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Comparative study of bone repair in mandibular body osteotomy between metallic and absorbable 2.0 mm internal fixation systems. Histological and histometric analysis in dogs: a pilot study

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Abstract. The objective of this study was to compare the bone repair along a mandibular body osteotomy stabilized with 2.0 mm absorbable and metallic systems. 12 male, adult mongrel dogs were divided into two groups (metallic and absorbable) and subjected to unilateral osteotomy between the mandibular third and fourth premolars, which was stabilized by applying two 4-hole plates. At 2 and 18 weeks, three dogs from each group were killed and the osteotomy sites were removed and divided equally into three parts: the upper part was labelled the tension third (TT), the lower part the compression third (CT), and the part between the TT and CT the intermediary third (IT). Regardless of the treatment system, union between the fragments was observed at 18 weeks and the CT showed more advanced stages of bone repair than the TT. Histometric analysis did not reveal any significant differences among the 3 parts or systems in the distance between bone fragments at 2 weeks. Although at 18 weeks the proportions of newly formed bone did not differ among TT, IT and CT, significantly enhanced bone formation was observed in all sections for the metallic group. The patterns of repair were distinct between treatments.

Key words: internal fixation; osteotomy; bone repair; absorbable system.

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Internal fixation (IF) systems have been widely used on the facial skeleton for the treatment of fractures and osteotomies. Since the early 1980s, IF systems manufactured with titanium (Ti) or its alloys have been developed to achieve adequate mechanical behaviour, biocompatibility and optimization of the bone repair process at the fracture or osteotomy site.[1] Although the treatment of mandibular fractures and osteotomies with metallic IF systems is a well-established method, several problems have been associated with the use of these permanent devices, including infection, breakage, intraoral exposure, osteoporosis or bone atrophy due to the stress shielding effect, changes in anatomical contour, palpability, sensitivity to cold, screw migration, corrosion, interference in imaging examinations and radiation therapy, allergic reaction, interference with the facial growth of paediatric patients and deposition of Ti particles in scar tissue and lymph nodes.[1–7] To address these concerns, efforts have been focused on the development of a new class of materials: absorbable polymers.[1,6,8]

IF systems manufactured with absorbable polymers were first used experimentally in monkeys and dogs,[9–11] with later use in humans for the treatment of zygomatic and orbital floor fractures.[12,13] Absorbable polymers with different molecular weights, crystallinities, mechanical properties and absorption times have been applied. Among the most commonly applied polymers are polylactic (PLA) and polyglycolic (PGA) acids, which have been manufactured separately or in combination with each other, with their isomers or with other substances such as hydroxyapatite (HA).[1,2,14–17]

The absorbable IF system must maintain the stability of bone fragments during the bone repair process, gradually transferring the stress function to the bone while the IF system is resorbing.[1,5,6] Some authors propose that absorbable IF systems have sufficient mechanical properties to allow effective bone repair at the fracture or osteotomy site,[1,3] others disagree.[15] Those in the latter group question the use of these systems in the mandible, where the forces resulting from muscular activity are greater than those sustained by the middle or upper third of the face.

The dynamics of bone repair in dogs were clearly affected by changes in the mechanical stimuli that occurred along the osteotomy site when a 2.0 mm metallic system was applied.[18] This effect was evident on histological analysis, although no statistically significant difference was detected in a histometric analysis. Although some clinical,[7,19,20] animal[1,2,16,21–23] and *in vitro* studies[3,24] have evaluated the mechanical behaviour of the absorbable IF systems, few studies have evaluated the influence of this behaviour on the bone repair process at the fracture or osteotomy site.[9–11,25] A better understanding of this relationship can help optimize procedures, techniques, instrumentation and system manufacturing.

The objective of this study was to conduct a histological and histometric analysis of bone repair along a mandibular body osteotomy stabilized with a 2.0 mm absorbable IF system and to compare the outcomes of these analyses with those obtained with the metallic IF system, which is considered to be the gold standard in the literature.

