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# Mechanical behavior of prosthesis in Toucan beak (*Ramphastos toco*)

R.S. Fecchio [a], Y. Seki [b,*], S.G. Bodde [b], M.S. Gomes [c], J. Kolososki [d], J.L. Rossi Jr. [a], M.A. Gioso [a,e], M.A. Meyers [b]

[a] *Laboratory of Comparative Dentistry, LOC-FMVZ-USP, University of São Paulo, Brazil*
[b] *Department of Mechanical and Aerospace Engineering, University of California, USA*
[c] *São Bernardo's Zoo, Brazil*
[d] *College of Industrial Engineering, FEI, Brazil*
[e] *FMVZ-USP, University of São Paulo, Brazil*

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## ABSTRACT

The purpose of this study is to characterize the structure of the beak of Toco Toucan (*Ramphastos toco*) and to investigate means for arresting fractures in the rhinotheca using acrylic resin. The structure of the rhamphastid bill has been described as a sandwich structured composite having a thin exterior comprised of keratin and a thick foam core constructed of mineralized collagenous rods (trabeculae). The keratinous rhamphotheca consists of superposed polygonal scales (approximately 50 µm in diameter and 1 µm in thickness). In order to simulate the orientation of loading to which the beak is subjected during exertion of bite force, for example, we conducted flexure tests on the dorso-ventral axis of the maxilla. The initially intact (without induced fracture) beak fractured in the central portion when subjected to a force of 270 N, at a displacement of 23 mm. The location of this fracture served as a reference for the fractures induced in other beaks tested. The second beak was fractured and repaired by applying resin on both lateral surfaces. The repaired maxilla sustained a force of 70 N with 6.5 mm deflection. The third maxilla was repaired similarly except that it was conditioned in acid for 60 s prior to fixation with resin. It resisted a force of up to 63 N at 6 mm of deflection. The experimental results were compared with finite element calculations for unfractured beak in bending configuration. The repaired specimens were found to have strength equal to only one third of the intact beak. Finite element simulations allow visualization of how the beak system (sandwich shell and cellular core) sustains high flexural strength.

## 1. Introduction

The avian beak is a continuously growing and dynamic vascularized structure composed of bone and keratin separated by a thin germinative dermal layer [1]. The keratinized sheath covering the upper and lower beaks is called rhamphotheca and can be divided into the rhinotheca, or maxillary keratin, and the gnathotheca, or mandibular keratin [2]. The edges of the rhamphotheca are called the tomia, and for the Toco Toucan as for other Toucans as for many other Ramphastids, are serrated or else pigmented to appear serrated or toothed with what was described as "Schaugebiss." [3].

The beak is used for foraging, feeding, social interaction, prehension of food or of nesting material, and in Psittacines, for locomotion [4]. Toucans are also known to engage in bill fencing behavior which is speculated to be an assertion of social dominance [4]. In large Parrots, the complete rhinotheca is entirely replaced in about six months, while in Toucans, the rhinotheca grows approximately 0.5 cm over a two-year period. The direction of growth is away from the dermis in the cranial-ventral plane out to the tomial edges of the bill where stresses due to abrasive wear of the rhamphotheca are presumed to be high [4]. The rate of growth of the gnathotheca is about two to three times faster than that of the rhinotheca [4]. Birds in captivity and in the wild may sustain injuries to the beak in the event of accidental collision, territorial aggression, or vitamin imbalance thus necessitating research of prostheses and reparation of such a vital appendage [4,5].

Understanding the mechanical response of Toucan bill requires investigation of both materials, primarily keratin and mineralized collagen, the conformation of those constituent materials or structure in which they are assembled, and the interaction between the material properties and structural features. Biological composites are for the most part composed of brittle (often mineral) and ductile (organic) components. Mechanical properties of biological composite structures are known to exceed those of the individual constituent materials [6–8]. The sandwich structure—thin, stiff exterior encasing a thick, low-density core—enables high flexural stiffness at low weight, where the requisite low weight presents a constraint for volant birds. Low weight of the bill allows for the Toucan to maintain center of mass in-line with the wings. Mechanical properties and structure of Toucan bill have been studied by Seki et al. [9,10], while methods for reparation of

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* Corresponding author. Tel.: +1 858 543 6091; fax: +1 858 534 5698.
*E-mail address:* yaseki@ucsd.edu (Y. Seki).

