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Stereological Analysis of Bone Architecture in the Pig Zygomatic Arch

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Abstract

Background—Stereological analysis of trabecular bone structure may reveal information about regional variations in stress distribution, especially in areas like the zygomatic arch in which those variations are difficult to assess mechanically. This study investigates regional differences in trabecular orientation, thickness, and density in the zygomatic and squamosal bones of pigs.

Methods—Zygomatic arches were serially sectioned frontally ($n = 4$), horizontally ($n = 4$), or parasagittally ($n = 4$), at a thickness of 0.8 mm. Sections were viewed under a stereomicroscope; video-images were digitized and analyzed with an automated program.

Results—All regions were anisotropic. Predominant orientation of trabeculae differed between and within bones. Three main patterns were seen. Anteriorly, zygomatic trabeculae were mainly arranged vertically and anteroposteriorly (relative to the occlusal plane). Posteriorly, including the jaw joint region, the squamosal featured primarily mediolateral trabeculae. In the midsection of the arch, where the two bones overlap, the trabeculae displayed a predominantly anteroposterior orientation with a secondary mediolateral peak. Trabeculae were typically 0.3–0.4 mm wide and occupied 40–50% of the area of the sections with few regional variations.

Conclusions—Trabecular bone in the pig zygomatic arch is arranged orthogonally, relative to the occlusal plane. In conjunction with information from strain gauge recording, these data suggest that the zygomatic bone is bent in the parasagittal plane whereas the squamosal is bent out-of-plane. The mediolateral trabeculae in the posterior regions are consistent with a cantilever effect at the jaw joint.

Keywords

trabecular structure; facial bones; stereology; pig

An understanding of stress distribution within bones requires three-dimensional information on bone strain and bone material properties. Material properties can be relatively easily obtained by mechanical (Turner and Burr, 1993) or ultrasonic (Ashman et al., 1984; Dechow et al., 1993) techniques for locations that are predominantly cortical. Trabecular bone is more difficult to assess, because the dimensions of the test samples strongly influence measured properties (Linde et al., 1992) and because failure at sample ends biases the overall results (Odgaard et al., 1989). These problems can be overcome if the sample of trabecular bone is large enough to allow strain to be measured specifically from the central section (Ashman et al., 1987; Odgaard et al., 1989; Teng and Herring, 1996).

Unfortunately, there are many cancellous areas in the irregularly shaped flat bones of the craniofacial skeleton that are too small for an adequate sample. Moreover, the orientation of the trabeculae can vary greatly between adjacent areas, suggesting that regional differences, which cannot be assessed experimentally, may be of great significance in the overall architecture of the bones. In such cases the best available technique is textural analysis of bone structure using stereological methods (Cowin, 1985; Goldstein, 1992). Such an analysis of the mandibular condyle of the pig (Teng and Herring, 1995) provided an accurate prediction of actual mechanical properties (Teng and Herring, 1996).

One location where analysis of trabecular architecture may help to elucidate loading is the zygomatic arch, a construct of two flat bones (zygomatic and squamosal) that are primarily cancellous in growing animals. In addition to providing the origin of the large masseter muscle, the arch mechanically links the tooth-bearing maxilla to the jaw joint and braincase. Loading in the arch is far from simple. A recent *in vivo* study of strain in the zygomatic arch of the pig (Herring et al., 1996) revealed the surprising finding that the lateral surfaces of the two component bones are deformed to different degrees and in different directions. Specifically, strain magnitudes were substantially higher in the squamosal bone than in the zygomatic, and the axes of the principal strains were rotated approximately 50°. Reasons for this striking variation are not clear. The two bones of the arch may be loaded differently. Specifically, the squamosal bone, but not the zygomatic bone, may be bent out-of-plane such that its lateral surface becomes more convex. Because strain gauges can be installed only on the lateral surface it is difficult to assess out-of-plane bending. Another possibility is that a relatively less dense and more anisotropic architecture of the squamosal bone was responsible for increased and reoriented strain even in the face of the same applied load. The present study on trabecular architecture was undertaken to investigate these possibilities.

