Anatomical and mechanical properties of swine midpalatal suture in the premaxillary, maxillary, and palatine region

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The mechanical properties of the midpalatal suture and their relationship with anatomical parameters are relevant for both tissue engineering and clinical treatments, such as in sutural distraction osteogenesis. Soft tissues were dissected from ten swine heads and the hard palate was sliced perpendicularly to the midpalatal suture. Thirteen specimens were collected from each animal and analysed with micro-computed tomography and 4-point-bending for sutural width (Sw), interdigitation (LII), obliteration (LOI), failure stress (σf), elastic modulus (E), and bone mineral density (BMD). Values of the premaxillary, maxillary, and palatine region were compared with Kruskal-Wallis one-way ANOVA and Spearman’s rank coefficient was used to analyse the correlation between parameters and their position along the suture (α = 0.05). LII had values of 1.0, 2.9, and 4.3, LOI had values of 0.0%, 2.5%, and 4.5%, and E had values of 12.5 MPa, 31.3 MPa, and 98.5 MPa, in the premaxillary, maxillary, and palatine region, respectively (p < 0.05). Failure stress and rigidity of the midpalatal suture increased from rostral to caudal, due to greater interdigitation and obliteration. These anatomical and mechanical findings contribute to characterise maxillary growth, and may help to understand its mechanical reaction during loading, and in virtual simulations.

During growth, a system of joints develops at the interface of the adjacent bones of the skull, most of which are a type of synostosis described as “suture”. Sutures allow stress distribution and bone remodelling during growth and through sutural distraction osteogenesis, as documented in human1,2 and swine3. Histologically, human sutures are characterised by a fibrous connective tissue at the interface between the bony fronts i.e., the sutural ligament, composed of several cellular layers and fibres4, whose general structure is similar between man and species such as rabbit, sheep, and swine5,6.

In humans, the hard palate originates during the intramembranous development of the nasomaxillary complex, and is formed by two lateral processes that grow toward the median line, and one anterior part known as primary palate8. Eventually, the hard palate consists of three pairs of bones connected along the midline by the midpalatal suture. This is divided into a premaxillary, a maxillary, and a palatine segment. Subsequently, the midpalatal suture further undergoes morphological changes progressively until adulthood9.

Although animal studies have reported that the midpalatal suture is a type of viscoelastic material10,11, quantitative information is very sparse and research studies had to assume the suture to behave as an empty space12, or to attribute to it the characteristics of other tissues13. Furthermore, studies on humans suggested that mechanical properties may be affected by its rostro-caudal gradient of ossification12,13, that is a relationship apparently still undocumented. Its reaction to loading is also relevant for regenerative medicine since sutural distraction is capable in inducing osteogenesis14, whose potential has found application in clinical treatments such as the maxillary expansion15,16 i.e., the widening of the upper jaw along the midpalatal suture.

The objectives of this study were to assess the change in the morphology and mechanical properties along the midpalatal suture of the swine, testing if any difference was present among the three analysed regions i.e., the premaxillary, the maxillary, and the palatine, and if any correlation existed with the position along the rostro-caudal direction.

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Results

Histology and scanning electron microscopy. Proceeding from rostral to caudal, the general anatomy of the suture in the premaxillary region was a simple linear connection between the bony borders. At the most rostral part, a Y-shaped structure was visible, resulting from the overlapping of different structures. This included the straight connection of the premaxillo-premaxillary suture, the zig-zag pattern of the maxillo-maxillary suture, and the concave profile of the vomer inserting on the premaxilla (Fig. 1G–I). In the maxillary region the suture was progressively serrated, changing to an extended and parallel sinusoidal arrangement (Fig. 1D–F). Further, in the palatine region the sutural complexity was greater, with evident physical interlocking. The two maxillo-palatal sutures were also visible on its left and right side (Fig. 1A–C).

The fibrous ligament of the suture presented five well-identifiable layers in the premaxillary region. In the most rostral part, cambial layers were thick, exhibiting several strata of proliferating osteogenic-like cells and appearing as growth sites. The capsular layers and the loose middle layer comprised of less cells and more fibres connecting the two sides. In this same region, two distinct patterns of fibre orientation were distinguishable: a parallel disposition in the most medial part, and a more radial orientation that was in contact with the bone (Fig. 2G–I). Compared to the premaxillary region, the ligament of the maxillo-maxillary suture was relatively dense with more cells, and the two distinct orientations of the fibres were not identifiable (Fig. 2D–F). The palatal region showed very thin cambial layers with a single layer of cells flattened against the bony surface. The capsular layers and the middle layer were not easily recognisable, forming the main bulk of the suture. The region showed more robust trabeculae and mature bone as indicated by the occasional presence of osteons-like cells (Fig. 2A–C). As a whole, the anatomy and the organisation of the sutural ligament of the premaxillary region were markedly different from the maxillary and the palatine.
Scanning-electron microscopy (SEM) pictures corroborated the histological findings showing a complex sinusoidal interface composed of two opposing and relatively flat fronts facing each other at a distance in the order of hundreds of microns. An intricate matrix of tubular-like fibrillar strings of 1 to 10 µm of diameter emerged converging towards the medial region of the interface following different orientations. In addition, a smooth and relatively uniform layer covered the surface of the fibres, which were not distinguishable as single structures and appeared as a net-like pattern (Fig. 3).

