Original Article

Titanium Screw Implants in Optimization of Radiographic Evaluation of Facial Growth in Longitudinal Animal Studies

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Abstract: The aim of this study was to develop a method that optimizes the reliability of longitudinal radiographic evaluation of small and fast-growing animals, such as the rabbit. Because the use of conventional cephalometric methods, including superimposition of serial radiographs, is more problematic in small animals than in humans, two titanium-alloy screws were placed 10 mm apart in the sagittal crest of the parietal bone in 10 growing New Zealand white rabbits. The anterior screw served as holder for a steel pin that, in turn, secured the fixation of the rabbit's head to a specially designed cephalostat. A lateral cephalogram of each animal was exposed on four occasions at one-month intervals. Computer-aided superimpositions were made of all four cephalograms from each animal using the screws in the calvarium as reference structures. To evaluate the method, the superimpositions were repeated after three to eight weeks, and the superimposition reproducibility was calculated. From the results, it can be concluded that the method allows congruent positioning of the animal skull relative to the film-focus assembly at repeated radiographic examinations. Furthermore, it introduces readily identified reference structures in the animal skull that can be used at high-precision superimposition of serial radiographs. (*Angle Orthod* 2004;74:610–617.)

Key Words: Radiographic superimposition; Cephalometrics; Rabbits

INTRODUCTION

In the evaluation of adverse or beneficial effects on facial growth induced by pathological conditions or different treatment modalities, the transfer of data and conclusions from experimental animal studies is commonly indispensable.[1] This can be exemplified by the large number of longitudinal radiographic experimental studies evaluating facial growth in the monkey.[2–5] With concern to accessibility and cost-benefit, alternative animal models have, however, been explored.[6,7] In recent years, an increasing number of publications from various research groups have focused on growth effects on the facial skeleton due to temporomandibular disorders, and in particular the effect induced by temporomandibular joint (TMJ) afflictions. The majority of these studies have been performed as radiographic evaluations in rabbits[8–14] because of the suitability of the rabbit TMJ for studying afflictions of this joint.[15–17] The rabbit is a small animal that shows low relative change in mandibular size from juvenility through adolescence. The size of the rabbit mandible increases only by approximately 15–20% during this period, as compared with a 70–75% increase of the human mandible during the same growth period.[6] Metric analysis of deviations in growth by longitudinal radiographic evaluation in a small animal with low increase in size, such as the rabbit, emphasizes the ultimate reliability of the cephalometric method.

If the deviations in growth to be studied are small, superimposition of the serial radiographs is the preferred method.[18] Such a radiographic evaluation must heed two basic objectives: (1) correct repositioning of the object relative to the film-focus assembly to avoid image distortion due to deviation in placement[19,20] and (2) identification of reference points, planes, or areas, as prerequisites for correct orientation of repeated radiographs relative to each other at superimpositioning.[21] In dental practice and in research that includes humans, the issues of repositioning and superimpositioning reliability are well known and usually not a problem, whereas difficulties occur when small and fast-

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Accepted: October 2003. Submitted: November 2002.

growing animals are studied in experimental models. Limitations due to positioning problems of an anaesthetized animal for repeated radiographic examinations as well as the difficulty in translating human anatomical landmarks to a small animal entail the risk of flawed results.[22,23]

Specially designed cephalostats are commonly used in animal studies.[11,24–27] However, the smaller the experimental animal, the less fit its exterior anatomy to aid accurate replacement. A study design that avoids the repositioning problem, comprehends the sacrifice of animals at different age before the radiographic examination.[9,28–30] Such a longitudinal investigation fails to follow each animal over time, and intraindividual differences in growth cannot be taken into account. Furthermore, inserted metallic implants are frequently used in animal models,[4,11,14,24,31,32] both to serve as readily identified landmarks and as reference points at superimposition. The latter is particularly useful at regional superimposition of single bones, such as the mandible.[1]

