Original article

Implant-based three-dimensional superimposition of the growing mandible in a rabbit model

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Summary

Background: The reliable assessment of craniofacial morphological changes during growth requires invariant regions for image registration. As these regions have not yet been identified in three dimensions, intra-osseous implants are required as fiducial markers for the reliable assessment of three-dimensional (3D) mandibular growth changes. The objective of this study was to develop an animal model for the assessment of the 3D morphological changes of the mandible during growth, using implants as fiducial markers.

Materials and methods: Titanium implants were placed in the body of the mandible of six New Zealand White rabbits. Cone beam computed tomography (CBCT) scans were taken 1-week following implant placement and after an additional 8-weeks of growth. Segmentations of CBCT images were exported into custom-made scripts, implant centroids were identified, implant stability during growth calculated, and the segmented mandibles were registered on the implant centroids.

Results: The buccal cortical bone of the body of the mandible was stable during growth and suitable for fiducial marker placement. Bilateral implants resulted in more accurate rigid registration of the growing rabbit mandible than only unilateral implants. 3D mandibular growth changes were visualised by means of semi-transparencies.

Conclusions: This animal model appears to be feasible for the assessment of the 3D morphological changes occurring during mandibular growth. To the best of our knowledge this is the first time that the implant superimposition method has been combined with 3D imaging to accurately reveal mandibular growth changes.

Introduction

Current knowledge of craniofacial growth is derived largely from clinical studies, where growth changes have been assessed using two-dimensional (2D) lateral cephalograms (1–4). This approach has limited accuracy, is prone to measurement errors (5, 6), and the information obtained is limited by the 2D nature of the image. Nowadays, high-resolution three-dimensional (3D) craniofacial imaging is possible using a multitude of imaging modalities. Furthermore, 3D imaging has been shown to be considerably more accurate than 2D radiographic films for the measurement of TMJ structures (7–9).

A number of different techniques for osseous surface registration of serial 3D craniofacial images of growing patients have been previously suggested (10–14). These techniques rely on invariant regions that remain stable during growth being appropriate for registration, for example the anterior cranial base. However, there is evidence
suggesting that these structures, including the anterior cranial base (15), undergo significant modelling changes during growth, which may result in slight structural changes ultimately invalidating these structures for use as stable regions for registration. Indeed, a complex mosaic pattern of osseous surface modelling of the craniofacial skeleton during growth has been identified (16), which masks subtle, but extremely important, growth changes on osseous surface-based superimposition (17). Furthermore, the mandible is not an integral part of the skull and its position varies with six degrees of freedom even when the mouth is closed in intercuspal position (18). A number of mandibular structures have been suggested to remain stable during growth and to be suitable for registration (19), however, these have also been shown to be unreliable (20, 21). Regional mandibular registration on implants is therefore required to accurately reveal mandibular growth changes (17), and remains the gold standard today.

The aim of this current research was to develop a method for 3D regional mandibular registration using implants as fiducial markers in an animal model, and to produce a fully automated method of rigid registration on implants with no examiner interaction. To the best of our knowledge this is the first time that the implant superimposition method has been combined with 3D imaging to accurately reveal mandibular growth changes.

Materials and methods

Ethical approval for this study was obtained from the Animal Ethics Committee of the University of Otago, New Zealand (61/12).

Fiducial marker selection

Testing for artefact and contrast with cortical bone on cone beam computed tomography (CBCT) of various materials was carried out by drilling osteotomies in the buccal cortical bone of a rabbit mandible and inserting material samples, followed by imaging of the mandible in a Galileos Comfort CBCT unit (Sirona, Bensheim, Germany). The CBCT appearances of the following dental ceramics were tested: sintered and unsintered alumina (InCeram Alumina, VITA, Bad Säckingen, Germany), unsintered zirconia and alumina combined (InCeram Zirconia, VITA), and lithium disilicate (IPS e.max press and IPS e.max CAD, Ivoclar Vivadent, Schaan, Liechtenstein). These were prepared into cubes or discs with a maximum dimension of approximately 1.5 mm, by manually cutting ceramic blocks or pressed ceramic rods. About 1-mm diameter spheres of the following materials were also tested: sintered alumina (Goodfellow, London, UK), grade 5 titanium alloy (R.G.P. International, Milano, Italy), and tantalum (X-Medics, Frederiksberg, Denmark). The CBCT appearance of the various materials was assessed subjectively and the material with optimal appearance was selected for use as the fiducial markers.