Anatomy. The data were not normally distributed (p < 0.05) and thus non-parametric tests were applied. 
Sw had median values of 1162 µm (IQR = 218 µm) and 1132 µm (IQR = 300 µm) in the premaxillary region, 229 µm (IQR = 98 µm) and 172 µm (IQR = 59 µm) in the maxillary region, and 137 µm (IQR = 50 µm) and 148 µm (IQR = 28 µm) in the palatine, in the axial (SwAX) and coronal (SwCOR) plane, respectively (Table 1, Fig. 4A,B). Sw showed a negative correlation with PosCON of −0.754 (p < 0.001) and −0.443 (p < 0.001), regarding the axial and coronal plane, respectively.

LII had median values of 1.0 (IQR = 0.0) and 1.0 (IQR = 0.0) in the premaxillary region, 2.1 (IQR = 1.2) and 2.9 (IQR = 1.5) in the maxillary region, and 7.3 (IQR = 6.2) and 4.3 (IQR = 1.0) in the palatine, in the axial (LIIAX) and coronal (LII COR) plane, respectively (Table 1, Fig. 4C,D). LII showed a positive correlation with PosCON of +0.718 (p < 0.001) and +0.473 (p < 0.001), regarding the axial and coronal plane, respectively.

LOI had median values of 0.0% (IQR = 0.0%) and 0.0% (IQR = 0.0%) in the premaxillary region, 3.4% (IQR = 6.4%) and 2.5% (IQR = 2.6%) in the maxillary region, and 5.2% (IQR = 3.9%) and 4.5% (IQR = 2.3%) in the palatine, in the axial (LOIAX) and coronal (LOICOR) plane, respectively (Table 1, Fig. 4E,F). LOI showed a positive correlation with PosCON of +0.513 (p < 0.001) and +0.725 (p < 0.001), regarding the axial and coronal plane, respectively.

BMD had median values of 0.54 g/cm³ (IQR = 0.07 g/cm³) in the premaxillary region, 0.65 g/cm³ (IQR = 0.24 g/cm³) in the maxillary region, and 0.67 g/cm³ (IQR = 0.12 g/cm³) in the palatine (Fig. 4J). BMD showed a positive correlation with PosCON of +0.532 (p < 0.001).

In general, besides each inter-group comparison, the anatomy of the premaxillary region resulted to be remarkably different from the others.
Mechanical properties. $E$ had median values of 12.5 MPa (IQR = 11.2 MPa) in the premaxillary region, 31.3 MPa (IQR = 29.6 MPa) in the maxillary region, and 98.5 MPa (IQR = 250.3 MPa) in the palatine (Table 1 and Fig. 4G). $E$ showed a positive correlation with $\text{PosCON}$ of $+0.494 (p < 0.001)$. 

Table 1. Values of the anatomical and mechanical parameters. IQR = interquartile range.

| Parameter  | Premaxillary region (PM) | Maxillary region (MA) | Palatine region (PA) |
|------------|--------------------------|-----------------------|----------------------|
| $S_w_{\text{AX}}$ $\mu$m | median 1162 | 229 | 137 |
| IQR | 218 | 98 | 50 |
| $S_w_{\text{COR}}$ $\mu$m | median 1132 | 172 | 148 |
| IQR | 300 | 59 | 28 |
| $LII_{\text{AX}}$ mm/mm | median 1.0 | 2.1 | 7.3 |
| IQR | 0.0 | 1.2 | 6.2 |
| $LII_{\text{COR}}$ mm/mm | median 1.0 | 2.9 | 4.3 |
| IQR | 0.0 | 1.5 | 1.0 |
| $\text{LOI}_{\text{AX}}$ % | median 0.0 | 3.4 | 5.2 |
| IQR | 0.0 | 6.4 | 3.9 |
| $\text{LOI}_{\text{COR}}$ % | median 0.0 | 2.5 | 4.5 |
| IQR | 0.0 | 2.6 | 2.3 |
| $\text{BMD}$ g/cm$^3$ | median 0.54 | 0.65 | 0.67 |
| IQR | 0.07 | 0.24 | 0.12 |
| $E$ MPa | median 12.5 | 31.3 | 98.5 |
| IQR | 11.2 | 29.6 | 250.3 |
| $\sigma_f$ MPa | median 3.8 | 3.2 | 11.1 |
| IQR | 1.7 | 2.2 | 10.7 |
| $\epsilon_f$ % | median 29.5 | 8.8 | 10.8 |
| IQR | 14.3 | 6.0 | 8.5 |

Figure 3. SEM images showing the coronal plane (rostro-caudal view) of a specimen from the maxillary region. The general view ($35\times$) illustrates the highly interdigitated suture along the triangular-shaped bone with the base facing the oral cavity, and the apex oriented towards the nasal cavity for connection with the vomer (A); bone-ligament-bone interface ($130\times$), (B); sutural ligament ($450\times$) (C); detail of sutural ligament fibres ($1000\times$) (D).
σ_f had median values of 3.8 MPa (IQR = 1.7 MPa) in the premaxillary region, 3.2 MPa (IQR = 2.2 MPa) in the maxillary region, and 11.1 MPa (IQR = 10.7 MPa) in the palatine (Table 1 and Fig. 4H). Although σ_f did not show a significant correlation with PosCON (+0.625, p = 0.144), it had significantly higher values in the palatine region.