The possibility to identify stable reference areas or planes outside the facial skeleton for superimposition is, however, limited in the rabbit model. In humans, accurate superimposition is commonly made on best anatomic fit of the anterior cranial base.[33,34] Although this area is assumed to be a stable configuration in humans from juvenility through adolescence, the anterior cranial base of the rabbit displays a substantial amount of growth during the corresponding period.[35] Superimposition on constructed reference planes as analogues to the anterior cranial base is moreover doubtful because the identification of stable and sufficient anatomical landmarks in the neural cranium may be hard or impossible in the growing rabbit.[22] The use of accurately identified anatomical landmarks in the visceral cranium is not applicable when studying facial growth. In a pilot study, we made tests of superimposition on best anatomic fit of the anterior cranial base and on a constructed reference plane as an analogue to the cranial base, as described by Tavakkoli-Jou et al[14] and Sinsel et al[27], respectively. From our assiduous attempts, we are inclined to perceive that the difficulties to find a reliable and stable reference area representing the anterior cranial base in the growing rabbit is one of the major drawbacks of the model.

The aim of this study was therefore to develop a method that by admitting congruent positioning at repeated radiographic examination and by introducing readily identified reference structures can be used at superimposition to optimize the reliability of longitudinal radiographic evaluation of small and fast-growing animals, such as the rabbit.

MATERIALS AND METHODS

Ten New Zealand white rabbits (*Oryctolagus cuniculus*) were studied, and the study was reviewed and approved by the local ethical committee on animal experiments (registration number A 128-00). The animals were 10 weeks old at the beginning of the study and were allowed to grow for 91–101 days, with a mean study period length of 98.6 days. The study period was therefore defined by the growth period of the rabbit.[25,36] Each animal had two titanium-alloy screws surgically inserted into the calvarium and one tantalum implant inserted in the anterior part of the maxilla. The calvarium was chosen as the site for the titanium-alloy screws for two reasons. First, only a minor amount of growth was expected in this part of the skull, and second, it was chosen to achieve as large a distance as possible between the titanium-alloy screws and the facial skeleton, where the largest amount of growth was expected.[15,31] Repeated radiographic examinations were performed on each animal. During the radiographic examinations the animal was positioned in a specially designed cephalostat,[4,11] modified for this study. The anesthetic, surgical, radiographic, and histological procedures were performed according to the following protocol.

Anesthesia

Insertion of screws and implants was performed under general anesthesia with 0.4 mL Dormicum®, Hoffman-LaRoche AG, Basel, Switzerland, (midazolam five mg/mL) per kilogram of body weight, intraperitoneally administered, and 0.2–0.3 mL of Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium, (fentanyl citrate 0.315 mg/mL, fluanisone 10 mg/mL) per kilogram of body weight, intramuscularly injected.

To achieve local anesthesia subcutaneous injection of 0.3–0.5 mL Citanest Octapressin®, AstraZeneca AB, Södertälje, Sweden, (30 mg/mL + 0.54 μg/mL) was made before incisions where made in the scalp and the alveolar mucosa.

At radiographic examination after one and two months, the animal was anesthetized intramuscularly with Hypnorm® according to the previously described procedure.

At the final radiographic examination after three months, the animal was sacrificed by an intravenous injection of approximately 1.2 mL Pentotal®, Abbott Scandinavia AB, Solna, Sweden, (pentobarbitalnatrium 60 mg/mL) per kilogram of body weight.

Surgery

A two-cm-long sagittal incision was made centrally on the scalp. The sagittal crest of the parietal bone was identified, and two square-fit head, self-tapping, 1.2 × 3-mm titanium-alloy screws (Stryker Corp.) were placed in holes drilled approximately two and 12 mm posterior to the sutura coronalis, respectively (Figure 1). The incision was closed with a continuous 4-0 silk suture. The implant in the anterior part of the maxilla was a tantalum ball with a diameter of 0.5 mm. At insertion of the implant, a minor incision was made in the alveolar mucosa, and an implant

FIGURE 1. Two titanium-alloy bone screws, with square-fit heads placed in holes drilled about two and 12 mm posterior to sutura coronalis of the parietal bones, respectively.

insertion instrument was used (Ole Dich Instrumentmakers, Hvidovre, Denmark).