Materials and methods

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of São Paulo (Process 07.1.188.53.9) and all animal care was conducted at the Experimental Animal Care Facility II under the care of the same veterinarian. Twelve healthy, male, adult mongrel dogs aged between 3 and 8 years old and weighing between 15 and 20 kg were used as subjects. The dogs were randomly divided into two groups after a simple draw according to which IF system was applied (metallic and absorbable groups). The previously mentioned veterinarian administered general anaesthesia, intravenous zolazepam/tiletamine (0.12 ml/kg), which was maintained with inhalation of isoflurane and oxygen *via* orotracheal intubation. After animals were assigned to groups, an iatrogenic osteotomy was performed between the third and fourth mandibular premolars through an intraoral approach using a surgical micro-reciprocating saw. A surgical blade with a thickness of 0.2 mm was positioned perpendicular to the mandibular body, and a continuous defect was created. The fragments were reduced manually and stabilized by applying 155 mm self-lock reduction forceps (Synthes, Oberdorf, Switzerland).

Metallic group

Two 4-hole Ti plates fixed with 2.0 mm Ti alloy self-tapping screws in the 2.0 mm non-locking system (Synthes, Oberdorf, Switzerland) were used according to the recommendation of the Association for Osteosynthesis–AO for a transverse fracture line without dislocation.[26] Two 1.5 mm drill bits with stop lengths of 6.0 mm and 20.0 mm were used to drill holes near the alveolar process and the mandibular base, respectively. The plate positioned along the alveolar process was fastened monocortically with 6.0 mm self-tapping screws first, and then the other plate was fastened bicortically along the mandibular base. To ensure bicortical engagement, the length of the self-tapping screws was selected according to the buccal–lingual dimension of the mandibular base, which varied from 8.0 to 12.0 mm (Fig. 1). After copious irrigation with sterile saline solution, the surgical wound was continuously sutured with a 4-0 absorbable thread (Poliglactina 910).

Absorbable group

Two 4-hole plates manufactured with a combination of L-polylactic acid (LPLA), D,L-polylactic acid (DLPLA) and trimethylene carbonate (TMC) were fixed with 2.0 mm non-locking screws composed of the same material (Inion, Tampere, Finland). With the exception of the handling of the plates and screw insertion, which were performed according to the

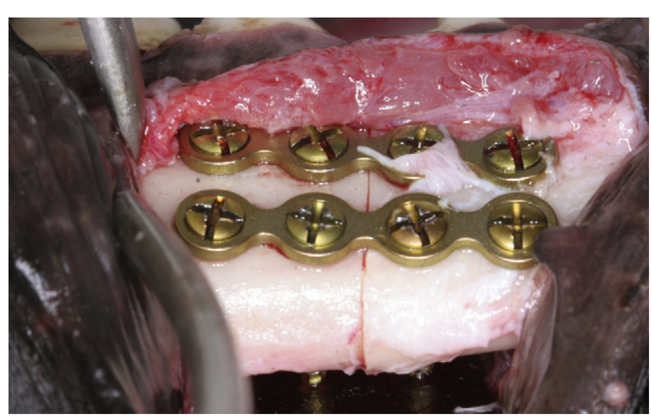

Fig. 1. Plates positioned and fastened in the metallic group. Two of the bicortically engaged screws passing through the lingual cortical surface can be observed.

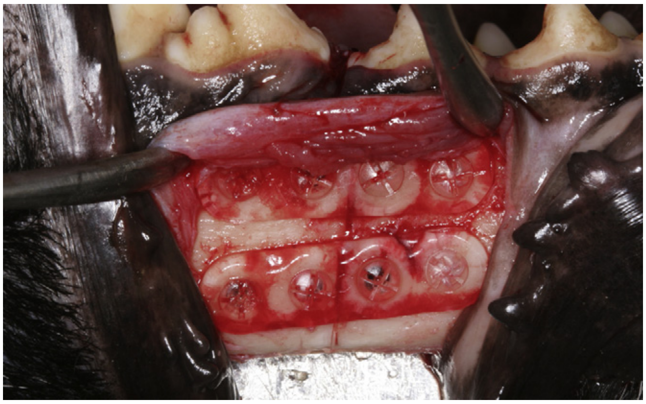

Fig. 2. Positioned and fixed plates in the absorbable group.

manufacturer's recommendations, and the manufacture of the dental splint, the surgical steps conducted in this group were identical to those in the metallic group.