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fractured beaks using acrylic resin have been investigated by Fecchio et al. [11].

## 2. Materials and methods

The beaks of Toco Toucan (*Ramphastos toco*) were obtained after the natural death of fully matured hosts and stored at room temperature. Both the maxilla and mandible were used for mechanical tests and structural analysis, although only the maxilla was used for flexure testing. Due to limited specimen availability or inadequate information about the host, no attempts were made to correlate results of mechanical tests or structural characterization with gender or age of host, in this study.

### 2.1. Structural and EDX analyses

For the structural analysis of the rhamphotheca and beak foam, samples were pre-coated with gold palladium and imaged using environmental scanning electron microscopy (FEI, Quanta 600). Energy dispersive X-ray (EDX) spectroscopy was used for composition analysis of rhamphotheca and trabeculae. The 3D structure of the interior foam was imaged by μ-CT (G.E. explore RS rodent CT scanner). The foam section was scanned by μ-CT (unfiltered X-rays) at a resolution of 93 μm. The set of images was used to reconstruct the 3D structure by VTK (Visualization Toolkit) software (Fig. 3(c)).

### 2.2. Hardness testing

Specimen preparation for nanoindentation and microindentation testing was identical. Sections of rhamphotheca on the exterior or trabeculae from the interior foam of Toucan beak were excised by razor blade and mounted in epoxy. The experimental procedure was the same as that employed for hardness measurement of starling beak keratin as implemented by Bonser [12]. A LECO M-400-H1 hardness testing machine and Hysitron nanoindentor were used. The micro-indenter was applied at a load 100 gf for 15 s, and a further 45 s was allowed to elapse before the diagonals of the indentation were measured. Vickers Hardness is determined by the following equation:

$$HV = \frac{0.00018544P}{d^2}[\text{GPa}] \tag{1}$$

where $P$ is applied load (N) and $d$ is the mean length of diagonal (mm). Nanoindentation specimens were polished with 0.05 μm alumina powder. The loads of 500 and 1000 μN. were applied using a Berkovich tip for nanoindentation and the indentation load was sustained for 5 s. The hardness value was calculated according to

$$H_{\text{nanoindentation}} = \frac{P}{24.5h_p^2}[\text{GPa}] \tag{2}$$

where $P$ is applied load [N], and $h_p$ [m] is the depth of the penetration.

### 2.3. Flexural testing

Flexure tests were performed in order to simulate the forces to which the beak is possibly subjected during foraging activities. The flexure tests were performed on specimens from five beaks, removed from Toucans presumed to have died by natural causes, in order to study the forms of fixation with the use of acrylic resin. Flexural testing was conducted using EMIC® universal testing machine, model DL 500 MF, equipped with a 300 N load cell. The proximal extremity of the beaks was immobilized with an epoxy resin while the distal extremity was fixed with a nylon fastener. This nylon fastener was connected to the movable headstock of a dynamometer through a brace of steel. The force was applied in the opposite direction of bite force, so as to imitate resistance presented by the object of bite force. Strain-rate was not varied significantly in this experiment; all tests were conducted at a cross-head speed of 5 mm/min. Fisher's exact test was applied for statistical analysis.

## 3. Results and discussion

### 3.1. Structure of the beak

Fig. 1 depicts a picture of beak structure and typical dimensions at the mid to near caudal cross-section of the beak. Remarkably, the beak comprises 1/3 the length (only the bill of one subspecies of Toucan exceeds that) yet only makes up about 1/30th to 1/40th of the total mass.

The beak of Toucan is a sandwich structured composite with remarkable sub-structure including foam at the interior and tiling or irregular laminate structure on the exterior. Fig. 2 depicts the hierarchical structure of the rhamphotheca from mesostructure by photograph to topical microstructure by scanning electron micrograph and schematic representation. Fig. 2 (b) shows the exterior shell consisting of multiple layers of keratin scales, which are polygonal in shape and superposed or overlapping in arrangement. The thickness of each keratin scale is approximately 1 μm and the diameter is approximately 50 μm (Fig. 2 (c)). At intertile surfaces, viscous adhesive was observed but not successfully characterized in this study. The average total rhamphotheca thickness is 0.5 mm with the thickness exceeding 1 mm at the gonys, tomia, and culmen (Fig. 1). Beak keratin contains a relatively small amount of sulfur [13], and this was verified by EDX. A minimal amount of calcium in the rhamphotheca has also been detected by EDX. These findings are in agreement with results by published by Pautard [14].