Figure 4. Sutural width (Sw) (A and B), linear interdigitation index (LII) (C and D), and linear obliteration index (LOI) (E and F) values in the axial (AX) and coronal (COR) plane. Elastic modulus (E) (G), failure stress (σ_f) (H), failure strain (ε_f) (I), bone mineral density (BMD) (J) values in the three analysed regions. Boxplot parameters: the top line of the upper box (dark grey) represents the 75th percentile (3rd quartile); the line between the upper box and the lower box represents the 50th percentile (2nd quartile); the bottom line of the lower box (light grey) represents the 25th percentile (1st quartile); the top of the upper whisker represents the maximum and the bottom of the lower whisker represents the minimum.
were found among regions (\( p_{LII} \) and 20.5 weeks, respectively)\(^1\), showing changes of sutural complexity in agreement with the present findings. Analysed with histology the interdigitation of the maxillo-maxillary suture in farm pigs and mini pigs of 18 weeks (SD = 0.443 on the coronal plane, \( p < 0.001 \)).


did not show this characteristics (Fig. 2D–F), which were confirmed by SEM imaging showing an unorganised fibrous matrix in the interface (Fig. 3B,C). This net of fibres also presented empty spaces (Fig. 3D), which may facilitate the fluid movement within the ligament during mechanical loading\(^8\), and potentially contributing to the viscoelastic behaviour reported by previous studies\(^9,11\).

Although previous histological reports of the midpalatal suture of both humans\(^4,7,12,13,17\) and animals\(^5,19\) exist in the published literature, the present work on a swine model provides further details on the anatomical variations along the rostro-caudal direction within the same subject.

Anatomy. The \( Sw \) of the maxillary region showed values between 231 \( \mu m \) (SD = 97 \( \mu m \)) and 201 \( \mu m \) (SD = 75 \( \mu m \)) in 18–38yo humans\(^1\), which are similar to the data of the same region assessed on the axial (229 \( \mu m \), IQR = 98 \( \mu m \)) and coronal planes (172 \( \mu m \), IQR = 59 \( \mu m \)) in the present study. Nevertheless, the premaxillary region (\( Sw_{AX} = 1162 \mu m \), IQR = 218 \( \mu m \); \( Sw_{COR} = 1132 \mu m \), IQR = 300 \( \mu m \)) was distinctly wider in the swine model (Fig. 4A,B). Although Knaup et al. reported the \( Sw \) of the midpalatal suture to be similar among different regions in humans\(^13\), the research summarised values of subjects 18–68yo, compared to the longitudinal analysis of the present study that revealed a negative correlation between \( Sw \) and the rostro-caudal gradient (\( -0.754 \) on the axial plane, and \( -0.443 \) on the coronal plane, \( p < 0.001 \)). Accordingly, it is worth noting that the length of the palate of the swine is greater than in humans\(^2\), and this may affect the analysis of the correlation.

The maxillary and palatine regions of the human midpalatal suture develop progressive interdigitation after birth to 18yo\(^7\), measurable through the interdigitation index (II) proposed by Rafferty et al.\(^3,11\), which corresponds to the \( LI_{CCOR} \) described in the present study. Beyond the age-dependency, the present work highlighted a region-dependency of the interdigitation. In fact, the sutures in the premaxilla was simple (\( LI_{CCOR} = 1.0, IQR = 0.0; LI_{LIIAG} = 1.0, IQR = 0.0 \)), whereas the maxillary region (\( LI_{LII} = 2.1, IQR = 1.2; LI_{CCOR} = 2.9, IQR = 1.5 \)) and the palatine region (\( LI_{LII} = 7.3, IQR = 6.2; LI_{CCOR} = 4.3, IQR = 1.0 \)) exhibited a greater complexity. Significant differences in \( LI \) were found among regions (\( p < 0.001 \)) (Fig. 4C,D), with a positive correlation of \( LI \) along the rostro-caudal direction in both the axial (+0.718, \( p < 0.001 \)) and coronal planes (+0.473, \( p < 0.001 \)). Burn et al. analysed with histology the interdigitation of the maxillo-maxillary suture in farm pigs and mini pigs of 18 weeks and 20.5 weeks, respectively\(^19\), showing changes of sutural complexity in agreement with the present findings. They reported increasing values for the anterior, middle, and posterior maxillary region from 5.08 (SD = 1.75) to 6.88 (SD = 1.54) to 7.66 (SD = 1.62), respectively, in animals exposed to soft diet, and from 3.61 (SD = 0.69) to 6.43 (SD = 1.3) to 7.60 (SD = 1.17), respectively, in animals exposed to hard diet\(^19\). Perhaps surprisingly, these values were higher than the \( LI_{CCOR} \) calculated in the present study for older animals, which should be related to greater sutural complexity instead\(^12\). Even so, the sutural length was measured by Burn et al. by tracing the margin of the cortical bone on one side of the interface, rather than along the midline of the interface, which may lead to a more intricate and longer line.