MATERIALS AND METHODS

The heads of twelve pigs aged approximately 4–6 months were obtained from a local slaughterhouse. Their zygomatic arches were removed and cleaned of soft tissues. Each arch was embedded in dental acrylic and serially sectioned at a thickness of 0.8 mm with a diamond saw in either the frontal, horizontal, or parasagittal (referred to below as sagittal) plane (Fig. 1). The frontal and sagittal sections were perpendicular to the occlusal plane, and the horizontal sections were parallel to the occlusal plane. Marrow was removed from each section with a water jet; then the sections were stained with hematoxylin. In order to enhance contrast and remove depth effects, the empty marrow cavities were filled with confectioner's sugar with the use of a small spatula. Sections were mounted, with wax, on glass slides.

These sections were viewed under incident light at 25X (Nikon stereomicroscope, SMZ-U). Images were captured (Panasonic 5100 videocamera) to the computer with a Scitex A/D board from three regions of each bone (Fig. 1). The zygomatic bone was subdivided into anterior, middle, and posterior regions; for frontal sections the middle region was further divided into superior and inferior portions. The squamosal bone was subdivided into a posteromedial region, consisting of the roof of the jaw joint, and posterolateral and anterior regions, which together comprised the zygomatic process of the bone. An effort was made to capture images from the entire region, resulting in a variable number of images (1–10) depending on the size of the region. A total of 482 images were analyzed. Noise was reduced, and images were thresholded and stored digitally as black-and-white TIF files with the use of the program NIH Image 1.52.

Each image pixel was assigned the value of 0 if it was white (i.e., marrow cavities) or 1 if it was black (i.e., trabeculae). A customized automated program, MacAZ, was used to count the total numbers of pixels both within trabeculae and marrow cavities and along the boundaries between trabeculae and marrow cavities. To analyze the orientation of the bony trabeculae, an

edge-tracing algorithm was used. The algorithm examined all strings of six contiguous boundary pixels, using the coordinates of the first and the sixth to calculate a slope (direction) relative to the reference line at 0° (occlusal plane for frontal and sagittal sections, anteroposterior axis for horizontal sections). These slopes, based on 6-pixel distances (0.085 mm), generated 24 possible directions between 0° and 180°, creating bins ranging in width from 5.2° to 9.5°. This method analyzed the entire image rather than a representative sample, and resulted in a frequency distribution of angles for each image. The frequency distribution served to identify the most common orientations of trabeculae in each image.

In order to quantify anisotropy (the degree of nonrandomness of orientation), standard deviations were calculated for the angle frequency distribution of each image (Korstjens et al., 1994). Higher standard deviations imply larger differences in the frequency at the different angles, and thus greater anisotropy. This procedure was validated by running the algorithm on progressively more elongated (more anisotropic) ellipses, with axial ratios from 1.0 to 3.0; axial ratio and standard deviation were almost perfectly correlated ($r = 0.992$).

The density of trabecular bone was calculated for each image by dividing the number of bone pixels by the total pixels of the image. Average trabecular thickness was estimated by dividing the number of bone pixels by half the number of boundary pixels; the quotient was multiplied by the true length of one pixel (0.0141 mm) to achieve an absolute measure.

RESULTS

The pig zygomatic arch consists of zygomatic and squamosal bones, separated by the zygomatico-squamosal suture (Fig. 1). Anteriorly, the zygomatic bone is linked to the maxilla by the zygomatico-maxillary suture, but posteriorly, the articular (posterior medial) region of the squamosal bone is linked to the braincase as a short cantilever. The lateral surface of the zygomatic arch is oriented at about 20–25° to the sagittal plane. A typical section is illustrated in Fig. 2. Most of the arch is composed of trabecular bone, but in the larger (presumably older) arches, the anterior region of the squamosal bone was almost entirely woven compact bone, and the interior of the anterior region of the zygomatic bone was partially excavated by the maxillary sinus.