Fig. 3 (a) is a photograph of beak cross-section in which the foam (consisting of membranes in a framework of fibers) is visible. Fig. 3 (b) is a scanning electron micrograph of foam in which trabeculae and membranes are observable and from which geometric characterization is possible. Most of the cells in the Toucan bill foam are sealed off by membranes having a thickness of less than 1 μm. Thus, it can be considered a closed-cell system of variable cell size and edge connectivity of three or four. The trabeculae range in thickness from 70 to 200 μm and have circular or elliptical cross-sectional shape (Fig. 3 (c)).

Seki et al. [10] reported the amino acid composition of the Toucan beak foam. Glycine, as is typically found in bone, constituted one fifth of the components by weight. The amino acid results also support the claim that the foam of the Toucan bill is collagen rich. Thus, the foam is mineralized collagen or bone. The trabeculae were found to have a Young's modulus twice as high as that of rhamphotheca; this disparity may be explained by the high calcium content of the trabeculae.

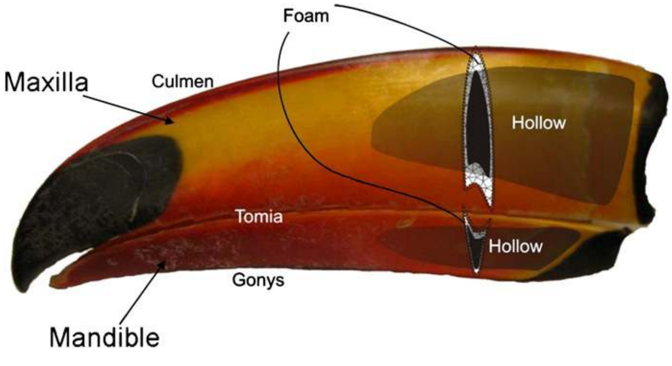

**Fig. 1.** Representation of Toco Toucan beak with different components. The median dorsal border of the rhinotheca is called the culmen, and the median ventral border of the gnathotheca is called the gonys.