Further, discrepancies found between the present study (\( LII_{LII} = 2.9 \)) and a previous analysis showing lower sutural interdigitation in humans (\( LII_{LII} = 1.4 \)) might be explained by inter-species differences such as the long maxillary complex of the swine, which can be subjected to greater cantilever forces during mastication\(^20\). Nevertheless, a previous animal study suggested that sutural interdigitation was not reduced in swine restricted to soft diet\(^19\), albeit the 12-weeks experimental exposure adopted might have not been long enough to allow macroskopical sutural remodelling.

Despite the fact that growth of the upper face finishes approximately two years before the body height ceases to increase\(^4\), sutural ossification continues beyond completion of growth\(^1,12,13,17,22\). With regard to this, data from the coronal plane (\( LOI_{COR} \) ) of the maxillary region of the midpalatal suture in 15–35yo humans revealed a greater suture obliteration in its caudal part compared to its rostral\(^12\). These results are in agreement with the present findings, which showed a positive correlation of \( LOI_{LII} \) with the rostro-caudal gradient (+0.513, \( p < 0.001 \)). Accordingly, from a visual qualitative evaluation of human dry skulls (\( LOI_{AX} \)) probably 11–68yo, a previous
Mechanical properties. Bone strain can reach 1000 μm during loading of the facial skeleton in mastication, whilst the opening of the midpalatal suture can be even greater than 5.0 mm during maxillary expansion. Such important deformations of the sutural interface make the mechanical behaviour of the sutural ligament and its surrounding bone of biological and clinical interest. Although the midpalatal suture has received historical and anatomical description in humans and animals, and it is the primary structure involved in maxillary expansion in humans and animals, the information regarding its mechanical properties is very sparse and it is probably confined to the analysis of few specimens in animals. This limited knowledge forced authors to design analytical models incorporating values of E from studies testing sutures other than the midpalatal, and to develop numerical models based on either arbitrary values or even representing the suture as an empty space.

In the present study, a decreasing εf (−0.691, p < 0.001) and an increasing E (+0.494, p < 0.001) were observed toward the caudal region of the suture, depicting a more rigid structure (Fig. 4G–I). Accordingly, an increasing sutural interdigitation (LIIAX + 0.718, p < 0.001; LIICOR + 0.473, p < 0.001) and obliteration (LOIAX + 0.513, p < 0.001; LOICOR + 0.725, p < 0.001) were present from rostral to caudal, which is representative of a more interlocked and mature suture.

Furthermore, peculiar mechanical properties arose for each region, suggesting to consider the midpalatal suture to be composed by three segments not only anatomically, but also mechanically. With regard to this, anatomical parameters such as sutural width, interdigitation, obliteration and bone mineral density, resulted to be notably different especially between the premaxilla and the two more caudal regions (Table 1). εf followed a similar trend, which might be explained by the greater width of the premaxillo-premaxillary suture, allowing larger soft tissue deformation during bending (Table 1). Whereas, it was the palatine region to be considerably different from the other two in terms of σf (Table 1), suggesting that the effect of the amount of obliteration on this parameter might not be linear.

Analogies with clinical treatments. In pathological conditions such as craniosynostosis i.e., the premature ossification of cranial sutures, not only obliteration but also mechanical properties have shown differences between synostosed and healthy sutures in humans. Thus, understanding the amount of physiological ossification is important. Further clinical interest in the ossification of the midpalatal suture is related to the maxillary expansion. For example, in humans, 5.0% of obliteration has been regarded as the limit for splitting the midpalatal sutures, which is not reached in most patients under 25 years of age. Furthermore, a non-parallel V-shaped opening of the maxillary halves has been reported by studies on humans, and the rostro-caudal pattern of the mechanical features shown in the present study (Fig. 4) may contribute to formulate research hypothesis about this phenomenon. However, maxillary expansion might be difficult to achieve in some patients despite low LOI. In fact, not only intrinsic sutural properties may be relevant during maxillary expansion, and the hindrance offered by surrounding structures may also restrain the opening of the midpalatal suture in its most caudal area, both in humans and animals.

Limitations. With regard to the estimation of the mechanical parameters, the use of the cross-section (S0) of the specimen may have influenced the value of σf. In fact, although the anterior region may have not been significantly affected, due to the low LII associated with a relatively flat sutural surface, the most caudal region may have received an over-estimation of the σf because of a more extended surface subsequent to a greater interdigitation.
Additionally, \(x_{\text{low}}\) was used for the calculation of \(\varepsilon\) and the deformation was assumed to be evenly distributed along the supported length of the specimen. Conversely, utilising \(S_{w}\) would have led to the opposite approximation to consider all the \(\varepsilon\) to happen within the sutural ligament and none in the bone. As a consequence, the values provided might be more representative of the entire structure rather than the sutural ligament alone, whose real \(\varepsilon\); might be of greater magnitude. Accordingly, \(E\) may be also more illustrative of the composite bone-suture-bone structure, instead of one single constituting material.

Further, although the specimens were considered to behave as linear beams, their shape was irregular. Nevertheless, beside some protruding structures such as in the area of the alveolar processes and the projection of bone-suture-bone structure, instead of one single constituting material.