To achieve adequate adaptation, the plates were immersed in a sterile saline solution, warmed to 55 °C in a water bath (Thermo, Inion, Tampere, Finland) for 5 min, and manipulated to the contours of bone surface of the buccal cortical bone *via* digital manipulation. Two 1.75 mm drill bits with stops of 6.0 mm and 22.0 mm were applied to generate holes near the alveolar process and the mandibular base, respectively. Afterward, a tap was manually inserted to the full drill depth to provide threading for the screws before insertion. The plate positioned along the alveolar process was fastened monocortically with 7.0 mm screws first, and then the other plate was fastened along the mandibular base. To ensure bicortical engagement, the length of the screws was selected according to the buccal–lingual dimension of the mandibular base, which varied from 9.0 to 13.0 mm (Fig. 2).

Similar to the metallic group, after copious irrigation with saline solution, the surgical wound was continuously sutured with a 4-0 absorbable thread (Poliglactina 910). Afterward, a dental splint was created using a 0.8 mm orthodontic wire and light-cured composite resin involving the lingual surface of the first molar and the buccal surfaces of the second, third and fourth premolars, as well as the canine (Fig. 3). The final configuration of the dental splint was obtained to avoid dental contacts during mandibular movements and, consequently, some form of occlusion interferences. Regardless of the group, no functional restriction was applied and all animals were placed on a regular chow diet (dry granulated fodder) immediately following the surgical procedure.

At 2 and 18 weeks post-treatment, three dogs from each group were killed per time period. The mandibular body was resected close to the plates' extremities and immediately placed in a 10% neutral buffered formalin solution. After fixation, the region containing the osteotomy site was carefully resected with a 0.10 mm thick double-faced flexible diamond disc (Sorensen, Cotia, Sao Paulo, Brazil) and decalcified in 10% trichloroacetic acid. Decalcified specimens from the osteotomy site and the adjacent bone were divided into three equal parts. The upper part that included the crest of the alveolar process was labelled the tension third (TT), the lower part that included the mandibular base was labelled the compression third (CT), and the part between the TT and CT was labelled the intermediary third (IT). For each specimen, the upper site of each part was oriented horizontally and embedded in paraffin. The 5.0 μm thick sections were obtained and stained with haematoxylin and eosin (HE) and Mallory's trichrome (TM) for light microscopic analysis.

Fig. 3. Dental splint created with 0.8 mm orthodontic wire and light cured composite-resin, involving the lingual surface of the first molar and the buccal surfaces of the second, third and fourth premolars and the cuspid.

Histological analysis

Histological analysis of the entire osteotomy area was performed using conventional and polarized light microscopy (DMLB2, Leica, Wetzlar, Germany).

Histometric analysis

Three randomly selected sections from each part were used. The images were acquired with a digital camera (DFC300FX, Leica, Wetzlar, Germany) coupled to an optical microscope (DMLB2, Leica, Wetzlar, Germany) and the IM50 Image Manager software (Leica, Wetzlar, Germany). The region of interest was restricted to the osteotomy site at the cortical level.

At 2 weeks post-treatment, the osteotomy osseous margins were identified based on the typical interface between the parent lamellar bone and the non-mineralized connective tissue. The distance between the bone fragments was measured linearly at the most external surface of the lingual and buccal cortical bone.

At 18 weeks, after clear identification of the interface between old and newly formed bone under polarized light at the osteotomy site, the total area delimited by the old bone surface in each cortical bone was determined using QWin Plus software (Leica, Wetzlar, Germany). The area of newly formed bone, including woven bone, parallel-fibred bone, and lamellar bone, was determined using the same software. Both area values (total and newly formed bone) were measured and the percentage of newly formed bone was determined. The endosteal and periosteal reactions were not included in this measurement.

To compare the percentages of newly formed bone at the buccal and lingual corticals, the references used to identify the cortical for the TT and IT, whether buccal or lingual, were the proximity of the tooth roots, which are closer to the buccal cortical than the lingual cortical. In contrast, for the CT, the proximity of the mandibular canal was used, which is closer to the buccal cortical. After testing the normality of the data distribution with the Shapiro–Wilk test, statistical analysis was performed using Student's *t*-test and two-way analysis of variance (ANOVA) with a significance level of 0.05.

Results

Histological analysis

2-Week period

Regardless of the experimental group, the amount of newly formed immature bone at 2 weeks was not sufficient to promote union of the bone edges created by the osteotomy (Fig. 4A–D).

The histological sections for metallic TT showed well-defined buccal and lingual cortical lamellar bone, with the medullary bone area predominantly characterized by loose connective tissue, different densities of collagen fibres, focal areas of inflammatory infiltrate and haemorrhage. The osteotomy site was partially filled with non-mineralized connective tissue. Bone remodelling was observed for the cortical bone (both buccal and lingual) as revealed by the presence of Howship's lacunae containing osteoclasts at the periosteal and endosteal surfaces, which was associated with a discrete endosteal reaction characterized by the presence of interconnected trabeculae of immature, woven bone (Fig. 4A).