Trabecular orientation was surprisingly regular in all three planes of the section. Generally speaking, trabeculae tended to be parallel with the occlusal plane or orthogonal to it. However, the predominant orientations differed not only between bones, but also within them. For example, in frontal sections (Fig. 3A), most regions showed a strong peak at 0°/180° (mediolateral and parallel to the occlusal plane); however, the anterior region of the zygomatic bone had primarily vertical trabeculae and the middle-superior zygomatic region peaked at 120°. Similarly, in horizontal sections (Fig. 2, Fig 3B), most regions showed anteroposteriorly arranged trabeculae, but the posterior regions of the squamosal had mediolateral trabeculae instead. Sagittal sections revealed at least three patterns (Fig. 3C). The posterior squamosal regions had relatively evenly distributed orientations, albeit with a minor vertical tendency. The center (anterior squamosal and posterior zygomatic regions) featured anteroposterior trabeculae. The anterior zygomatic region had a strong vertical peak. The middle zygomatic region was intermediate between anterior and posterior zygomatic regions with a peak at 140°.

Data on anisotropy are presented in Table 1 and depicted in Figure 4. If trabecular architecture were isotropic, then the standard deviations of the distribution frequencies would be close to zero. Such a result was never found; all regions had moderate to strong anisotropy, with standard deviations ranging from 0.9 to 2.9. Within regions, there were differences among individual arches, most notably in the posterior zygomatic region. However, all arches showed similar regional variations in anisotropy. Within bones, significant differences among regions

were seen in all comparisons except squamosal bone in horizontal section and zygomatic bone in frontal section. Each region, except for the anterior zygomatic, showed differences in anisotropy among the planes.

Planar and regional variations in anisotropy and directionality are synthesized in Figure 4 and Figure 5. The most anisotropic region (values of 2.5–2.9) in the arch was the posterior part of the zygomatic bone, where anteroposteriorly aligned trabeculae were prominent in both horizontal and sagittal sections. However, all regions were strongly anisotropic (values of 1.5 or greater) in at least one plane of section (Table 1, Fig. 4).

Trabeculae were typically 0.3–0.4 mm wide and occupied 40–50% of the area of the sections (Table 1). In contrast to trabecular orientation and anisotropy, which showed regional variations but were fairly similar among individual arches, thickness and density showed few regional variations (5 of 12 ANOVAs were significant) but did show many differences among individuals (25 of 38 ANOVAs were significant).

In vivo strains are known from the anterior squamosal region and from the body of the zygomatic bone. In order to compare these areas, the zygomatic anterior and middle regions were combined (Table 2). T-tests revealed similar levels of anisotropy in the two areas except for frontal sections, which showed greater anisotropy in the zygomatic body than the anterior squamosal. Trabecular thickness and density were similar in the two areas.

DISCUSSION

In the present study we relied on techniques recently developed for anatomically exploring the lattice structure of trabecular bone (Cowin, 1986; Goldstein, 1992; Korstjens, 1994; Kuhn et al., 1990). This cancellous architecture may be as important as bone density in determining the mechanical properties of trabecular bone (Goulet et al., 1994; Snyder and Hayes, 1990; Teng and Herring, 1996; Turner et al., 1990) and thus is expected to provide valuable information on the loading pattern of the bone. For instance, a parallel, unidirectional trabecular structure is thought to indicate unidirectional loading, and the angle between two crossed systems is thought to indicate the extent of joint movements (Fiala and Hert, 1993).

Our study of the pig zygomatic arch shows a consistent density and robustness throughout the structure, suggesting comparable levels of loading. The architecture of the trabeculae is basically orthogonal, with one predominant and one minor direction in each plane of section. However, there are striking differences in orientation between the two bones and even within each bone, as illustrated in Figure 5. In the posterior portion of the squamosal bone, mediolateral trabeculae predominate, with a minor vertical component. In the central part of the arch where the bones overlap, trabeculae run predominantly anteroposteriorly and secondarily mediolaterally. In the anterior zygomatic region, vertical trabeculae become more prominent, with anteroposterior as the minor component.