In the discussion of the mechanical data it should also be considered that specimens from the premaxilla were cut with a different method compared to the other regions. Nevertheless, all specimens were analysed with \(\mu\)CT and no fracture or damage was present before mechanical testing.

Lastly, animal age was estimated according to local regulations on edible animals and by dental-age assessment, and it should be considered as approximate. This said, a domestic swine reach maturity at about the age of 5–6 months\(^{38}\) and a gross inter-species comparison may allow approximately 9 to 12-month-old swine to be analogised to young humans at their last growth stages\(^{37}\) with completed suture growth in the maxillary complex. However, associating the age with changes in the sutural parameters, or achieving translational application to clinical treatments, was beyond the scope of the present study.

**Concluding remarks.** Despite its clinical relevance in craniofacial development and orthopaedic treatments, hardly any information exists on the mechanical properties of the midpalatal suture, which should be analysed as a composite structure formed by three distinct segments.

The midpalatal suture was characterised by reduced elasticity and increased failure stress proceeding towards its most caudal part. The mechanical findings were supported by concordant anatomical features describing a progressively more mature structure along the same gradient. Nevertheless, the conspicuous anatomical and mechanical distinction of the premaxilla compared and contrasted with the rest of the maxillary complex suggests caution in attributing the highlighted differences only to the sutural maturation, since this structure remains simple and almost unossified throughout swine entire ontogeny\(^{38}\).

The present study emphasised the mechano-anatomical topographical variation of the midpalatal suture, on top of the already known age-related changes. These features can be of primary relevance with regard to sutural distraction osteogenesis, and for the understanding of the growth and post-growth modifications of the maxillary complex.

**Methods**

**Sample preparation.** Animals were purchased from the market after slaughter for human consumption, in agreement with regulations of the Committee on the Use of Live Animals in Teaching and Research (CULATR) of the University of Hong Kong. Eleven palates were harvested with high-speed hand-piece from swine heads (approximately 9 to 12-month-old) under water irrigation. One palate was used for histology, and ten for \(\mu\)CT and 4-point-bending (4PB). Samples were sliced parallel to the coronal plane (Fig. 5) via cutting-machine (IsoMet 5000, Buehler\(^{6}\), USA), under water irrigation. Specimens from the premaxilla could not be cut via automatic cutting machine and were sliced with a circular hand saw (9710, Robust\(^{6}\), Hong Kong) under water irrigation. According to its position in the palate, the width of the specimen between its right and left extremity (\(w_{\text{AX}}\), mm) and the width between its nasal and oral extremity (\(w_{\text{COR}}\), mm) were left according to the anatomy, whereas the width between its rostral and caudal extremity (\(w_{\text{ALG}}\), mm) (Fig. 6) was standardised (≈2.5 mm). Alternate specimens were selected for \(\mu\)CT and 4PB, keeping the remaining for SEM (VP-SEM SU1510, Hitachi\(^{7}\), Japan). Thirteen specimens were tested from each palate (Fig. 5B). Specimens were immersed in saline solution (0.9% NaCl), refrigerated at 4 °C, and evaluations were performed within 48 h from the death of the animal.

**Histology.** One palate was fixed with 4% paraformaldehyde (HO(CH₂O)ₙH) for 24 h. After decalcification with formic acid (HCOOH) for five days, \(\approx\)3.0 mm slices were prepared using a surgical blade on the coronal plane. Auto tissue processing (Excelsior ES, Thermo Scientific\(^{8}\), UK) was applied following standard steps in ethanol and xylene. Specimens were embedded in paraffin wax and cut into 6 μm sections using a microtome (RM 2155, LEICA\(^{9}\), Germany) on the same plane. Sections were stained with haematoxylin (Sigma H9627) and eosin (Merck 15935), and mounted with a mounting medium (Fisher Scientific, SP15–500, Permount\(^{10}\)). Images were captured with an optical microscope (Eclipse LV 100 POL, Nikon\(^{8}\), Japan).

**\(\mu\)CT.** \(\mu\)CT scans (SkyScan\(^{11}\)1722, Bruker, US) were acquired at 640 × 512 pixel resolution, 80 kV voltage, 100 mA current, 1° rotation step, and 25 μm pixel size. Two standardised phantoms of hydroxyapatite (\(\text{Ca}_5(\text{PO}_4)_3(\text{OH})\)) with bone mineral density (\(\text{BMD},\ \text{g/cm}^3\)) of 0.25 g/cm\(^3\) and 0.75 g/cm\(^3\) were simultaneously scanned in each acquisition. BMD calibration was obtained for each specimen from the average attenuation coefficient of a volume of one-hundred layers of the \(\mu\)CT of each phantom and a round region of interest with 4.0 mm diameter. BMD was calculated for a cylinder of 6.0 mm length and 1.0 mm diameter oriented on the latero-lateral axis (CTAnalyser\(^{12}\), Bruker, US) after aligning the specimen (DataViewer\(^{12}\), Bruker, US). The length of the suture (\(l\), mm) i.e., the line traced along the midline of the sutural interface, was measured on both planes (axial and coronal) (Fig. 6). The linear interdigitation index (\(\text{LII}, \text{mm/mm}\)) i.e., the ratio between the length of the suture and the width of the specimen, was calculated for both planes (axial and coronal):

\[
\text{LII}_{\text{AX}} = l_{\text{AX}}/w_{\text{AX}}
\]
The sutural width ($Sw$, $\mu m$), i.e., the shortest distance between the two cortical plates enclosing the sutural ligament, was calculated as the average among four equidistant measurements along the suture on both planes (axial and coronal) (Fig. 6). The obliterated length of the suture ($lob$, mm), i.e., the sum of the obliterated segments along the suture, was calculated as the difference between the total length of the suture and the length of the sutural ligament.