The anterior screw served as an attachment for a stainless square-fit screwdriver blade (Stryker Corp, Kalamazoo, MI, USA), a steel pin, inserted in the square screw head hole. The steel pin was aimed to secure the fixation of the rabbit's head to the cephalostat.

After the insertion of screws and implants, the animal was given 0.1 mL Temgesic®, Reckitt and Colman, Hull, UK, (buprenorphine 0.3 mg/mL) subcutaneously per kilogram of body weight for analgesia, and approximately 15 mL of saline per kilogram of body weight to prevent dehydration.

For repeated radiographic examinations after one and two months, reincisions were made on the scalp to uncover the anterior screw at fixation to the cephalostat. After sacrifice of the rabbit at the end of the study period, all screws were checked with the screwdriver for stability on lateral movement and were classified as stable or otherwise.

Radiographic examinations and analysis

Lateral cephalograms of each animal were exposed on four occasions: at the beginning of the study, after one month, after two months, and after three months. Hence, a total of 40 radiographs were taken. During the examinations, the animal was placed in a supine position with the left side of the head facing the radiographic focus. The head was fixed in the cephalostat by attaching the steel pin to the anterior screw in the calvarium, with ear pins bilaterally in the external auditory canals, and with a nose fork on the anterior aspect of the nose. At the first examination, the individual angulation (in degrees) of the steel pin and the position (in mm) of its holder were registered with the aid of a protractor and a ruler on the cephalostat. These data were then used at each subsequent examination. The cephalograms were exposed using a Philips Practix dental X-ray unit (Philips, Amsterdam, The Netherlands) with 60 kV, 10 mA, and 0.4 to 0.6-second exposure time. The focus-to-film distance was 100 cm and the object-to-film distance 11 cm, implying an average magnification of the object of about 12%. The film-screen combination was chosen to allow for detailed measurements in the cephalograms and consisted of DuPont Cronex Hi-plus ZJ screens (DuPont, Bad Hamburg, Germany) and CEA blue-sensitive film (CEA, Strängnäs, Sweden).

All cephalograms were digitized in an Agfa Arcus II scanner (Agfa-Gevaert NV, Mortsel, Belgium) with 600 dpi resolution and 16-bit resolution of gray scale. The software used in handling the digitized cephalograms was Adobe Photoshop v. 5.0.2 (Adobe Systems Incorporated, San Jose, CA, USA).

Three tests were performed to evaluate whether the screws in the calvarium could be used as reliable reference structures at superimposition.

The first test was to evaluate whether the geometric structure of a titanium-alloy screw is sufficiently complex to be used at superimposition. Ten cephalograms were randomly chosen, one from each animal, and duplicated in the computer. After adjustments of contrast and density, one of the paired images was inverted regarding gray scale, reduced to approximately 50% transparency, and randomly rotated and displaced relative to the other. Individually created black masks were laid over the 10 pairs of cephalograms, leaving only the posterior screw and approximately one mm of the surrounding bone and soft tissue visible on each cephalogram. The paired images were finally superimposed in each of the 10 cases, only by the aid of the visible posterior screw. The superimpositions were made with up to 15 times magnification on the computer screen. After superimposition was completed, the black masks were removed, and the discrepancy in position of the tantalum implant in the maxilla was measured in each pair of cephalograms.

The second test was to evaluate the reproducibility of superimposition of serial cephalograms, using the posterior titanium-alloy screw in the calvarium as a single reference structure. The images of examinations two, three, and four were superimposed on the image of examination one in each of the 10 animals. Hence, a total of 30 different superimpositions were performed. Every superimposition was then repeated after three to eight weeks. Precision was calculated by measuring the discrepancy in position of the tantalum implant in the anterior part of the maxilla, at each of the repeated superimpositions.