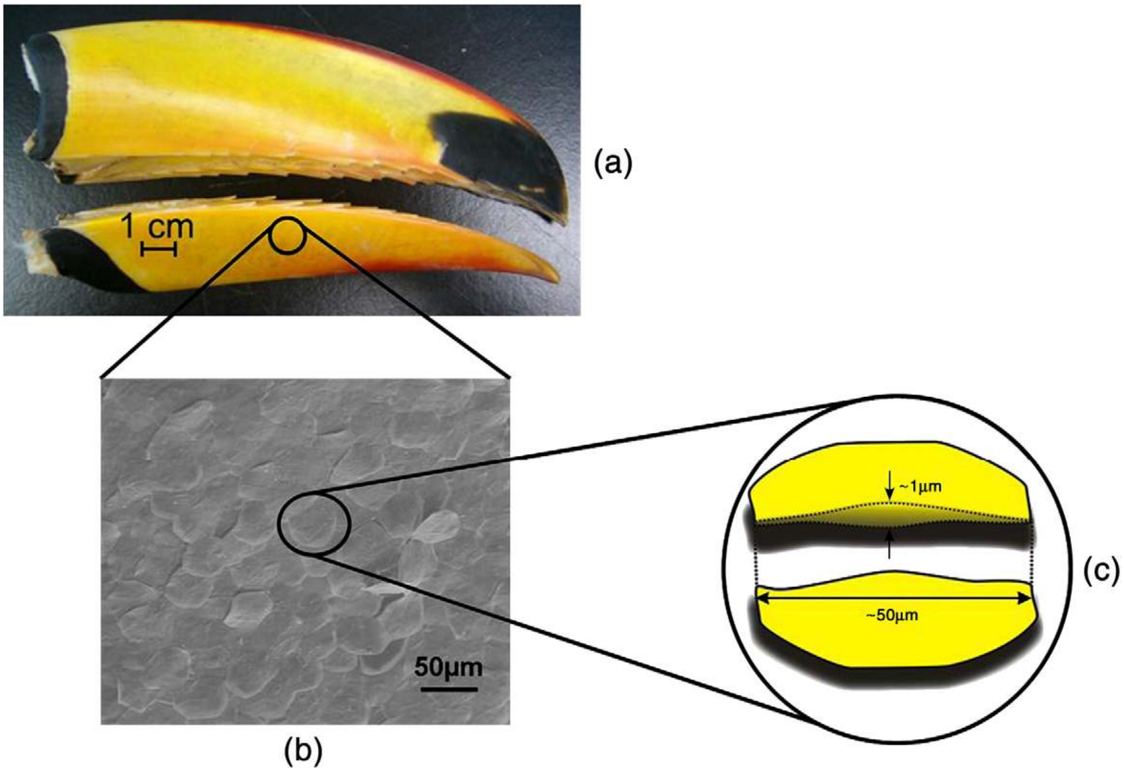

**Fig. 2.** Structure of rhamphotheca (a) photograph of beak; (b) scanning electron micrograph of exterior keratin; and (c) a schematic of each keratin scale.

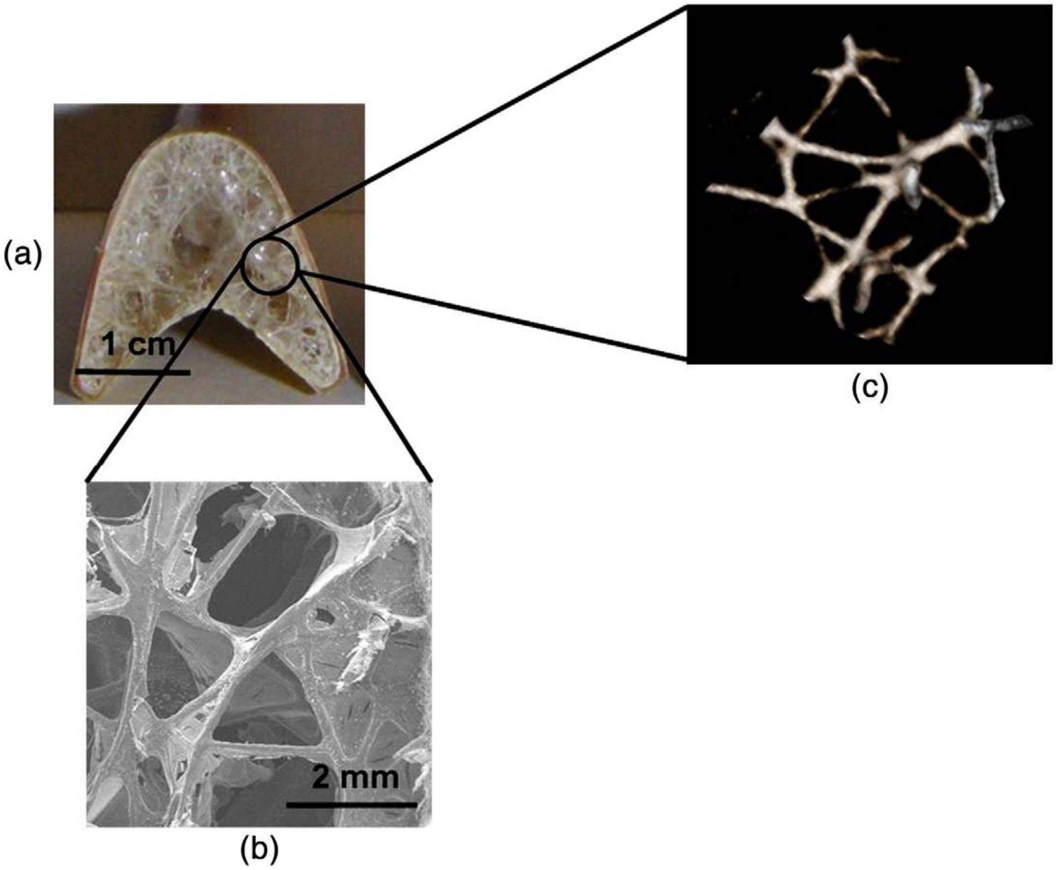

**Fig. 3.** (a) Photograph of beak cross-section in which foam (consisting of membranes in a framework of fibers) is viewable; (b) Scanning Electron Micrograph of foam (b) and (c) 3-D visualization of structure of trabeculae constructed from computed tomography (CT) images.