\[
LI_{\text{COR}} = \frac{l_{\text{COR}}}{w_{\text{COR}}}
\]

The sutural width ($Sw$, $\mu m$) i.e., the shortest distance between the two cortical plates enclosing the sutural ligament, was calculated as the average among four equidistant measurements along the suture on both planes (axial and coronal) (Fig. 6). The obliterated length of the suture ($lob$, mm) i.e., the sum of the obliterated segments along the suture, was calculated as the difference between the total length of the suture and the length of the sutural ligament.
The suture (where obliterated means that no radiotransparent space was present between the two cortical plates) (Fig. 6), was calculated on both planes (axial and coronal). The linear obliteration index \((\text{LOI}, \text{mm/mm})\) i.e., the ratio between the length of obliterated suture and the total length of the suture, was calculated for both planes (axial and coronal):

\[
\text{LOI}_{AX} = \frac{\text{lob}_{AX}}{w_{AX}}
\]

and

\[
\text{LOI}_{COR} = \frac{\text{lob}_{COR}}{w_{COR}}
\]

The surface of the cross-section of the specimen \((S_0, \text{mm}^2)\) was measured on the sagittal plane (Fig. 6). Anatomical parameters were measured with graphical software (ImageJ39).

Four-point-bending. 4PB tests till failure were performed using a testing machine (Instron® 4444, UK) with a ±100 N load cell (Fig. 5C). Specimens were positioned with the oral side facing the lower span \((x_{\text{low}})\), similar to the bending during maxillary expansion, and loaded at 1.00 mm/min at room temperature \((\approx 25 ^\circ \text{C})\), while kept moist with saline solution spray. Data were acquired at 0.1 KHz recording deflection \((\delta, \text{mm})\) and force \((F, \text{N})\). Specimens were assumed to be solid linear beams with a constant cross-section \((S_0)\). After 4PB, specimens failed along the suture were identified with an optical stereo-microscope (UFX-II, Nikon ©, Japan) at 40 \(\times\) magnification40. Only these specimens were included in the mechanical calculation and only geometry at the failure site was considered \((S_0 = S_{0f}, w_{AX} = w_{AXf}, \text{and } w_{COR} = w_{CORf})\). Because of the static bending, the mass of the beam was assumed to be negligible and only \(x_{\text{line}}\), and not the actual beam length \((W)\), was considered. Because of a variable anatomy on the oro-nasal direction (Fig. 6K), a mathematical \(w'_{\text{COR}}\) rather than the actual \(\mu\text{CT measurement} (w_{\text{COR}})\) was used to better comply with the assumption of uniform \(S_0\):

\[
w'_{\text{COR}} = \frac{S_0}{w_{AX}}
\]

The elastic modulus \((E, \text{N/m}^2)\) was calculated:

\[
E = \frac{F_f}{\delta_f} \left(\frac{2c(3x_{\text{line}}^2 - 4c^2)}{8w_{AX}w'_{\text{COR}}^3}\right)
\]

where \(\delta_f (\text{mm})\) is the deflection at failure.

Failure strain \((\varepsilon_f, \text{mm/mm})\), at \(\frac{1}{2} S_{0f}\) and \(\frac{1}{2} w_{\text{CORf}}\), was calculated:

| symbol | list of the variables |
|--------|----------------------|
| \(F\) | force \(\text{N}\) |
| \(\delta\) | deflection \(\text{mm}\) |
| \(r\) | radius of the roller \(\text{mm}\) |
| \(c\) | horizontal distance between upper and lower roller on one side \(\text{mm}\) |
| \(x_{\text{upp}}\) | upper span length \(\text{mm}\) |
| \(x_{\text{low}}\) | lower span length \(\text{mm}\) |
| \(l\) | length of the suture \(\text{mm}\) |
| \(w\) | width of the specimen \(\text{mm}\) |
| \(W\) | length of the beam \(\text{mm}\) |
| \(S_0\) | cross-section of the specimen \(\text{mm}^2\) |
| \(\text{lob}\) | obliterated length of the suture \(\text{mm}\) |
| \(E\) | elastic modulus \(\text{Pa}\) |
| \(\sigma\) | stress \(\text{Pa}\) |
| \(\varepsilon\) | strain \(\text{mm/mm}\) |
| \(S_w\) | width of the suture \(\mu\text{m}\) |
| \(LII\) | linear interdigitation index \(\text{mm/mm}\) |
| \(\text{LOI}\) | linear obliteration index \(\text{mm/mm}\) |
| \(\text{PosCAT}\) | region of the specimen \(\text{PM, MA, PA}\) |
| \(\text{PosCON}\) | position of the specimen \(1\text{ to }13\) |

Table 2. List of the variables.
\[ \varepsilon_f = \frac{12w'_{\text{COR}}}{(3x_{\text{low}}^2 - 4c^2)} \delta_f \]  

(7)

Failure stress \( (\sigma_f, \text{N/m}^2) \) was calculated:

\[ \sigma_f = F_j \frac{3c}{w_{\text{Ax}}W_{\text{COR}}^2} \]  

(8)

where \( F_j \) (N) is the force at failure.