Animals

A total of six New Zealand white rabbits (Oryctolagus cuniculus) were used; two males (labelled Rabbits A and B) that were 8-weeks-old at study commencement and four females (labeled Rabbits C, D, E, and F) that were 10-weeks-old at study commencement. During the study period, the animals were kept in individual pens and had access to water and food ad libitum. Body weight was recorded throughout the study period. All six animals had implants placed surgically, to act as fiducial markers for CBCT registration.

Surgical procedure

Anaesthesia was induced using subcutaneous Ketamine (30 mg/kg; Parnell Laboratories NZ Ltd) and Domitor (0.25 mg/kg; Pfizer Animal Health®). Mepivacaine 2% local anaesthetic (Scandonest®) with 1:100 000 adrenaline was administered prior to the surgical incisions, to achieve local anaesthesia and haemostasis. Grade 5 titanium alloy spherical fiducial markers of 1.0 mm diameter (R.G.P. International) were surgically implanted superficially in the buccal cortical bone of the body of the mandible (Figure 1), under general anaesthesia. Prior to performing animal surgery the aim was to place six implants per hemi-mandible. Implants were placed under sterile conditions via extra-oral incisions at the surgical sites, incisions were kept as small as possible while enabling adequate access. An osteotomy was drilled into the bone and the titanium sphere tapped into place, using a custom-made instrument. The area was closed with 5-0 resorbable sutures (©Ethicon Inc., USA). Following the surgical procedures, anaesthesia was reversed using intravenous Antisedan (2.5 mg/kg; Pfizer Animal Health®). Post-operatively the animals were given subcutaneous Carprofen anti-inflammatory analgesic (4 mg/kg; Norbrook®) and enrofloxacin antibiotic (Baytril, 5 mg/kg; Bayer NZ Ltd®) once daily for 3 days.

Three-dimensional imaging

Full head field of view CBCT scans of the animals were taken using a Galileos Comfort CBCT unit (Sirona). A custom-made radiolucent vertical cephalostat was used to support the animal's body and head during imaging, which has been described in a previous publication (22). The initial CBCT scan (T1) was taken 1-week after implant placement surgery and the second CBCT scan (T2) was taken 8-weeks after the T1 scan. At each imaging session, animal sedation was obtained using a subcutaneous injection of ketamine (15 mg/kg, Parnell Laboratories NZ Ltd) and Dormitor (0.25 mg/kg, Pfizer Animal Health NZ Ltd), and was reversed using intravenous Antisedan (1.25 mg/kg, Pfizer Animal Health NZ Ltd) following completion of the CBCT scans. The Galileos CBCT exposure parameters used were 85 kV and 42 mAs (high-contrast mode).

Figure 1. Images to illustrate the implant site. (a) Photograph of a dry rabbit skull, implant site highlighted by black line; (b) CBCT lateral cephalogram reconstruction showing the spatial arrangement and location of the implants at T1 in one rabbit mandible.
Image segmentation and rigid registration

The Sidexis XG software package (version 2.52; Besniheim, Germany) and Galaxis 3D Viewer (version 1.7.2.1; Besniheim, Germany) were initially used to handle and export the scans as Digital Imaging and Communications in Medicine (DICOM) files into TurtleSeg software (version 1.2.2.1776, Andrew Topp) which was used for semi-automatic segmentation. The mandibles and individual implants were segmented from the T1 and T2 CBCT data as separate labels and exported as masks into custom-made software written in C++. This was used for implant mask centroid determination, implant stability estimation, and rigid registration of the mandible masks on the stable implant mask centroids.

To estimate implant stability the Euclidean distance between each pair of implant mask centroids within each scan was calculated, and then compared between the T1 and T2 scans. A threshold of more than 0.3 mm pairwise distance change in Euclidean distance between implant mask centroids was set for exclusion from the registration process. An implant was excluded from the registration process only if the threshold was exceeded between that implant and all of the other implants in the same hemi-mandible. To determine the optimal implants for registration the software then calculated the total summed error in the distances between the implant centroids in the registered T1 and T2 scans, for every possible combination of implants that remained eligible. Using this method the accuracy of fit of all possible implant combinations for rigid registrations was assessed. The implant combination with the smallest summed error was selected for rigid registration.

