods are summarized with respect to the five groups of study objectives in Table 4.

Several parameters are investigated to evaluate the bone reaction around the devices and conclude about their tissue integration.

Table 4. **Assessment methods regarding the groups of objectives. The numbers between brackets indicate the references using the specified methods**

Themes	Detailed objectives	Parameters	Methods
Clinical procedure (group I)	Immediate-/early- or delayed-loading	Immediate vs. Early vs. No loading (17, 24, 35)	**MicroCT** [(BIC (17), BV/TV (17)] **Histomorphometry** [osseointegration (35), BIC (17, 20, 21, 23, 24, 27, 28), BV/TV (17, 21, 23, 27),MS/BS (23, 27), ES/BS (27), WV/TV (23)]
		Immediate vs. No loading (20, 27, 28)	**Histology** [slide observation (24)] **Radiography** [displacement (24, 28)] **Clinical evaluation** [displacement (24), gingival inflammation (24), success rate (23, 35), mobility (20, 28), periodontal pocket depth (20)]
		Early vs. No loading (21)	**Fluorescent bone labeling** [bone apposition (21, 28, 35), osteodynamic changes (20), bone remodeling (21, 28), Bone Formation Rate/day (23, 28),MAR/day (23)]
		Multiple healing periods (23)	**Digital micrometer** [displacement (20)] **Energy dispersive X-ray spectroscopy** [chemical element dispersion (28)]
	Drill procedure	Self-drilling vs. Self-tapping (18, 19)	**Histomorphometry** [BIC (18, 19, 26), bone area between threads (26)] **Fluorescent bone labeling** [bone remodeling and apposition (18, 19)] **Clinical evaluation** [mobility (18), success rate (18), gingival inflammation (18)]
		Drilling vs. Not drilling (26)	**PIT and PRT digital measurement** [PIT/PRT values (18)] **Periotest** [mobility (26)]
	Insertion with root contact	Devices with root contact vs. inserted in the middle of the alveolar bone (25)	**Clinical evaluation** [success rate (25), mobility (25)] **Histology** [observation of bone deposition (25)]
Devices characteristics (group II)	Metal and alloys	Ti vs. Ti6Al4V (31*,32*,33*)	**Finite Element Analysis** [bone stress (33*)] **Fluorescent bone labeling** [bone remodeling (32*), MAR/day (32*)] **SEM** [marginal bone level (31*), BIC (31*)]
	Length	6 vs. 10 mm (24)	**Histomorphometry** [BIC (24)] **Histology** [slide observation (24)] **Radiography** [displacement (24)] **Clinical evaluation** [displacement (24), gingival inflammation (24)]
	Surface characteristics	Threaded-surfaced vs. Porous-surfaced devices (14, 31*,32*,33*)	**Finite Element Analysis** [bone stress (33*)] **Fluorescent bone labeling** [bone remodeling (32*), MAR/day (32*)] **SEM** [marginal bone level (31*), BIC (31*)] **Histomorphometry** [BIC (14)] **Histology** [bone remodeling (14)]
		Sand-blasted vs. Acid-etched (14)	**Radiography** [displacement (14)] **Clinical evaluation** [displacement (14), gingival inflammation (14), mobility (14), periodontal pocket depth (14)]

***Table 4.* Continued**

Themes	Detailed objectives	Parameters	Methods
Specific system of devices (group III)	Newly designed devices	Immediate-loading vs. No loading (38)	**Histology** [slides observation (38)] **Histomorphometry** [BIC (38)] **Radiography** [displacement (38)] **Clinical evaluation** [displacement (38), gingival inflammation (38)]
	Length-reduced devices	Loading vs. No loading (36, 37) Immediate-loading (15)	**Histomorphometry** [BIC (15, 37), bone density (36), osteon density (37)] **Fluorescent bone labeling** [bone apposition (15), osteodynamic changes (36), bone remodeling (36)] **Clinical evaluation** [success rate (15), displacement (37), mobility (36, 37), plaque deposition (37), gingival inflammation (37)]
Specificclinicalindication (group IV)	Molar intrusion	Loaded vs. Unloaded (22, 30)	**Histology** [root resorption (22, 30), inflammation (22)] **Histomorphometry** [osseointegration (22)] **Fluorescent bone labeling** [bone remodeling (22, 30)] **Radiography** [displacement (22, 30), root resorption (22)] **Clinical evaluation** [displacement (30), gingival evaluation (22, 30)] **SEM** [BIC (30)]
Alveolar adaptation (group V)	Loaded devices	Loaded vs. Unloaded (29, 34)	**Histomorphometry** [BIC (16, 29), BV/TV (16),MS/BS (16), Fractional resorption surface (16, 29), Fractional formation surface (16, 29), Fractional Resting surface (16), bone density (29)]
		Loaded devices (16)	**Fluorescent bone labeling** [osteodynamic changes (16)] **Radiography** [bone level (34)] **SEM** [BV (34)] **Clinical evaluation** [displacement (29, 34), mobility (16, 34), gingival inflammation (29, 34), periodontal pocket depth (29)] **Finite Element Analysis** [stress and strain area in the peri-implant bone (16, 29)]