For metallic CT, a remarkable endosteal reaction was observed adjacent to the osteotomy site, consisting of interconnected trabeculae of immature bone in a continuum with the internal cortical surface that was previously resorbed by osteoclasts. The osteotomy site and the medullary bone area showed dense connective tissue with focal areas of inflammatory infiltrate and haemorrhage (Fig. 4B).

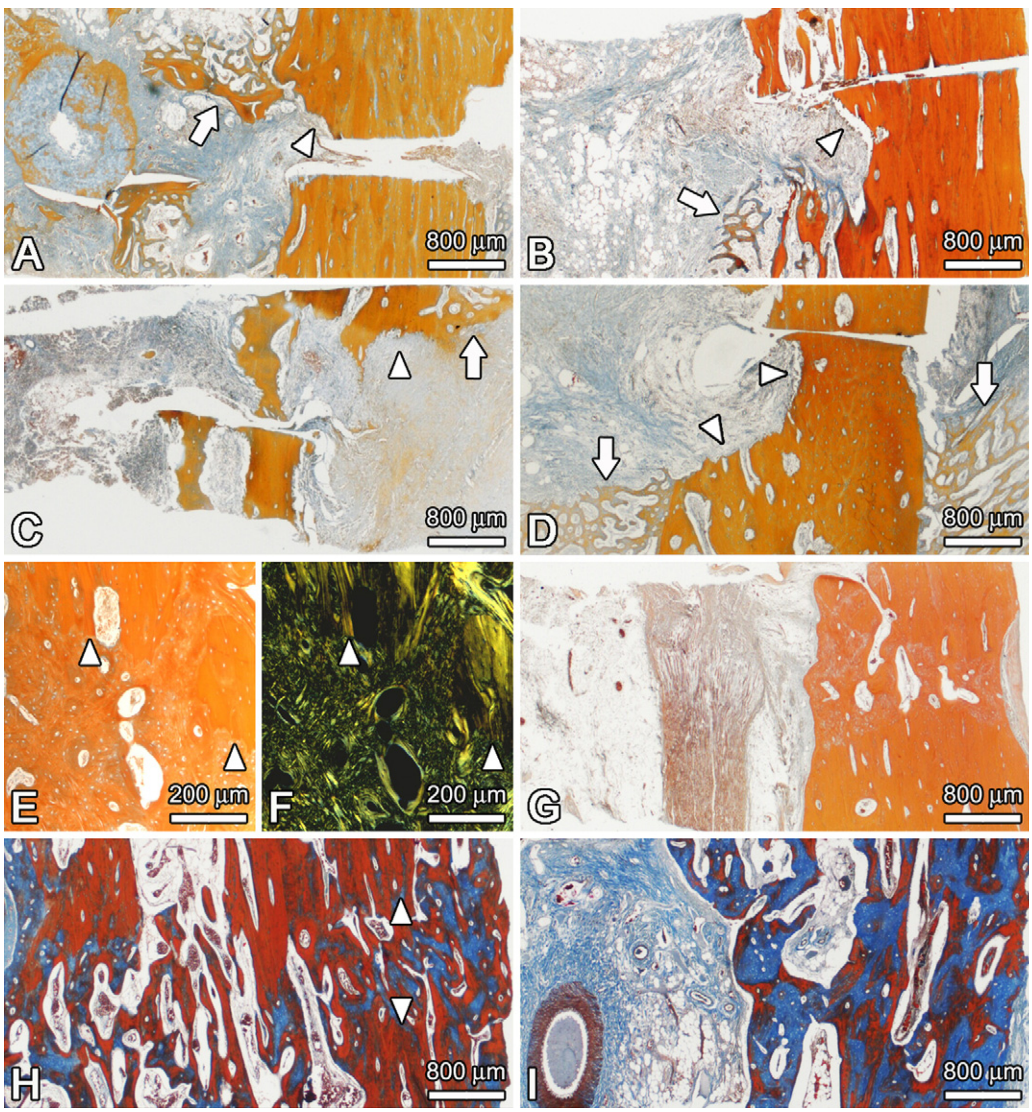

Fig. 4. Conventional (A–E and G–I) and polarized (F) light microscopy of histological sections of 2 week results for metallic TT (A) and CT(B), and absorbable TT(C) and CT (D). 18 week results for metallic TT (E and F) and CT (G), and absorbable TT (H) and CT (I). (A–D) At 2 weeks, the cortical lamellar bone adjacent to the osteotomy site showed areas of bone resorption (arrowheads) and apposition of immature bone (arrows) as a result of the periosteal and endosteal reactions. (E and F) The interface between parent lamellar bone and parallel-fibred bone (arrowheads) at 18 weeks under conventional (E) and polarized (F) light. (G–I) The metallic group showed more compact cortical bone at the osteotomy site at 18 weeks (G). The absorbable group exhibited newly formed bone with typical features of spongy bone (H and I) at 18 weeks. Mallory's trichrome. Objectives: 2.5× for A–D, G–I; 10× for E and F.

Metallic IT shared histological features with metallic TT and CT, as described above.

For absorbable TT, the osteotomy site and the medullary bone area were primarily characterized by loose connective tissue with moderate to intense inflammatory infiltrate and small haemorrhagic foci. The endosteal and periosteal reaction exhibited areas of bone resorption and newly formed immature bone with interconnected trabeculae in a continuum with the parent lamellar bone (Fig. 4C).

Adjacent to the osteotomy site for absorbable CT, the inner surface of buccal and lingual cortical bone exhibited early, immature bone formation and resorption lacunae. The osteotomy site and the medullary bone area showed dense connective tissue with focal areas of inflammatory infiltrate and haemorrhage. Some sections also revealed a periosteal reaction characterized by newly formed immature bone (Fig. 4D).

Absorbable IT shared histological features with absorbable TT and CT, as described above.

18-Week period

Regardless of the IF system applied, the gap created by osteotomy was completely filled with newly formed bone (Fig. 4E–I). For both the metallic and absorbable groups, the TT, IT and CT shared histological features, with the exception of the presence of the mandibular canal in the CT.

For the metallic TT, IT and CT, the bone edges created by the osteotomy were connected by compact parallel-fibred bone exhibiting areas of both immature and lamellar bone and Haversian canals of varying diameter (Fig. 4E–G). The interface between the parent lamellar bone and the newly formed bone was clearly identified under polarized light (Fig. 4E and F).