The third test was to evaluate the reproducibility of superimposition of serial cephalograms, using both titanium-alloy screws in the calvarium as paired structures. The 30 superimpositions described above were made again and repeated after three to eight weeks. Precision was calculated as described in the previous paragraph.

The formula used for calculating precision(s) in all three tests was $s = \sqrt{\Sigma d^2/2n}$ where d is the discrepancy in position of the tantalum implant at the repeated superimpositions, measured to the nearest tenth of a mm, and n is the number of superimpositions performed.

Histological examination

To study the screw-to-tissue contact, a parietal bone block containing the paired titanium-alloy screws was removed from the skull of four animals at the end of the study period and stored at 20°C in formaldehyde solution. Histological examination of the bone surrounding the anterior screw, which had served as attachment for the steel pin during the radiographic examinations, was carried out. Using a cutting grinding technique, principally in accordance with the method described by Donath,[37] a section of the selected screws and surrounding bone was obtained with a thickness of 30 μm. The sections were stained with Stevenel's blue.[38]

RESULTS

The insertion of the screws and implants was uneventful, and the repeated skin incisions to uncover the anterior screw at the radiographic examinations were easily performed.

Clinically, all anterior screws but one were stable and resisted sideway forces when tested at the end of the study. It was, however, noticed that all the anterior screws could be rotated within their sockets at the monthly examinations during the study. The posterior screws were never manipulated during the study. At the last examination, all posterior screws were stable but could be rotated within their sockets.

A match between the steel pin and the hole in the head of the anterior screw was achieved in each animal at every examination. The positioning of the skull in the cephalostat was thereby constant relative to the film-focus assembly, giving periodically congruent cephalograms.

A substantial amount of growth was observed in the facial skeleton during the study period. The mean distance from the posterior screw to the tantalum implant in the maxilla increased from 72 mm to 85 mm, implying an average growth of approximately 13 mm in the part of the maxilla where the tantalum implant was situated. No changes in the overall distance between the paired screws in the calvarium were registered (Figure 2). Minor variation in angulation between examinations was, however, noticed in the anterior screw in three of the animals.

The 10 computer-aided superimpositions of the masked pairs of cephalograms revealed a precision of superimposition only by the aid of the geometric structure of a screw, with an s value less than 0.06 mm at the site of the maxillary implant.

The 30 computer-aided superimpositions of serial cephalograms using the posterior screw as a single reference structure revealed a precision of reproducibility of $s = 0.39$ mm at the site of the maxillary implant.

The 30 computer-aided superimpositions of serial cephalograms using both screws as paired structures revealed a precision of reproducibility of $s = 0.41$ mm at the site of the maxillary implant.

The histological examination revealed delicate trabeculae and large marrow spaces of the parietal bone. The neck of the screws was surrounded by fibrous connective tissue, and the apex was in contact with the dura. The larger part of the screws was adjacent to bone marrow and fibrous tissue, whereas direct screw-to-bone contact was noted in minor areas. Occasionally, a slight inflammatory reaction was seen adjacent to the screws (Figure 3).

DISCUSSION

The method presented in this study allows congruent positioning of the animal skull relative to the film-focus assembly at the repeated radiographic examinations and introduces readily identified reference structures in the animal skull that can be used at high-precision superimposition of serial radiographs.