BIC, bone-to-implant contact; BV/TV, bone volume/tissue volume; MS/BS, mineralized surface/bone surface; ES/BS, eroded surface/bone surface; WV/TV, woven bone volume/tissue volume; MAR, mineral apposition ratio; PIT, peak insertion torque; PRT, peak removal torque; SEM, scanning electron microscopy.

*Same study.

Osseointegration was evaluated, in most of the studies (n = 18), by the estimated percentage of bone-to-implant contact (BIC) using histomorphometry (14–21, 23, 24, 26–29, 37, 38) or Scanning Electron Microscopy (SEM) analysis (30, 31). The extent of marginal bone level – evaluated by SEM (31) or radiography (34) – the measurement of peak removal torque (PRT) values (18), and/or the ease to removal (30, 35) were used rarely to assess indirectly the amount of osseointegration. Whatever the objectives groups, most of the studies assessed this parameter (group I, n = 11; group II, all studies n = 5; group III, n = 3; group IV, all studies n = 2; group V, n = 3).

Dynamic and static bone parameters were assessed too. The evaluation of osteodynamic changes was observed and quantified in many studies (n = 12) by either vital staining (a sequence of different fluorochromes) (15, 16, 18–22, 27, 28, 30, 32, 35, 36), leading to highlight the nature of the newly formed bone and the chronology of bone contact, or by labeling with two injections of the same fluorochromes solution (23). Fluorochromes were used in numerous studies, whatever the studies objectives (group I, n = 7; group II, n = 1; group III, n = 3; group IV, all studies n = 2; group V, n = 1). Bone remodeling was observed on histological slides in one study (14). Concerning static parameters, they were most of time quantified using histomorphometry (16, 17, 21, 23, 27, 29, 36, 37) and using microCT in only one study (17). Research work of group II and IV did not measure these parameters, and only few studies of the other groups of objectives investigated it [group I, n = 4; group III, n = 2 (bone density); group V, n = 2]. The aspect of peri-implant bone was observed on histological slides in some works (14, 19, 22, 24, 25, 38), belonging to all groups of objectives (except group V).

The stability of the devices is evaluated by measuring the mobility or checking the stability; qualitative measures (14, 16, 18, 25, 28, 34, 36), or quantitative measurements evaluated by Periotest® (26) or mobility scores (20, 37). Only studies belonging to group IV did not assess this parameter (group I, n = 5; group II, n = 1; group III, n = 2; group V, n = 2). The device displacement during loading was also measured in few studies (whatever the groups may be). It is measured using Xrays (14, 22, 24, 28, 30, 38), digital caliper (20, 31), calibrated slide gauge (37), or through impressions (29).

Gingival inflammation was observed clinically (14, 18, 22, 24, 29, 30, 34, 37, 38) and by histological analysis (22, 38), in few studies belonging to the five groups of objectives.

Finally, the stress level at the tissue–implant interface was evaluated by Finite Element Analysis (FEA) (29, 33) after reconstitution by microCT (16) and by measuring the peak insertion torque (PIT) (18). Studies using the FEA correlate bone resorption and tension applied to bone and allow to identify specific area of maximum stresses (33). These methods were rarely encountered and concerned mainly studies of group V.

Whatever the parameters are, the most frequent methodologies used are histomorphometry analysis with or without intravital bone labeling and clinical evaluation. The other methods are scarcely used (one to six studies).

Discussion

The present paper reviews and compares the different methods reported in the literature to assess the periodontal reaction induced by the insertion and loading of metallic devices for orthodontic anchorage in animal models. Data were collected and analyzed from January 1999 to and including December 2009. This period corresponds to the increased use of endosseous anchorage in orthodontics and the increased number of experimental studies concerning these types of devices. Previous relevant review papers corresponding to the criteria of this electronic literature search evaluated the success rates of various implant systems for orthodontic anchorage (39), cell culture, and animal models for studying the effects of mechanical loading on periodontal cells (40), and functional and morphological tissue reaction around orthodontically loaded devices (41). But none of them analyzed the assessment methods used in experimental studies to evaluate tissue reaction around orthodontic implants and/or miniscrews and microimplants. A standardization of methodology is now required to evaluate and to compare such devices for these specific indications (42).