**Data analysis.** Categories were created for the premaxillary (PM), maxillary (MA), and palatine (PA) region i.e., \( (\text{PM}_{\text{COR}}, \text{MA}_{\text{COR}}, \text{PA}_{\text{COR}}) \), and specimens were also numbered from rostral to caudal (from 1 to 13) i.e., \( (\text{PM}_{\text{COR}1}, \text{MA}_{\text{COR}1}, \text{PA}_{\text{COR}1}) \). Anatomical parameters \( (W, L, \text{LOI}, \text{BMD}) \) were calculated including all the data, whereas mechanical parameters \( (E, \varepsilon_f, \sigma_f) \) were calculated including only data from specimens failed along the suture. Normality of the data distribution was assessed with the Shapiro-Wilk test. The Kruskal-Wallis one-way ANOVA with the Mann-Whitney’s post hoc test were used to compare \( E, \varepsilon_f, \sigma_f, W, L, \text{LOI}, \text{BMD} \) relatively to \( \text{PM}_{\text{COR}} \), and the Spearman’s rank coefficient was used to analyse correlations with \( \text{PM}_{\text{COR}} \). Data analysis was performed with statistical software (SPSS® V23.0, IBM, US) at significance level \( \alpha = 0.05 \).

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**References**

1. Mao, J. J., Wang, X. & Kopher, R. A. Biomechanics of craniofacial sutures: orthopedic implications. *Angle Orthod 73*(2), 128–135 (2003).

2. Savoldi, F., Tsoi, J. K. H., Paganelli, C. & Matinlinna, J. P. The Biomechanical Properties of Human Craniofacial Sutures and Relevant Variables in Sutural Distraction Osteogenesis: A Critical Review. *Tissue Eng Part B Rev*, https://doi.org/10.1089/ten.TEB.2017.0116 (2017).

3. Haas, A. J. Rapid expansion of the maxillary dental arch and nasal cavity by opening the midpalatal suture. *The Angle Orthodontist 31*, 73–90 (1961).

4. Persson, M., Magnusson, B. C. & Thilander, B. Sutural closure in rabbit and man: a morphological and histochemical study. *J Anat 125*, 313–321 (1978).

5. Pritchard, J. J., Scott, J. H. & Girgis, F. G. The structure and development of cranial and facial sutures. *J Anat 90*, 73–86 (1956).

6. Björk, A. Sutural growth of the upper face studied by the implant method. *Eur J Orthod 29*, 882–888 (2007).

7. Melsen, B. Palatal growth studied on human autopsy material. A histologic microradiographic study. *Am J Orthod 68*(1), 42–54 (1975).

8. Herrng, S. W. Mechanical influences on sutural development and patency. *Front Oral Biol 12*, 41–56 (2008).

9. Tanaka, E., Miyawaki, Y., de Pozo, R. & Tanne, K. Changes in the biomechanical properties of the rat interparietal suture incident to continuous tensile force application. *Arch Oral Biol 45*, 1059–1064 (2000).

10. Lee, H., Ting, K., Nelson, M., Sun, N. & Sung, S. J. Maxillary expansion in customized finite element method models. *Am J Orthod Dentofacial Orthop 136*, 367–374, https://doi.org/10.1016/j.ajodo.2008.08.023 (2009).

11. Romanyk, D. L. et al. Towards a viscoelastic model for the unfused midpalatal suture: development and validation using the midsagittal suture in New Zealand white rabbits. *J Biomech 46*, 1618–1625, https://doi.org/10.1016/j.jbiomech.2013.04.011 (2013).

12. Persson, M. & Thilander, B. Palatal suture closure in man from 15 to 35 years of age. *Am J Orthod 72*, 42–52 (1977).

13. Knaup, B., Yildizhan, F. & Wehrbein, H. Age-related changes in the midpalatal suture. A histomorphometric study. *J Orofac Orthop 65*, 467–474, https://doi.org/10.1007/s00056-004-0415-y (2004).

14. Caprioglio, A. et al. Cellular Midpalatal Suture Changes after Rapid Maxillary Expansion in Growing Subjects: A Case Report. *Int J Mol Sci 18*, https://doi.org/10.3390/ijms18030615 (2017).

15. Garrett, B. J. et al. Skeletal effects to the maxilla after rapid maxillary expansion assessed with cone-beam computed tomography. *Am J Orthod Dentofacial Orthop 134*, 8–9 (2008).

16. Provatisid, C. G., Georgiopoulou, B., Kotinas, A. & McDonald, J. P. Evaluation of craniofacial effects during rapid maxillary expansion through combined in vivo/in vitro and finite element studies. *Eur J Orthod 30*, 437–448, https://doi.org/10.1093/ejo/cjn046 (2008).

17. Wehrbein, H. & Yildizhan, F. The midpalatal suture in young adults. A radiological-histological investigation. *Eur J Orthod 23*, 105–114 (2001).

