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Research Article

Effect of Microstructure of Spongy Bone in Different Parts of Woodpecker's Skull on Resistance to Impact Injury

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Natural biological materials such as bone, teeth and nacre are nano-composites of protein and mineral frequently exhibit highly superior strength for self-assembly and nanofabrication. Mineral mass and microstructure/nanostructure of bone are susceptible to stimulation by mechanical loads, ensuring that its mechanical behavior and strength are adapted to environmental changes. Woodpeckers repeatedly drum tree trunks at a speed of 6-7 m s^{-1} and acceleration of ~1000 g with no head injuries. The uneven distribution of spongy bone has been founded on woodpecker's skull in our previous study. More knowledge of the distribution of the shock-absorbing spongy bone could be incorporated into the design of new safety helmets, sports products, and other devices that need to be able to resist the impact. In this study, the effect of microstructure of spongy bone in different parts on woodpecker's skull compared with other birds was observed and analyzed. It was found that the unique coordinate ability of micro-parameters in different parts of woodpecker's skull could be one of the most important roles of its resistance to impact injury. Better understanding of the materials would provide new inspirations of shock-absorbing composite materials in engineering.

1. Introduction

Head injuries sustained in sports or lifesaving in space ejection and car crash accidents commonly cause serious brain injury or death [1–3]. But woodpeckers repeatedly bash their heads against tree trunks at a speed of 6-7 m s^{-1} and acceleration of ~1000 g without any head injuries [4–7]. The woodpecker rhythmically drums surfaces such as dead tree limbs and metal poles with its beak to catch worms to eat and attract a mate or announce its territorial boundaries [7, 8]. The woodpecker's resistance to head impact injury was a prime example of adaptive natural evolution by Natural Selection mentioned by Darwin who commented it was so admirably adapted to catch insects under the bark of tree [9]. Over the ensuing decades, the adaptiveness and evolution of the feature have been examined by many researchers not only ornithologists and biologists but also whom in the fields of mechanical engineering, medical engineering, material science, and electronics engineering [4–16]. But few studies have been done in view of materials. A major problem was that logically explanations were little based on the observation and analysis of microstructure and nanostructure of woodpecker's bone in view of biomaterials.

Natural biological materials such as bone, teeth, and nacre are nanocomposites of protein and mineral frequently exhibit highly superior strength for self-assembly and nanofabrication that have been designed by natural evolution over millions of years [17–19]. There was also overwhelming evidence that the mineral mass and microstructure and nanostructure of bone are susceptible to stimulation by mechanical loads, ensuring that its mechanical behavior and strength are adapted to environmental changes [20–29].

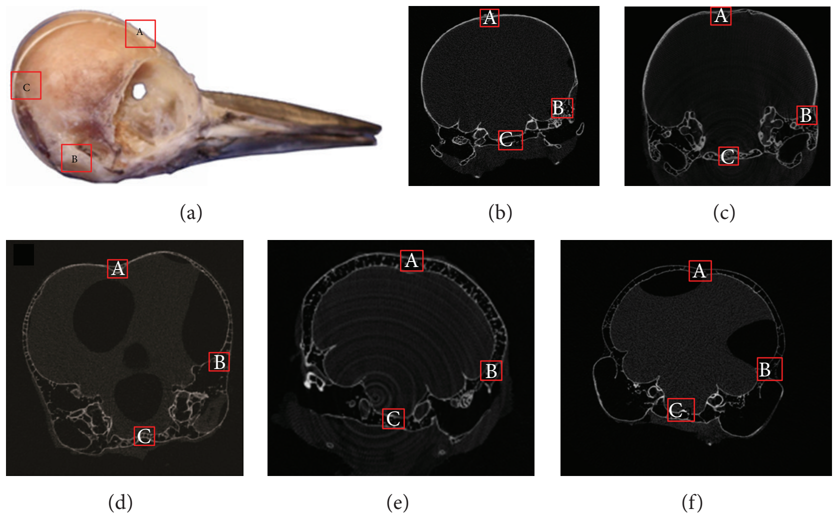


Figure 1: (a) The three parts on the skull of birds and micro-CT scan images of cranial bone of (b) Great Spotted Woodpecker; (c) Grey Headed Woodpecker; (d) Eurasian hoopoe; (e) Mongolian skylark; (f) Great Tit: (A) forehead; (B) temporomandibular; (C) occiput.

For example, the shape of the bill was adapted to the forces on it during drilling [30], and in vivo bite force was reflected in skull morphology and geometry and in the capacity for contraction of the jaw muscles [31, 32]. The hyoid bone of woodpecker has unique strength and flexibility owing to its unique micro/nanohierarchical composite structures. It consists of a flexible cartilage and bone skeleton covered with a thin tissue layer having high strength of 136 MPa and elasticity of 3.74 GPa. At the interface between the cartilage-bone skeleton and the tissue layer, there is a hierarchical fiber connection [15]. The cranial bone of the woodpecker achieved a higher ultimate strength of 6.38 MPa compared with the Lark of 0.55 MPa [6, 33, 34], which suggested that the mechanical properties are sensitive to the shape of individual trabeculae [35]. Materials that contain more organic material are expected to exhibit greater flexibility under load [36–40].

The uneven distribution of spongy bone was founded on woodpecker's skull in our previous study [6, 33]. More knowledge of the microstructure distribution of the shock-absorbing spongy bone could be incorporated into the design of new safety helmets, sports products, and other devices that need to be able to resist the impact. Therefore, the microstructure and nanostructure of spongy bone in different parts of woodpecker skull should be associated with woodpeckers' resistance to impact injuries. In this study, the effect of microstructure of spongy bone on the different parts of woodpeckers' skull compared with that of other birds was observed and analyzed quantitatively. Better understanding of the materials would provide new inspirations of shock-absorbing composite materials in engineering.