Study objectives

Most studies aimed to answer a clinical question (n = 22), and particularly, the ability of immediate loading in orthodontics (n = 8) was a topic of interest. Three publications focused on the understanding of the alveolar adaptation to the implanted devices. This is probably due to the already existing extensive knowledge about the healing process around prosthetic dental implants, and that even if the forces applied are different in nature and values, the healing process may be considered as quite identical. Thus, there is a lack of information about the real mechanisms of healing around orthodontic devices. On the contrary, main studies tried to answer a clinical question, such as the clinical procedure, the device design, and characteristics (i.e., diameter, surface characteristics), which are mostly different from conventional prosthetic dental implants. Concerning studies of group IV, they all investigated devices for molar intrusion. Other orthodontic indications should be investigated, such as tooth proximity, the implantation site and the nature, direction and values of forces applied as they can be different from one indication to another.

Protocol parameters

Most recent studies used miniscrews for orthodontic purposes. The number of miniscrews per animal was increasing leading to more data per animal. This is possible as orthodontic miniscrews are smaller in length and diameter than dental implants, and their placement position is more variable. Therefore, determining the number of devices with preliminary statistical analyses prior to the experiment is equally important as determining the number of animals to be included in the experiment. Only three studies retrieved the devices (miniscrews) before histological analysis. This can be interesting especially when microCT analysis is performed, as it avoids artifacts for the bone interface assessment (43, 44).

Canine models were used in 88% of the studies included in this systematic review. The similarities of bone composition with human (45) and the good compliance of this model explain this choice. However, owing to their status of companion animals, many ethical issues about their use for implant biomaterial research in bone (46) were raised. Similarities in bone composition and bone remodeling mechanism are found between humans and pigs, making the latter the subject of choice for studying bone during growth and implant design (46, 47). Furthermore, Wang et al. (48) showed the histological similarity of the inflammatory process in pigs to that observed in human periodontal diseases. Thus, the use of porcine models when studying periodontal reaction to insertion and orthodontic loading of screws or dental implant is of great interest, especially when growing animals are used. The major interest of pig compared with the dog resides in the fact that the pig is omnivorous and therefore has similarities with humans regarding chewing and digesting food and the masticatory system development as a whole. It is of interest to notice that studies of group V mainly used an omnivorous animal model.

In more than 92% of publications in the review, adult animals were used in the protocol, which is an appropriate methodology for studying dental implants for prosthetic use. However, in orthodontics miniscrews are often used in growing individuals, and therefore, the use of younger animals may be biologically relevant. Moreover, there is a lack of information of the bone reaction around orthodontically loaded miniscrew implants in growing subjects. The number of animals used in the studies varied significantly (from 2 to 10). A power analysis should always be conducted prior to the start of an experiment.

From the 25 studies, 12 did not include an oral hygiene program, which diminished the methodological quality of the study. Plaque control is essential to prevent periodontal inflammation. However, when subgingival ultrasonic scaling was performed (31, 32), anesthesia was needed, which is difficult to repeat during the experimental period. Scaling during the surgical insertion of the devices (under anesthesia) and then frequent tooth brushing with an antiseptic solution is advised. This hygiene protocol has to be carried out because gingival inflammation is a major risk factor when using temporary anchorage devices (27, 49). In the study of Wehrbein et al. (37), bleeding on probing was reported despite the tooth brushing and rinsing with antiseptic solution was employed, but only once a week. We advice a daily hygiene program if devices are inserted in the alveolar mucosa.

Tissue response criteria and assessment methods

Whatever the type of devices, the number of methods used to assess tissue reaction ranged from 2 (17, 21, 24–27, 37) to 5 (16, 28, 30). The most frequently used methods were clinical evaluation and histology/histomorphometry (with or without fluorescence labeling). This combination of methods is found in most of the publications and appears to be the basis for periodontal reaction evaluation. Another method commonly used was intravital bone labeling (14 among the 25 reviewed publications), which is the only method to measure the dynamic parameters of bone formation and remodeling. Other techniques used were SEM (in only five studies), FEA (three studies), and microCT (one study). It is important to note that microCT appeared in only two studies, despite the ability of non-destructive 3D evaluation of bone tissue around the implants (43, 44, 50, 51). This methodology is as reliable as histology for determining trabecular bone parameters (43) and BIC (51). Probably, this can be explained by the cost of this kind of instrumentation. In addition, there are some doubts about the quality of the radiographs of the direct BIC as some studies revealed a metallic halation artifact around the devices (43, 44). However, other publications did not exhibit metal artifacts at the implant–bone interface (50, 51).