The chosen implant mask centroids in the T2 CBCT scan were rotated and translated to align with the corresponding implant mask centroids in the T1 scan. An exact match between implant centroids was not possible, as the centroids were not exactly coincident between the T1 and T2 scans, so a method of registration which minimised the sum of squares of residual errors was used. The residual error in this sense was the remaining Euclidean distance between an implant centroid from T1 and the corresponding implant centroid from T2 after the registration transform had been applied. An implementation provided by Visualization Toolkit software (VTK, Kitware Inc., NM, USA) based on the closed-form approach (23), was then used, giving a direct solution rather than an iterative approximation. The transform provided by this method was then applied to the T2 mandible, resulting in the rigid registration of the mandibles using the implant centroids as landmarks. The implementation provided by VTK would also have provided similarity and affine transform solutions if desired and it could have been extended to allow for independent weighting of implant centroid pairs, although this was not used in this study. Rigid registration output files were viewed as semi-transparencies in ParaView software (version 4.1.0, Kitware Inc.) to enable visualisation of growth changes.

Euthanasia, specimen harvesting and preparation

All animals were euthanased 1 day after the T2 CBCT scans using intravenous pentobarbitone (100 mg/kg Pentobarb 300, Provet Pty Ltd, Auckland, NZ) following sedation with Dormitor (0.5 mg/kg; Pfizer Animal Health NZ Ltd). Following euthanasia, the mandibles were dissected from all rabbits, and immediately placed in a sealed container of 10% neutral buffered formalin for two days and were then stored in 20% ethanol. The specimen from Rabbit B was used for histological examination of the bone-implant interface to confirm healing of the implant site without inflammation, thus eliminating inflammation as a potential cause of implant instability. The tissues were processed according to a protocol previously described (24). A single 500 µm section from the mandible of Rabbit B was prepared and after superficial etching and decalcification with 20% ethanol and 1% formic acid in an ultrasonic bath, the section was stained with one part MacNeal’s tetra-chrome and two parts toluidine blue. Light microscopy (Olympus binocular, variable magnification settings) was used to examine the section.

Results

The CBCT appearance of some of the fiducial marker samples tested is shown in Figure 2. Of all the implant materials tested, grade 5 titanium alloy was subjectively determined to have the optimal CBCT appearance, with high contrast with cortical bone and minimal artefact and was selected as the fiducial markers for this study.

Implants were successfully placed in all animals and the scans of mandibles obtained at T1 and T2 could be successfully registered (Figure 3). Two of the animals had five or six implants placed unilaterally and four of the animals had implants placed bilaterally, between three and six implants per hemi-mandible. All animals recovered uneventfully following surgery, there were no post-operative complications regarding healing of the implant sites and all animals completed the study. Mean body weight at the start of the study was 1.8 kg (range 1.7–1.9 kg), and at the end of the study was 3.6 kg (range 3.3–4.0 kg).

Two of the implants placed were unusable for rigid registration, one of which was placed in Rabbit A and was dislodged from the osteotomy into soft tissues, and the other was placed in Rabbit B and was unstable during growth. Excluding these two implants, the mean pairwise distance change between ipsilateral implants was 0.26 mm [standard deviation (SD) = 0.44 mm], whereas the mean pairwise distance change between contralateral implants was 0.81 mm (SD = 0.31 mm). With the exception of the two implants that have already been mentioned, the change in pairwise distances between all other implants was below the 0.3 mm stability threshold for rigid registration.

Rigid registration on segmented implant surface centroids was successfully completed for all animals. An example of a semi-transparency from Rabbit D is shown in Figure 4. Asymmetry was evident in some of the registrations, most notably in the registrations only on ipsilateral implants (Rabbits B and C). Some of the details of asymmetry in the registrations are shown in Figure 5.

Three implants were included in the histological section from Rabbit B (Figure 6); one of these implants had been unstable during growth, while the other two had been stable. The unstable implant was shown to be located deeper than the stable implants, and was surrounded by periodontal ligament cells. The two stable implants in the histological section were located within cancellous bone, and were surrounded by bone marrow cells with areas of direct contact of bony trabeculae with the implant surface.

Figure 2. CBCT sagittal and axial slices of a wet rabbit mandible. Implants are indicated with arrows: (a) sintered alumina; (b) lithium disilicate; (c) unsintered alumina/zirconia; (d) tantalum; (e) grade 5 titanium alloy.
Discussion

The current study has demonstrated the feasibility of an animal model for implant-based 3D superimposition of a growing mandible. Small spheres of titanium were successfully implanted in the mandibles of six rabbits using minimally invasive surgery. The growing mandibles and the spherical implants could be identified and segmented using low radiation CBCT, and the implants could be used as fiducial markers for fully automated rigid registration of the mandibles. A preliminary study was carried out in two rabbits using tantalum implants, which however, resulted in severe artefact on CBCT, thus limiting the accuracy of the registration procedure on implants which could not be fully automated in the preliminary study (22, 25).