### 3.2. Hardness of the beak

The plot in Fig. 4 represents the micro- and nanohardness of rhamphotheca (beak keratin) and trabeculae. The hardness of trabeculae is higher compared to that of rhamphotheca in both micro- and nanoindentation. The hardness from nanoindentation is approximately double with that deduced from microindentaion measurements. The microhardness of beak keratin is 0.22 ± 0.01 GPa and that of trabecula is 0.28 ± 0.03 GPa. The nanohardness is consistently higher than microhardness of either component of the bill, and this is believed to correspond to a size effect and scale of mineral interactions. The nanohardnesses of beak keratin and trabeculae are 0.48 ± 0.06 GPa and 0.55 ± 0.12 GPa, respectively. The hardness of trabeculae is higher than that of rhamphotheca probably due to increased mineral content.

### 3.3. Rhinotheca prosthesis

The flexure tests were conducted in order to investigate forms of fixation with the use of acrylic resin. In each trial, the proximal extremity of the beaks was immobilized in epoxy resin while the distal extremity was held with a nylon fastener. This nylon fastener was connected to the movable headstock of a dynamometer (EMIC DL500MF) through a brace of steel (Fig. 5 (a)). The intact beak was fractured in the central portion (Fig. 5 (b)) when subjected to a force of 270.4 N, with displacement of 23.3 mm. The application of force, while center aligned with the axis along which bite force is expected to be exerted during the act of plucking fruit, for example, does not simulate the natural loading condition that might be experienced during bill fencing, for example.

All other bill specimens were treated with resin according to varied protocol or else at varied regions on the beak. All force–displacement data are plotted in Fig. 6. The second beak was resin treated on both lateral surfaces and fractured at 70 N at a displacement of 6.4 mm. The third beak was conditioned in acid for 60 s before application of resin, and it resisted a force of up to 63.3 N at a displacement of 6 mm. The other two tests were performed on the fourth and fifth maxillary beak specimens, the entire lateral rhinotheca (excluding the palate) of which were coated in resin. The fourth beak, which was not pre-conditioned in acid, resisted up to 134 N with displacement of 12.6 mm and, the fifth maxillary beak sample after undergoing acidification resisted up to 102 N with a displacement of 9.7 mm.

Finite element calculations were performed using LS-DYNA for modeling the bending behavior of unfractured, intact maxilla. The force–displacement response was modeled using an elastic-plastic constitutive equation with kinematic hardening (material model 3) for shell and the crushable foam model (material model 63) for interior foam. We used a Young's modulus of 1.5 GPa and yield stress of 120 MPa for shell and assumed a uniform thickness of 1 mm. The Young's modulus for the foam was taken as 5 MPa. The calculation predicted lower buckling force and displacement than the values verified by experiment. This discrepancy may be accounted for by differences in geometry of actual beak specimens compared to that of the CAD based beak model and non-uniformity in shell thickness that was not reproduced in the model. The bending behavior of the beak is