Analysis of trabecular architecture permits a choice between the two alternative explanations of why bone strain is different in the two bones of the arch (Herring et al., 1996). The first possibility, that the zygomatic is primarily bent in the parasagittal plane but the squamosal is bent out-of-plane, is supported by the data (see below). The second possibility, that the squamosal is less dense and/or more anisotropic than the zygomatic, is clearly refuted.

The present results on cancellous architecture yield some surprising contrasts with our previous study on surface strain of the zygomatic arch (Herring et al., 1996). Both lines of investigation indicate that the arch does not behave like a simple beam and that stress distribution varies regionally. However, the orthogonal orientation of trabeculae in the interior of the arch does not reflect the oblique orientation of surface strain measured during contraction of the masseter

muscle, the major source of loading on the arch. Below we combine both sources of information in order to construct a more complete model of loading.

In the previous study we recorded strain from the lateral surface of two of the regions studied here: the anterior region of the squamosal bone and approximately the intersection of the anterior and middle parts of the zygomatic bone. The squamosal was under primarily tensile strain, with the major principal axis directed from anteroinferior to posterosuperior. In contrast, the major principal strain (tension) on the zygomatic surface was oriented along the line of action of the masseter, anterosuperior-to-posteroinferior. This bone also showed evidence of bending in the sagittal plane, with the superior edge becoming more concave.

Considering the zygomatic bone first, the relative paucity of mediolateral trabeculae in the anterior and middle/superior regions supports the notion that the body of this bone is bent in the parasagittal plane. The source of this loading is presumably the inferior pull of the masseter on a bone braced by anterior and posterior sutures. The vertical and anteroposterior disposition of trabeculae implies, however, that many of the cancellous struts are loaded in bending or shear.

No strain recordings were made from the posterior region of the zygomatic, but its internal architecture strongly suggests that its loading resembles that of the anterior squamosal rather than that of the zygomatic body. The anteroposterior and mediolateral trabecular orientation in the anterior squamosal and posterior zygomatic regions is consistent with the hypothesis that this part of the arch is bent in the horizontal plane (which is out-of-plane with respect to the parasagittally oriented arch). Such bending would also explain the tensile strain observed on the lateral surface of the squamosal. The source of the bending load may be the medial pull of the musculature or loads transmitted through the jaw joint. As in the case of the zygomatic body, however, the orthogonally arranged trabeculae are not aligned with the principal axes of strain, and so must be under shearing or bending loads.

The most surprising finding of the current study is the predominant mediolateral orientation of trabeculae in the posterior sections of the squamosal, including the articular eminence of the jaw joint. Our earlier studies on the other element of the joint, the mandibular condyle, all suggested vertical loading (Teng and Herring, 1995; 1996). Clearly, the two elements of a joint need not have similar trabecular or stress distributions. In retrospect, the gross anatomy of the pig articular eminence (Fig. 6) also gives evidence that vertical loading will not predominate: (1) this bony plate is narrow superoinferiorly; (2) although the eminence is attached to the braincase medially, its lateral surface is free; and (3) the eminence is not parallel with the occlusal plane but is tilted 15–25° superolaterally. Thus, even a vertical force from the condyle would load the eminence in cantilever bending; this could include a substantial mediolateral component of force.

In conclusion, trabecular architecture provides important complementary data to in vivo strain recordings. In the pig zygomatic arch, the cancellous bone is arranged orthogonally relative to the occlusal plane. All regions are similar in density and trabecular thickness, but vary in anisotropy and in the predominant orientation of trabeculae. The most anisotropic region was the midsection of the arch where the squamosal and zygomatic bones overlap; out-of-plane bending may account for both the trabecular structure and strain patterns observed. Vertical and anteroposterior trabeculae in the anterior region of the arch suggest in-plane bending, whereas mediolateral trabeculae in the posterior region are consistent with a cantilever effect at the jaw joint.

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Fig. 1.