18. Mao, J. J. Mechanobiology of craniofacial sutures. *J Dent Res 81*, 810–816, https://doi.org/10.1177/154405910208101203 (2002).

19. Burn, A. K. et al. Dietary consistency and the midline sutures in growing pigs. *Orthod Craniofac Res 13*, 106–113, https://doi.org/10.1111/j.1601-6343.2010.01483.x (2010).

20. Herrng, S. W., Rafferty, K. L., Liu, Z. J. & Marshall, C. D. Jaw muscles and the skull in mammals: the biomechanics of mastication. *Comp Biochem Phys A 131*, 207–219 (2001).

21. Rafferty, K. L. & Herrng, S. W. Craniofacial sutures: growth and in vivo masticatory strains. *J Morphol 242*, 167–179 (1999).

22. Korbmacher, H., Schilling, A., Püschel, K., Amling, M. & Kahl-Nieke, B. Age-dependent three-dimensional micro-computed tomography analysis of the human midpalatal suture. *J Orofac Orthop 68*, 364–376 (2007).

23. Mann, R. W., Jantz, R. L., Bass, W. M. & Willey, P. S. Maxillary suture obliteration: a visual method for estimating skeletal age. *J Forensic Sci 36*, 781–791 (1991).

24. Grunheid, T., Larson, C. E. & Larson, B. E. Midpalatal suture density ratio: A novel predictor of skeletal response to rapid maxillary expansion. *Am J Orthod Dentofacial Orthop 151*, 267–276, https://doi.org/10.1016/j.ajodo.2016.08.043 (2017).

25. Herrng, S. W. & Teng, S. Strain in the braincase and Its sutures during function. *Am J Phys Anthropol 112*, 575–593 (2000).

26. Haas, A. J. The treatment of maxillary deficiency by opening the midpalatal suture. *Angle Orthod 35*, 201–217 (1965).

27. Angeleri, F. et al. Midpalatal suture maturation: classification method for individual assessment before rapid maxillary expansion. *Am J Orthod Dentofacial Orthop 144*, 759–769, https://doi.org/10.1016/j.ajodo.2013.04.022 (2013).

28. Southard, K. A. & Forbes, D. F. The effects of force magnitude on a sutureal model: a quantitative approach. *Am J Orthod Dentofacial Orthop 93*, 460–466 (1988).

29. Turley, P. K., Shapiro, P. A. & Moffett, B. C. The loading of bioglass-coated aluminium oxide implants to produce sutural expansion of the maxillary complex in the pigtail monkey (Macaca nemestrina). *Arch Oral Biol 25*, 459–469 (1980).

30. Savoldi, F., Tsoi, J. K. H., Paganelli, C. & Matinlinna, J. P. Biomechanical behaviour of craniofacial sutures during distraction: An evaluation all over the entire craniofacial skeleton. *Dent Mater 33*, e290–e300, https://doi.org/10.1016/j.dental.2017.04.025 (2017).
31. Grau, N. et al. Nanostructural and nanomechanical properties of synostosed postnatal human cranial sutures. *J Craniofac Surg* **17**(1), 91–98 (2006).
32. Atac, A. T. A., Karasu, H. A. & Aytac, D. Surgically assisted rapid maxillary expansion compared with orthopedic rapid maxillary expansion. *Angle Orthod* **76**, 353–359 (2006).
33. Wertz, R. A. Skeletal and dental changes accompanying rapid midpalatal suture opening. *Am J Orthod* **58**, 41–66 (1970).
34. Isaksen, R. J. & Ingram, A. H. Forces produced by rapid maxillary expansion. Part II. Forces present during treatment. *Angle Orthod* **34**, 261–270 (1964).
35. Savoldi, F., Tsoi, J. K., Paganelli, C. & Matinlinna, J. P. Evaluation of rapid maxillary expansion through acoustic emission technique and relative soft tissue attenuation. *J Mech Behav Biomed Mater* **65**, 513–521, https://doi.org/10.1016/j.jmbbm.2016.09.016 (2017).
36. Reiland, S. Growth and skeletal development of the pig. *Acta Radiol Suppl* **358**, 15–22 (1978).
37. Margulies, S. S. & Thibault, K. L. Infant skull and suture properties: measurements and implications for mechanisms of pediatric brain injury. *J Biomech Eng* **122**, 364–371 (2000).
38. Herring, S. W. Sutures – a tool in functional cranial analysis. *Acta Anat* **83**, 222–247 (1972).
39. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9**, 671–675 (2012).
40. Baumer, T. G., Powell, B. J., Fenton, T. W. & Haut, R. C. Age dependent mechanical properties of the infant porcine parietal bone and a correlation to the human. *Journal of Biomechanical Engineering* **131**, 111006–111001–111006 (2009).

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**Author Contributions**

F.S. contributed to the design of the study, preparing the samples, performing the experiments, analysing the data, and writing the manuscript; B.X. contributed to preparing the samples, performing the experiments, analysing the data, and drafting the manuscript; J.K.H.T. contributed to the design of the study, provided expert advice, and reviewed the manuscript; C.P. provided expert advice and reviewed the manuscript; J.P.M. provided expert advice and reviewed the manuscript. All authors approved the final version of the manuscript.

**Additional Information**

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