Concerning histological sections, numerous studies analyzed the sections parallel to the long axis of the devices, but in five papers, cross-sections were made (16, 21, 27, 29, 38), and only two made both sagittal and cross-sections (28, 30). The sections perpendicular to the long axis of the devices facilitate the evaluation of the peri-implant tissues that have been subjected to pressure, compression, and shearing forces. Both types of sections can give information of interest. However, if dental implants are used, parameters such as osseointegration are important to determine, and parallel sections are relevant to evaluate bone–implant contact all the length of the device. But, concerning miniscrews, as these devices are inserted nearby the dental roots, an evaluation all around the devices at the bone–implant interface and in a larger area is needed. In this last case, perpendicular sections seem to be more relevant. Even if it only concerns bone tissue, microCT technology allows examination of both parallel and perpendicular sections. Thus, a microCT analysis may be utilized before preparing sections for histological analysis.

Several parameters are observed in different studies to assess the level of osseointegration. The measurement of the BIC rate and more particularly that of the percentage of peri-implant bone volume with SEM are accurate methods to estimate the degree of osseointegration. Measuring the marginal bone level, which also gives an indication of osseointegration only applies to conventional dental implants, but has no interest in miniscrews that are not to be placed on the ridge. The PRT value can be used, as the BIC, to indicate the degree of osseointegration. However, it cannot provide information about the distribution of bone contact or the presence of area of inflammation in the bone at the interface. Finally, the parameter 'ease to removal' is implemented in both studies; the N = 1 case study of Ohmae et al. (30) and the one of Vande Vannet et al. (35) provide only qualitative data. When histological assessment of tissue samples is needed as well, the 'ease to removal' variable cannot be measured of course.

The injection of a single fluorochrome is only an indicator for the rate of bone formation. The advantage of injecting a sequence of fluorochromes is to evaluate rate, stages, and chronology of bone formation. Several fluorochromes are particularly of interest to understand mechanisms of bone healing. It should be systematically used when studying alveolar adaptation to orthodontically loaded devices. Complexity, cost, and number of interventions in each animal have to be considered. So the choice of one or the other methodology is a function of the objectives of the study. Structural bone parameters are rarely assessed, even in studies of group V. However, data coming from bone microstructure in relation to the orthodontic loading are required. Moreover, the specific design of such devices could be improved by including bone parameters.

With regard to the device mobility, the assessment is carried out qualitatively in the studies of

Chen et al. (18–20) (using tweezers) and Wehrbein et al. (36) and quantitatively using Periotest® in Kim's et al. study (26) or by applying mobility scores in the studies of Wehrbein et al. (37) and of Chen et al. (20). The interest of the quantitative measurement is to discriminate several levels of mobility, although low mobility is not necessarily considered as miniscrew failure. The levels of mobility are not clinically quantifiable when using tweezers, except when using indices of mobility as in clinical assessment of tooth mobility in periodontal examination, but this is not the case in the two mentioned studies (18, 36). Thus, it is necessary either to use a Periotest® or, at less, to use scores of mobility.

Displacement of loaded implants is objectified in some studies, using digital calipers or radiographs. Digital measurement is preferred because the method seems to be accurate enough without need for other costly and complicated techniques. For clinicians, the amount of movement through the issues is important as they can anticipate this movement and be careful in inserting devices away from dental roots. Gingival inflammation might be correlated with mobility and could be a confounder in studies on mobility. The fact that all studies indicate a slight inflammation is probably due to the difficulties to perform daily brushing and rinsing in animal studies. There is a lack of information about evolution of the inflammation during the study period. It should be interesting to check it regularly during all the study period (42).

Finally, finite element analysis could constitute an informative method for biomechanical studies about induced stress and strain. The PIT (peak insertional torque) does not make it possible to determine precisely the localization of the stress. Nevertheless, the complexity of FEA limits its use in orthodontic implant studies.

Conclusion

The use of endosseous anchorage devices in orthodontics differs from prosthetics in terms of clinical procedure, design, and loading. It leads to different tissue reactions and to specific assessment methods of the peri-implant area. Some parameters that need to be taken into consideration when studying tissue response to orthodontically loaded devices are as follows:

- Dogs are the preferred model regarding the number of studies. However, porcine models are of interest when growing animals are used or when studying the remodeling mechanism.
- A oral hygiene program should be part of the study protocol.
- MicroCT analysis is advisable for 3D evaluation of the peri-implant bony area.

Clinical relevance

Biointegration of dental implants or miniscrews used as osseous anchorage is fundamental to successful orthodontic treatment. A great variety of systems and clinical procedures exists. Studies evaluating the tissue response of systems and/or procedures allow the clinicians to make the best therapeutic choice. The aim of this review is to identify key endpoints for methods standardization, when studying the biointegration of orthodontically loaded implants. This might help clinicians in their assessment of available studies

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