For the absorbable TT, IT and CT, the osteotomy site was completely filled with bone tissue exhibiting typical features of spongy bone. Interconnected parallel-fibred bone trabeculae defined bone marrow areas filled with highly vascularized connective tissue (Fig. 4H and I). Some early Haversian systems could also be noticed. The interface between the parent lamellar bone and the newly formed bone was visualized under polarized light.

Histometric analysis

2-Week period

The distances between the bone edges created by the osteotomy for the metallic and absorbable groups and the thirds (TT, IT and CT) are presented in Fig. 5. Student's t-test did not reveal any significant differences ($p = 0.67$) among the cortices (buccal and lingual) or thirds (TT, IT and CT). Two-way ANOVA did not reveal any significant differences among the variables analysed, which were the IF system applied ($p = 0.99$), the thirds ($p = 0.07$) and the interaction of the IF system and thirds ($p = 0.66$).

18-Week period

The percentages of newly formed bone in the metallic and absorbable groups and thirds (TT, IT and CT) are presented in Fig. 6. Two-way ANOVA did not demonstrate a statistically significant difference among the thirds ($p = 0.14$); but there were statistically significant differences between the IF groups ($p = 0.01$) and when the data from thirds and two groups were crosschecked ($p = 0.03$).

Discussion

Studies of the absorbable IF system have revealed a series of advantages over metallic systems, including noncorrosive material, easier plate bending, elimination of an additional surgery to remove the system, low incidence of infection, lack of interference with imaging examinations and radiotherapy, gradual transference of the functional stress to the bone (preventing the stress shielding phenomenon) and the absence of cold sensitivity.[1–3,6,19–21,25,27–30] Some disadvantages have also been associated with these systems, such as the high cost, the need for a warming bath for plate moulding and the use of a thread formation device prior to screw insertion, which increases the intraoperative surgical time.[6,20,27–29,31] Some of the properties of the absorbable IF systems have also been discussed, such as resistance, rigidity, stability, quality of bone repair, absorption time, foreign body reaction and treatment predictability.[6,7,19,20,27,28,30] Currently, the absorbable IF system should not be considered a substitute for the metallic system, but an additional alternative in select cases. Some contraindications for the use of absorbable IF systems are cited in the literature, such as alcoholism, smoking, illicit drug use and high risk for failure to follow postoperative recommendations.[6,19,20,27,32]