A longitudinal study of rabbit mandibular growth (26) found that the main pubertal acceleration in mandibular length increments and intercondylar width increments for all rabbits were between 10 and 14 weeks. They also found additional peaks in acceleration in those same increments in some rabbits at 8 or 16 weeks. Therefore, in an attempt to maximise the amount of mandibular growth during the study period, we decided to begin the study with 8 and 10-week old rabbits.

When selecting the material for the implanted fiducial markers, biocompatibility was imperative, so as to prevent a compromise to the health of the animals (e.g. allergic reaction) as well as to enable a favourable healing response for implant stability. Another requirement was that the CBCT image of the implants was accurately segmentable, that is they needed to have high contrast with the surrounding bone, minimal artefact and be of a size and shape appropriate for the resolution of the CBCT scan. Dental ceramics and some metals were tested as they had the potential to meet these criteria. None of the materials tested completely satisfied all of the ideal requirements for CBCT appearance of fiducial markers in this study; a compromise was required between the level of contrast with cortical bone, and amount of artefact. The 1-mm diameter grade 5 titanium alloy spheres had high contrast with rabbit mandibular cortical bone and the level of artefact on CBCT was considered adequate for the purposes of this study. Grade 5 titanium alloy has shown excellent in vivo biocompatibility following implantation into the rabbit femur (27).

During the planning phase of the study the risk of implant instability during growth was considered to be fairly high, mainly due to the proximity of the implant site to the continuously growing rabbit tooth roots. Also, as perfect coincidence between implant centroids in the T1 and T2 scans was impossible due to discrepancies in implant...
centroid determination combined with transverse mandibular growth and potentially slight implant instability, a best-fit of implant centroids was required for registration. Therefore, although a minimum of three spherical implants were required for rigid registration, the aim was to place six implants per hemi-mandible. This not only maximised the number of implants to facilitate rigid registration even if some implants were unstable, but also reduced the impact of individual implant centroid discrepancies on the accuracy of registration. However, the placement of this number of implants was not achievable in all animals due to anaesthetic complications limiting the duration of anaesthesia available for surgery in some animals. In Rabbit B and Rabbit C only unilateral implant placement was achieved for these reasons.

It was impossible to precisely determine implant stability because there is no stable reference point in a growing animal with which to compare implant position. Therefore, implant stability was estimated, based on the change in pairwise distances between implant mask centroids in the T1 and T2 scans, that is the stability of individual implants was determined in relation to all of the other implants. The threshold for implant stability of less than 0.3 mm change in pairwise distance between the T1 and T2 scans was determined a priori, based on the voxel size of the CBCT machine used in this study. As the change in pairwise distance was higher for contralateral implants (mean 0.81 mm, SD = 0.31) compared with ipsilateral implants (mean 0.26 mm, SD = 0.44), it is likely that the increase in pairwise distance between contralateral implants was mainly due to transverse cartilaginous growth at the symphysis. Therefore, the implant stability threshold was applied only to ipsilateral implants. The changes in pairwise distances between ipsilateral implants were likely to have been largely due to implant centroid determination discrepancies. These discrepancies may have resulted from a number of factors, such as, the small size of the implants in relation to the limited resolution of the CBCT scans, the density of the implants resulting in artefact on the CBCT scans and human/computer error in segmentation of the implant surfaces.

The histological examination showed that the unstable implant in Rabbit B was located deeper than the stable implants and was surrounded by periodontal ligament cells. It is likely that this implant was displaced into the dental follicle and moved due to tooth eruption. Direct contact of bony trabeculae and bone marrow cells with the stable implants confirmed healing without inflammation and, presumably, osseointegration.

Although there were subtle differences between the semi-transparencies from different rabbits and there was asymmetry in some of the semi-transparencies, it was possible to identify a general growth pattern of the rabbit mandible (Figures 3 and 4). In summary, the mandible increased in length by both apposition at the posterior border of the ramus as well as the edges of the incisors moving anteriorly. The antero-posterior length of the ramus increased by apposition posteriorly. The condyle grew upwards and backwards, which together with molar eruption and apposition at the interior border of the mandible, resulted in an increase in the vertical height of the mandible. The incisor region moved downwards and forwards. The transverse width of the entire mandible increased. The upwards and backwards direction of condylar growth evident on the semi-transparencies is supported by previous research that examined rabbit mandibular growth histologically (28), and showed that the condyle grows upwards and backwards by endochondral bone formation in the condyle itself.