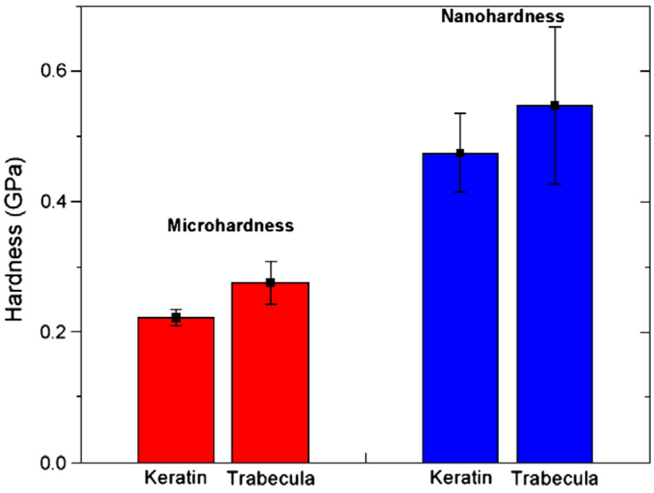

**Fig. 4.** Micro- and nanoindentation hardness of Toucan beak keratin and trabeculae.

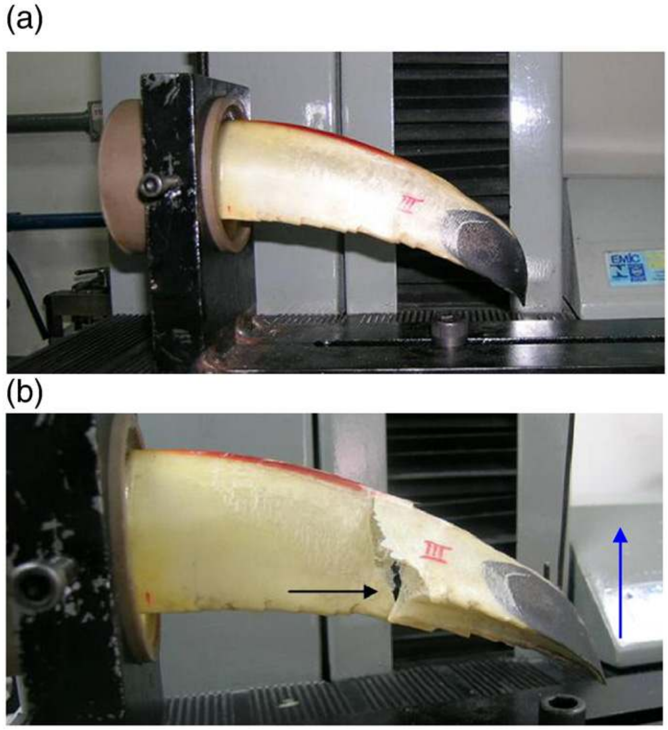

**Fig. 5.** Flexure testing configuration of beak maxilla with repair (a) immobilized proximal end prior to testing; (b) after fracture (cracked indicated by arrow). The blue arrow indicates the direction of applied force.

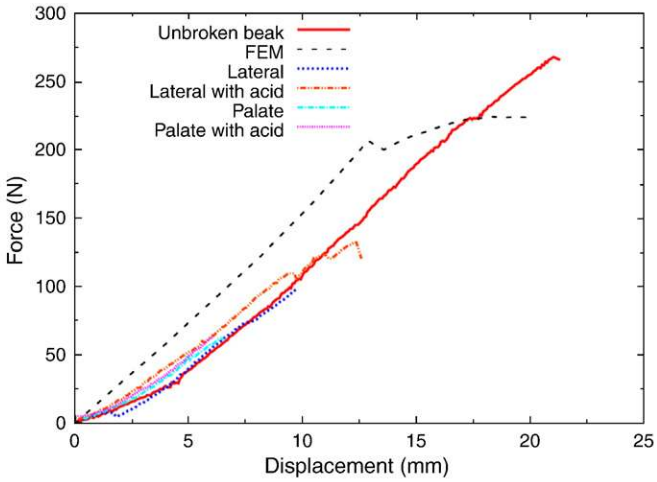

**Fig. 6.** Experimentally determined and calculated (by FEM) force (N) vs. displacement (mm) curve for the maxilla, the maxilla from FEM calculation, the maxilla reinforced laterally with resin, maxilla reinforced laterally after acidification, maxilla in which entire rhinotheca inclusive of the palate was reinforced with resin, and maxilla in which palate rhinotheca was reinforced after acidification.

dominated by the shell, in this case, the rhamphotheca and surface treatment techniques thereof, whereas foam stabilizes the deformation and resists the buckling of the beak during bending. Testing and simulation of loading in different orientations relative to the longitudinal axis of the bill should be completed in subsequent studies.

## 4. Conclusions

The following are the principal conclusions.

The Toucan beak can be modeled as a sandwich structured composite. The external shell, or rhamphotheca is composed of keratin scales with a diameter of approximately 50 μm and thickness of 1 μm. These keratin scales are fixed by an organic adhesive in a staggered pattern, leading to a total thickness of 0.5 mm on average. The low-density core of the beak, or trabecular foam, is a closed-cell foam. The trabeculae are composed of mineralized collagen. In addition to the composite construction, the beak has substructural elements that were tested in this study as well. The hardness of trabecula exhibits higher hardness than that of rhamphotheca.

Statistical correlation between results in flexure trials could not be reported, as different procedures were employed for each sample. Furthermore, force data as a function of displacement for the beak modeled by FEM are discrepant from all experimental trials. Nevertheless, qualitative behavior of the structure in flexure was reproducible as fracture occurred in the center of the bill in experimental trials as well as in the FEM simulation. Fixation at the palate, with or without acidification, consistently sustained lower forces than when fixation occurred at lateral regions of the rhinotheca, excluding the palate. Repaired specimens pre-treated with acid sustained higher forces than specimens to which resin was applied without acidification.

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