Although clinical and experimental *in vivo* evaluation has demonstrated good results with absorbable IF systems,[1,2,22–24,27,32] some authors warn that the stability between fragments is still lower than that

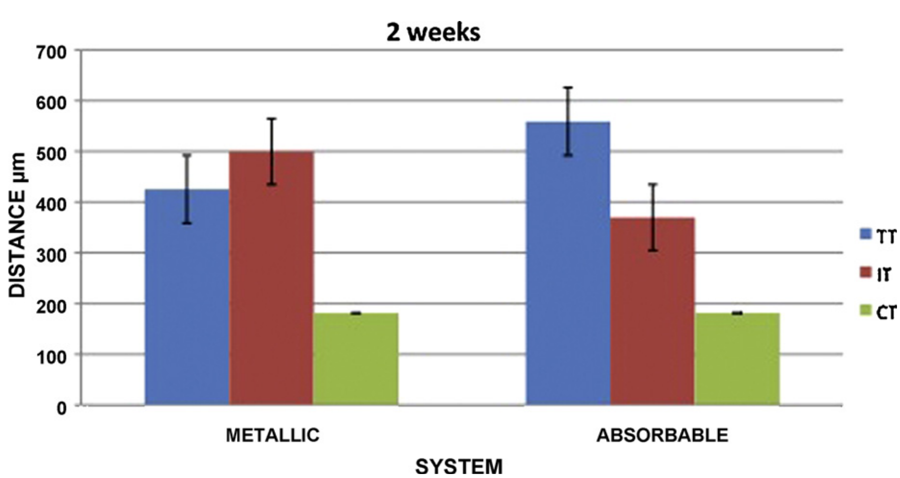

Fig. 5. The mean ± SD of the distance (μm) between the bone fragments at 2 weeks post-treatment for the metallic and absorbable groups and the thirds (TT, IT and CT). No statistically significant differences were detected.

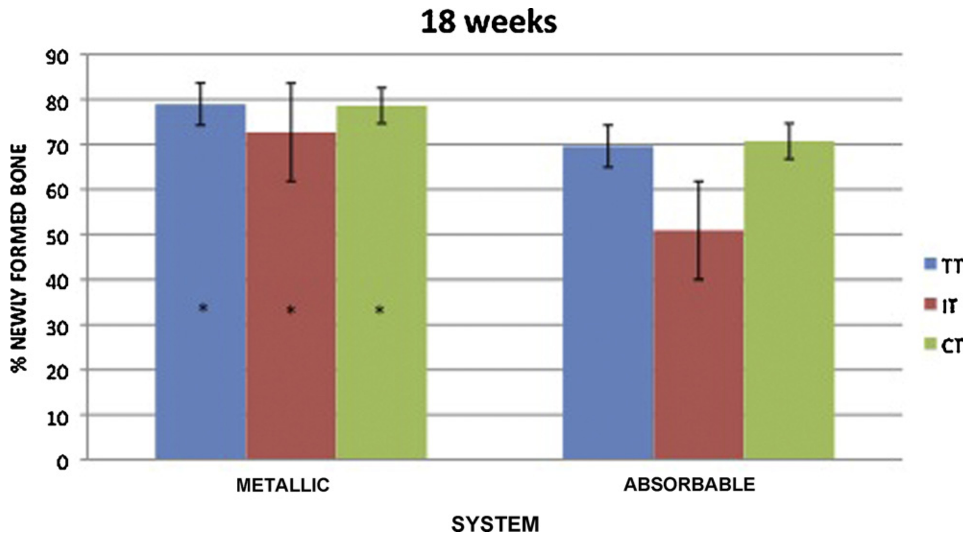

Fig. 6. The mean ± SD of the percentage of newly formed bone at 18 weeks post-treatment for the metallic and absorbable groups and the thirds (TT, IT and CT). Asterisks (*) indicate statistically significant differences at $p = 0.01$.