A frequent finding of new bone formation around titanium screws with a mean ratio of direct contact between bone and screws of 64.4% has been reported.[39] Because 60% bone contact or more is required for satisfactory function of dental implants, it should not be possible to unscrew such titanium screws.[40] In this study, it was possible to rotate all anterior screws at the monthly examinations, and after three months at the end of the study, one of the anterior screws was found unstable for lateral movements. Histological examination of the parietal bone block from four of the animals demonstrated bone-to-screw contact only in minor areas. Thus, the examined screws were not osseointegrated during the study period. Only four anterior screws were included in the histological evaluation because there was no intention to study the histological conditions systematically, but merely to illustrate the bone-screw relationship. However, because all 20 screws could be rotated in their socket, it is reasonable to assume that no screw had more than limited bone contact. This limited contact might be due to the vicinity of the screws to the interparietal osteosuture. Another reason could be a discrepancy between

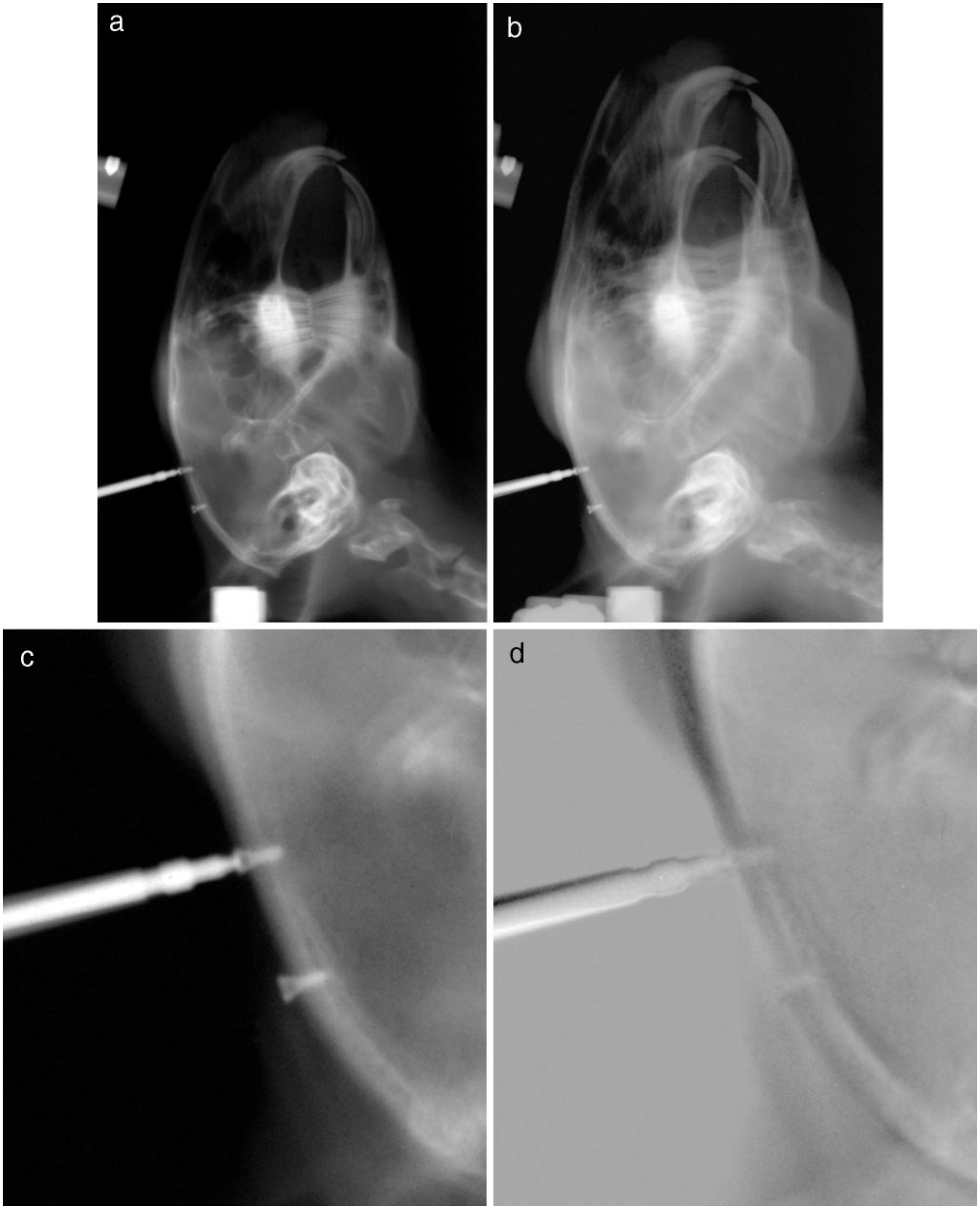

FIGURE 2. Cephalograms using titanium-alloy screws as reference structures. (a) Initial examination. (b) Initial examination and examination after three months superimposed. Note match between screws in spite of major craniofacial growth. (c) Close-up view of screws and steel pin in b. (d) Close-up view of screws and steel pin in b. Examination after three months inverted regarding gray scale. Note the extinction of matching structures.

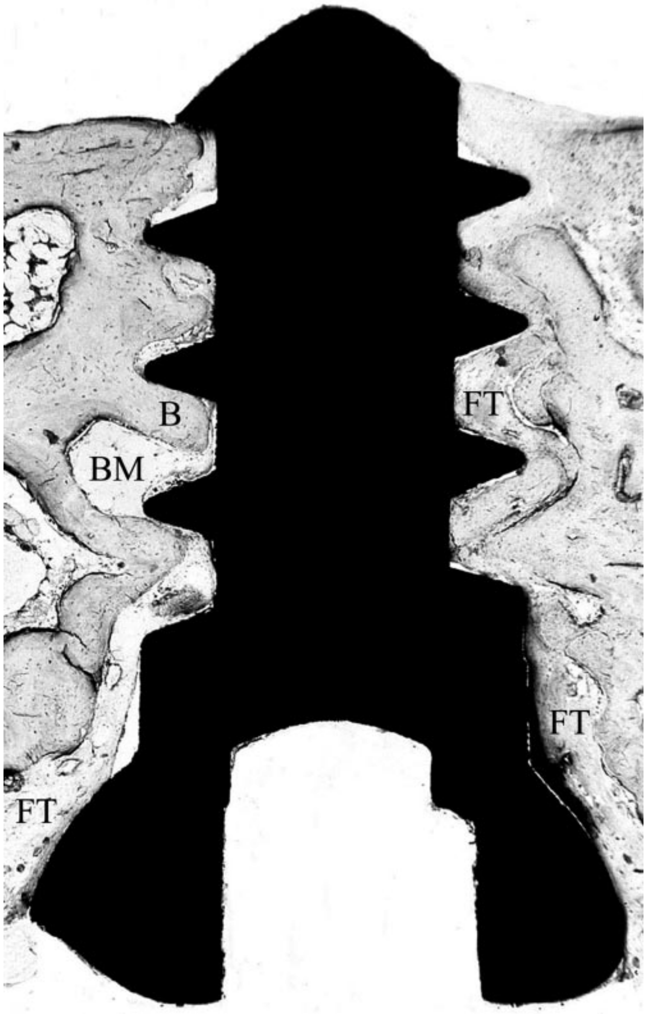

FIGURE 3. Histologic section of a titanium screw in the parietal bone three months after insertion. Apex of screw adjacent to cerebral dura. Fibrous connective tissue (FT), bone marrow (BM), bone (B). Stevenel's blue.

the prepared cylindrical holes in the bone and the conical shape of the screws. Titanium alloy instead of pure titanium might also have had a negative effect on the osseointegration. Irrespective of this, the stability of the screws in this study was sufficient to permit congruent positioning of the animal skull at the repeated radiographic examinations.