Right zygomatic arch, showing test regions and sectioning planes. **A:** Lateral and slightly superior view. The zygomatic bone is hatched and the squamosal bone is white. **B:** Superior view. Note that the squamosal bone is L-shaped in this view. The transversely oriented posterior medial region forms the articular eminence of the jaw joint. The abbreviations are those used in Figure 2–Figure 4.

Fig. 2.

Right zygomatic arch sectioned in the horizontal plane, showing regions examined. The arrow points anteriorly; lateral is to the right. Sa, anterior region of the squamosal bone, consisting mainly of woven compact bone; Spl and Spm, posterolateral and posteromedial regions of the squamosal; Za, anterior region of the zygomatic bone, mostly occupied by the maxillary sinus (asterisk); Zm and Zp, middle and posterior regions of the zygomatic bone.

Fig. 3.

Trabecular orientation frequency distributions. **A:** frontal sections; **B:** horizontal sections; and **C:** sagittal sections. S, squamosal (dotted lines and open symbols); Z, zygomatic (solid lines and symbols); a, anterior; i, inferior; m, middle; p, posterior; pl, posterolateral; pm, posteromedial; s, superior. Note a tendency for orthogonal orientation ($0/180^\circ$ or 90°) relative to the occlusal plane in all planes of section.

Fig. 4.

Comparison of anisotropy. Results from each plane are indicated by the hatching pattern of the bars. ANOVAs show there are differences (*: $p < 0.05$) among planes in all regions except the anterior zygomatic. Abbreviations above each bar give the most frequent orientation of trabeculae (see Fig. 3). A-P, anteroposterior; M-L, mediolateral; V, vertical. Trabeculae in the middle-superior zygomatic region were oblique in frontal section, as were those in the middle-inferior zygomatic region in sagittal section. Other abbreviations as in Figure 3.

A Lateral / Superior view

B Horizontal section

Fig. 5.

Summary of the trabecular architecture found in the zygomatic arch. The lines show the predominant orientation observed in each plane of section (F, frontal; H, horizontal; S, sagittal). The length of the lines is proportional to the degree of anisotropy. The scale bar indicates a value of 1.0. Except for the middle zygomatic region, trabeculae were oriented orthogonally, i.e., vertically, anteroposteriorly, mediolaterally. In the middle zygomatic region, two exceptions were seen (asterisks in A and B, and dashed line in A; for further details see Fig. 3 and text). **A:** Lateral/superior view, showing anteroposteriorly oriented (visible in H and S planes) and vertically oriented (visible in F and S planes) trabeculae. **B:** Horizontal section,

showing anteroposteriorly oriented and mediolaterally oriented (visible in F and S planes) trabeculae. The two bones of the arch have been separated for clarity

Fig. 6. Suggested loading of the posterior portion of the squamosal bone (articular eminence). Vertical loading from the condyle (C) causes cantilever bending (b) in the eminence, which is mounted on the braincase (bc). This loading condition could result in mediolateral stress within the eminence. Note the angulation of the jaw joint surface is 15–25° relative to the occlusal plane.

TABLE 1

Trabecular anisotropy, thickness and density (mean $\pm$ s.d.) for each plane of section