offered by the metallic systems.[6,7,19–22,24,32,33] In the present study, at the 2-week period, the distances between bone fragments did not differ based on the IF system applied. Nonetheless, moderate mobility between the bone fragments was clinically observed for the absorbable group only. During the weekly assessment, this mobility gradually decreased until it was absent at 4 weeks post-treatment. Therefore, some type of additional stabilization, such as a dental splint, should be recommended with the absorbable IF system during the first weeks to prevent malocclusion and interfragmentary instability.[6,19,20,27,32]

At 2 weeks, qualitative differences between the IF systems were detected in terms of histological features. The findings of increased areas of previous osteoclastic activity and more abundant inflammatory infiltrate in the medullary region, with a more discrete endosteal reaction associated with the cortical bones, for the absorbable group are consistent with those described in both early studies[9–11] and more recent reports.[1,23,25] Despite these differences, the amount of newly formed bone in both groups was insufficient to connect the bone edges created by the osteotomy; thus, the bone repair process was not completed at this point.

At 18 weeks, the newly formed bone filled the osteotomy site, connecting the bone edges created by the osteotomy for both groups. Differences were observed in terms of bone quality and quantity. The metallic group exhibited compact bone and the absorbable group showed spongy bone (23.3% and 36.3% of non-mineralized spaces, respectively), with marrow spaces filled with a highly vascularized connective tissue, which is consistent with the definition of spongy bone.[34–36] Although the presence of woven bone or lamellar bone has been shown in the bone repair area at 18 and 24 weeks in different experimental models,[1,23,24] no description of the quality of the newly formed bone along the osteotomy site has been provided to date.

Previous studies evaluating the pattern of bone repair process after using a metallic IF system showed the occurrence of the gap healing type and, less frequently, the presence of bone callus.[18,37] Bone callus formation has also been reported after the use of absorbable IF systems, and in some cases, the bone callus volume increased on completion of the repair process.[21–23] Differences between buccal and lingual periosteal reactions are expected. According to Reitzik and Schoorl[38] and Rasubala et al.,[39] the enhanced periosteal reaction at the lingual cortical could be due to interfragmentary movements, which were greatest at the lingual site. Conversely, at the buccal site, movements would be restricted by the fixation device to rotation about a transverse axis, resulting in a less periosteal callus formation. Freitag and Landau[40] stated that the amount and distribution of callus varied according to the known mechanical effects of plates and screws on the bone fragments, while Rasubala et al.[39] also considered surgical trauma (i.e. stripping of the periosteum and the surrounding soft tissue) to be an important factor in this phenomenon. In the present study, bone callus formation was observed in both groups, but the periosteal reaction was more marked in the absorbable group.

Although early studies evaluating the use of an absorbable system in the treatment of facial fractures reported complete bone repair process with mature, lamellar bone formation, the presence of multinucleated giant cells has also been described.[9–11] More recent studies of IF systems manufactured with LPLA or DLPLA combined with PGA have revealed a minimal inflammatory process and no foreign body reaction during the first 24 postoperative months. This finding is probably a result of the absorbable polymer applied, which is degraded more slowly than the systems manufactured with only PGA or PLA.[7,14,28,29,32,36,41] The abundance of giant cells and macrophages may represent reduced biocompatibility of the absorbable polymer, and an acute inflammatory process and foreign body reaction may occur.[6,9–11,28] In the present study, no multinucleated giant cells were observed at either 2 or 18 weeks, a finding that could be explained by three hypotheses: the absorbable polymer applied undergoes slow degradation, which prevents the excessive accumulation of residue; the time points evaluated did not span the degradation phase that involves the presence of these cells; and/or the analysed areas were relatively distant from the polymer surface where these cells were present. Some authors have suggested that when LPLA is combined with DLPLA, the giant cells and macrophages appear only 1 year after the surgical procedure.[2,6,21,22]

In conclusion, although the IF systems used here were able to facilitate the bone repair process at the osteotomy site after 18 weeks post-treatment, differences in bone quality and quantity were detected. More compact bone was observed in the metallic group, and spongy bone was observed at the osteotomy site in the absorbable group. Although the low number of animals indicates the need to interpret the statistical analysis with caution, these findings led the authors to question whether they represent different processes, or two phases of the same phenomenon. The authors propose that two different patterns of bone repair occurred, depending on the distinct biomechanical environments provided by the two IF systems. Further studies are needed to evaluate whether the present results can be reproduced consistently and are clinically relevant.

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Competing interests

None declared.

Ethical approval

Institutional Animal Care and Use Committee of the University of São Paulo (Process 07.1.188.53.9).

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