Although mild asymmetry in wild-type rabbit mandibular growth has been reported in the literature (29, 30), the asymmetry evident in some of the registrations was likely to have occurred due to registration error and not asymmetrical growth. The error in the registration process was most likely due to discrepancies in implant centroid determination as already described. The effect of these discrepancies on registration was magnified at the peripheries of the mandible because of the proximity of the implants to each other and the distance of them all from the peripheries of the mandible.

Subtle localised growth and modelling changes were evident from the rigid registrations that would have been impossible to detect by using less robust methods of registration without implants. Most of the morphological changes evident from the semi-transparencies corresponded to the histological modelling pattern identified in previous research (28), including the localised area of resorption immediately postero-superior to the incisors (Figure 7). However, the semi-transparencies from this current study showed the inferior portion of the anterior margin of the ramus as resorptive and the superior portion as depository, which is opposite to the histological modelling pattern identified in previous research (28).

Growth changes evident from the semi-transparencies could be quantified by using shape change analysis to identify correspondence between the T1 and T2 mandible surfaces. An alternative method, which has been used to quantify growth changes in orthodontic research (31–33), is the iterative closest point (ICP) algorithm. These two methods both have limitations and result in quite different growth measurements, especially for structures that elongate during growth (e.g. the incisor region of the rabbit mandible), or undergo surface modelling to maintain their shape while growing (e.g. the condyle region). For example, the ICP algorithm would not be accurate for quantifying the incisal edge position change in this study as although the incisal edges moved approximately 20 mm anteriorly, the distance between the incisal edges in the T1 scan and the superior surface of the incisors in the T2 scans were only approximately 4 mm. Whereas the main limitation of using shape change analysis to identify correspondence between surfaces is that correspondence is only estimated and not precisely determined. Tetracycline staining and histological examination may be used in future studies to enable more precise determination of correspondence.

Some important limitations of this study should be acknowledged. Firstly, both general somatic growth and local mandibular growth were likely to have been adversely affected to some extent by the surgical placement of implants, even though the surgical field was restricted to a very limited site. This is a limitation as this animal model was designed to investigate growth. However, the surgical approach to implant placement used in this study seemed to have been

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**Figure 7.** Comparison of modelling changes evident on the semi-transparencies with those found histologically (from Bang and Enlow (28), image redrawn with permission). Plain arrow annotation highlights similarities; Patterned arrow annotations highlight differences. (A) Semi-transparency growth overlay from Rabbit D; (B) Histological modelling pattern (from Bang and Enlow (28), image redrawn with permission), light grey stipple pattern indicates depository surfaces and black areas indicate resorptive surfaces (numbers relate to the original article).
favourable for post-operative recovery as evident from the animal weights, which showed a steady increase throughout the study period. Secondly, the slight asymmetry found in some of the registrations must be interpreted with caution, as it may reflect small registration errors rather than an asymmetric modelling pattern. We can speculate that registration asymmetry would have been vastly reduced if implant centroid determination accuracy was improved by using a higher resolution CBCT or larger implants. The use of larger implants would have required a thicker layer of bone superficial to the tooth roots to prevent them from being displaced by tooth eruption (i.e. a bigger animal, or identification of an alternative location for stable implant placement). Alternatively, guided implant placement may have been used to precisely locate implants between the roots of the teeth.

New methods involving 3D imaging and robust registration techniques, as developed in this study, have many potential future applications in preclinical research and may enable long-standing controversies to be solved, such as the effects of functional appliances on mandibular growth. There is also potential to collect high quality evidence regarding many conditions affecting mandibular growth and their treatments, such as TMJ arthritis or idiopathic condylar resorption, through the use of animal models similar to the one developed in this study. However, the results of animal research have limited applicability to human situations. Previous human research that has been conducted by means of implant-based superimposition techniques has considerably increased our understanding of craniofacial growth mechanisms (1, 17, 19). Ethical restrictions currently limit the application of implant-based 3D registration methods in human studies due to surgical implant placement and the radiation levels associated with CBCT (34). Nonetheless, these restrictions may be overcome in the future by adapting the methods for use with magnetic resonance imaging or if the radiation levels associated with CBCT decline due to technological advancements. Furthermore, the development of minimally invasive injectable resorbable implants would make implant fiducial marker placement in humans purely for image registration more feasible.

Conclusions
This research has successfully developed an animal model for the reliable assessment of mandibular growth in 3D. The buccal cortical bone of the body of the mandible superficial to the tooth roots was stable during growth and suitable for fiducial marker placement. A number of limitations were identified; however, these may be shown to be insignificant in future studies if higher resolution imaging is used. Although a minimum of three stable fiducial markers were required for accurate rigid registration, the placement of more than three implants accommodates for instability of some implants during growth and also may improve the accuracy of rigid registration.

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