The relationship between the screws in the calvarium and the tantalum implant in the maxilla could be described as a triangle where the screws represent the base of the triangle and the maxillary implant is the tip of the opposing angle. When using both screws as paired structures, the length of the base was about 10 mm. This should be compared to a base of only three mm (the length of the screw) when using the posterior screw as a single reference structure. The mean distance from the posterior screw to the maxillary implant among the experimental animals increased from 72 to 85 mm during the study period. The precision of reproducibility was expressed as the discrepancy in position of the maxillary implant at repeated superimpositions. This implies that the precision of about 0.4 mm at the site of the maxillary implant corresponds to dissimilarity in the match of the single posterior screw of about 0.015 mm. From a geometrical point of view, we expected to achieve even higher precision at superimposition with a wider triangle base, ie, using both screws as paired reference structures.

The unexpected equivalence of the s values using one or two screws as reference structures must be interpreted as a poorer match of the paired screws than of the single posterior screw at the repeated superimpositions. Minor positional changes of the anterior screw were in fact registered in some of the animals, and at the end of the study, one of the anterior screws was found unstable for lateral movements. These findings are most likely the result of the observed lack of osseointegration, in combination with the repeated manipulation, and the loading of the steel pin during the examinations.

Direct comparison between the superimposition reproducibility presented in this study and precision or error measurements presented in other studies is difficult. Different studies have used different procedures for calculation or no calculations at all. Sinsel et al[27] reported intraindividual coefficient of variations (CV) ranging from 0.01% to 0.44% in defining metal indicators and anatomical landmarks on lateral cephalograms in rabbits. However, no computations of the difficulty in defining the landmarks constructing the chosen reference plane at superimposition are presented in their study. The influence of the superimpositioning precision in the reported CV of 1.5% as an expression of the reproducibility of the cephalometric procedure is therefore obscure. Tavakkoli-Jou et al[14] visualized growth changes in rabbits evaluated with linear measurement by superimposing on best anatomic fit of the anterior cranial base, although without any calculations of superimposition precision. Rosenberg et al[41] also used superimposition to visualize growth changes in rabbit, however, without any information of the used reference plane, or the precision of superimposition. Other studies have calculated errors of measurement or measurement reliability. Mooney et al[26] reported a 5.45% error of measurement on lateral and dorsoventral cephalograms in rabbits. Masoud et al[25] presented CV of up to 1.3% for linear measurement on lateral and dorsoventral cephalograms in rabbits. Comparison with these studies is furthermore difficult because our study has focused on the reproducibility of superimposition as the first step of the analysis and not on the precision of measurements conducted on the serial radiographs. Alberius et al[22] compared the variability of measurement among cephalometry, osteometry, and stereophotogrammetry in a rabbit model and concluded that differences among studies may be due to the limitations of, in particular, the cephalometric and osteometric techniques. Alberius et al[22] also confirms our opinion that surprisingly many experimental investigations fail to report any computations on this matter or describes them only vaguely.

The s value reported in this study of less than 0.06 mm at the site of the maxillary implant can be interpreted as the accuracy of the method. The larger s value of about 0.4 mm at the site of the maxillary implant expresses the cumulated effect of different errors in the method when used on serial radiographs in a longitudinal model. Furthermore, it can be concluded that the introduced reference structure in the calvarium, ie, the titanium screw, could be defined with a precision of about 0.015 mm in serial radiographs on growing animals, and that the resulting discrepancy of about 0.4 mm in the part of the maxilla where the tantalum implant was situated represents less than a 0.6% error of the distance from the chosen reference structure in the neural cranium, ie, screw in the calvarium, to the area in the visceral cranium where growth can be of interest to register.

CONCLUSIONS

The described method allows congruent positioning of the animal skull relative to the film-focus assembly at repeated radiographic examinations.

Titanium-alloy screws can be regarded not only as metal indicators but also as readily identified and complex geometrical structures that can be used as reference structures at high-precision superimposition of serial radiographs.

ACKNOWLEDGMENTS

We thank Professor Jesper Reibel, Department of Oral Pathology and Medicine, Institute of Dentistry, Faculty of Health Sciences, University of Copenhagen, for help with histological interpretation and histological photographs. This project was supported by grants from The Swedish Research Council, the County Council of Västerbotten, and the Swedish Dental Society.

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