		Zygomatic											
		Squamosal				ANOVA				Middle			
		Anterior	Post/lat	Post/med	by region	Anterior	Superior	Inferior	Posterior	ANOVA by region			
No. arches		3	4	4	4	4	4	4	4		4	4	4
<i>Frontal: No. images</i>													
		10	34	29		30	19	16	32				
Anisotropy (S.D.) [†]		0.90 $\pm$ 0.23	1.65 $\pm$ 0.38	1.23 $\pm$ 0.29	*	1.54 $\pm$ 0.22	1.80 $\pm$ 0.32	1.17 $\pm$ 0.44	1.30 $\pm$ 0.36	NS			
ANOVA		NS	**	NS		NS	NS	*	**				
Thickness (mm)		0.30 $\pm$ 0.04	0.29 $\pm$ 0.04	0.37 $\pm$ 0.02	*	0.32 $\pm$ 0.01	0.29 $\pm$ 0.05	0.27 $\pm$ 0.04	0.31 $\pm$ 0.04	NS			
ANOVA		*	**	NS		NS	**	*	**				
Density		0.48 $\pm$ 0.05	0.40 $\pm$ 0.03	0.41 $\pm$ 0.03	*	0.46 $\pm$ 0.01	0.50 $\pm$ 0.05	0.38 $\pm$ 0.05	0.47 $\pm$ 0.03	*			
ANOVA		*	*	NS		NS	**	*	NS				
<i>Horizontal: No. images</i>													
		8	34	29		31		34	24				
Anisotropy (S.D.) [†]		1.74 $\pm$ 0.43	1.42 $\pm$ 0.10	1.48 $\pm$ 0.28	NS	1.66 $\pm$ 0.20		1.95 $\pm$ 0.06	2.47 $\pm$ 0.20	**			
ANOVA		NS	NS	NS		NS		NS	NS				
Thickness (mm)		0.36 $\pm$ 0.06	0.30 $\pm$ 0.04	0.30 $\pm$ 0.04	NS	0.29 $\pm$ 0.05		0.28 $\pm$ 0.04	0.34 $\pm$ 0.05	NS			
ANOVA		NS	**	*		**		**	**				
Density		0.45 $\pm$ 0.07	0.40 $\pm$ 0.06	0.41 $\pm$ 0.06	NS	0.41 $\pm$ 0.05		0.38 $\pm$ 0.06	0.42 $\pm$ 0.06	NS			
ANOVA		NS	**	**		**		**	**				
<i>Sagittal: No. images</i>													
		7	36	32		29		27	21				
Anisotropy (S.D.) [†]		1.74 $\pm$ 0.26	1.00 $\pm$ 0.09	0.89 $\pm$ 0.09	**	1.54 $\pm$ 0.27		2.07 $\pm$ 0.32	2.92 $\pm$ 0.91	*			
ANOVA		NS	NS	NS		NS		NS	**				
Thickness (mm)		0.33 $\pm$ 0.04	0.30 $\pm$ 0.05	0.33 $\pm$ 0.05	NS	0.30 $\pm$ 0.05		0.31 $\pm$ 0.03	0.38 $\pm$ 0.01	*			
ANOVA		NS	**	**		**		NS	NS				
Density		0.50 $\pm$ 0.07	0.44 $\pm$ 0.06	0.40 $\pm$ 0.08	NS	0.44 $\pm$ 0.04		0.43 $\pm$ 0.06	0.51 $\pm$ 0.03	*			
ANOVA		NS	**	**		NS		*	NS				
<i>ANOVAs among planes</i>													

Zygomatic											
Squamosal						ANOVA					
Anterior			Post/lat			Post/med			by region		
3			4			4			4		
No. arches											
Anisotropy (S.D.) [†]											
*			*			*			NS		
Thickness (mm)											
NS			NS			NS			NS		
Density											
NS			NS			NS			NS		
ANOVA by region											

TABLE 2

Comparison of anterior squamosal with anterior/middle zygomatic areas

	Frontal plane			Horizontal plane			Sagittal plane		
	Squamosal	Zygomatic	t	Squamosal	Zygomatic	t	Squamosal	Zygomatic	t
n	3	12		3	8		3	8	
Anisotropy (S.D.)	0.9 ± 0.23	1.5 ± 0.41	*	1.74 ± 0.43	1.66 ± 0.2	NS	1.74 ± 0.26	1.81 ± 0.39	NS
Thickness (mm)	0.30 ± 0.04	0.30 ± 0.04	NS	0.36 ± 0.06	0.29 ± 0.05	NS	0.33 ± 0.04	0.31 ± 0.04	NS
Density	0.48 ± 0.05	0.45 ± 0.06	NS	0.45 ± 0.07	0.41 ± 0.05	NS	0.50 ± 0.07	0.43 ± 0.05	NS

t: t-test;

* $P < 0.05$;

NS: not significant.