2. Materials and Methods

This study was approved by the Science and Ethics Committee of School of Biological Science and Medical Engineering in Beihang University, China (*Approval ID: 20090301*).

Two woodpeckers were selected including the Great Spotted Woodpecker (*Dendrocopos major*; length 23 cm *and* weight 70 g) and Grey Headed Woodpecker (*Picus canus*; 27 cm, 130 g) for their wide distribution in Northern China, which peck regularly and efficiently. For comparison, the other three kinds of birds with comparable size were also selected including Eurasian hoopoe (*Upupa epops*; 26 cm, 62 g) that pecks on insects inside the soil mainly, Mongolian skylark (*Melanocorypha mongolica*; 21 cm, 66 g) does not peck as a songbird, Great Tit (*Parus major*) does not also peck, to be compared with woodpeckers. They are widely distributed in Northern China. Dead specimens of specimens were collected from bird feeders for the microparameters measurements and observation.

The microparameters of spongy bone of five birds were measured three-dimensionally by micro-computed nondestructive tomography (micro-CT, Skyscan 1076, Skyscan, Belgium) at a spatial resolution of 35 μm. Three different parts from the forehead, temporomandibular, and occiput on their skull were selected, respectively, as shown in Figure 1. Microparameters were calculated as indicated in Table 1.

In order to know the microstructure on the three parts of two kinds of woodpeckers, three cranial samples measuring 4 mm × 4 mm were cut from the forehead, temporomandibular, and occiput of the Great spotted woodpecker and Grey Headed woodpecker, respectively, as indicated in Figure 1(a). The microstructure of these specimens was observed by scanning electron microscopy (SEM, JSM-6490, JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV and a working distance of 10–15 mm, at room temperature. Specimens were washed with normal saline to remove blood, mucus, and tissue fluid, dehydrated in an ascending ethanol series (30% to 100%) for 20 min at each concentration, and then sputter-coated with an approximately 20 nm layer of gold before observation. Values are presented as means and standard deviation (SD). Differences of the spongy bone on three parts of five birds were analyzed using paired Student's t-tests (SPSS

Table 1: Definitions of various microstructural parameters analyzed in this study.

Parameters abbrev.		Definition (units)
Bone volume fraction	BV/TV	Relative percentage of bone within 3D ROI (%); BV: bone volume; TV: tissue volume
Structural model index	SMI	Quantification of relative shape of trabeculae from rod-like to plate-like
Trabecular thickness	Tb.Th	Quantification of relative thickness of individual trabeculae within 3-D ROI (μm)
Trabecular number	Tb.N	Quantification of relative number of individual trabeculae within 3-D ROI (1/mm)
Trabecular separation	Tb.Sp	Quantification of relative spacing between individual trabeculae within 3-D ROI (μm)
Bone mineral density	BMD	3-D derivation of mineral density (g/cm^3)

version 16, Chicago, IL, USA), with $P < 0.05$ being accepted as significant. All reported P values are two-sided.

3. Results and Discussions

Microstructural parameters of the spongy bone on the forehead, temporomandibular, and occiput of the Grey Headed woodpecker, Eurasian hoopoe, Mongolian skylark, and Great Tit compared with Great spotted woodpecker are shown in Figures 2(a)–2(f).

Bone volume fraction (BV/TV) of spongy bone for Eurasian hoopoe and Mongolian skylark was lower than that of Great spotted woodpecker ($P < 0.05$) which was consistent with our previous study [6, 30, 31]. BV/TV of great Tit was lower than that of Great spotted woodpecker ($P < 0.001$). In addition, BV/TV of spongy bone on the temporo-mandibular was lowest compared with that of forehead and occiput for Great spotted woodpecker and Grey Headed Woodpecker, but there was no consistent tendency for other kinds of birds. BV/TV on the occiput was higher than that of forehead and temporo-mandibular except for Mongolian skylark. There was no significant difference of BV/TV between Great spotted woodpecker and Grey Headed Woodpecker.

The structural model index was introduced to quantify the characteristic form of three-dimensional structures in terms of the quantity of plate-like and rod-like structures. For ideal plate and rod structures, the SMI values are 0 and 3, respectively, and are independent of their physical dimensions. For a structure with both plates and rods of equal thickness, the value lies between 0 and 3, depending on the volume ratio of the rods and plates. Here, spongy bone on the forehead, temporo-mandibular, and occiput for Great spotted woodpecker's (SMI = 0.89~1.19), Gray-headed woodpecker (SMI = 1.06~1.61), and Eurasian hoopoe (SMI = 1.21~1.60) had more plate-like structures than Mongolian skylark (SMI = 1.30~2.79) and Great Tit (1.36~1.96). SMI of the spongy bone on Mongolian skylark and Great Tit's skull was more than 1.5, which means that more rod-like spongy bone was distributed on lark and Tit's skull. SMI of the Great Spotted Woodpecker, Grey Headed Woodpecker, and Eurasian hoopoe was less than 1.5, which means more plate-like spongy bone on Great Spotted Woodpecker, Grey Headed Woodpecker and Eurasian hoopoe's skull. SMI of Mongolian skylark and Great Tit was higher than Great Spotted Woodpecker, Grey Headed Woodpecker, and Eurasian Hoopoe significantly. But there was no significant difference among Great Spotted Woodpecker, Grey Headed Woodpecker, and Eurasian Hoopoe. It was suggested that pecking behavior in long term might be resulted in more plate-like spongy bone. For Great Spotted Woodpecker, there was little difference among the three parts. There was no obvious trend on the three parts for other four birds. For the significant difference of Great Spotted Woodpecker, the scanning electron microscope (SEM) images of Great Spotted Woodpecker were described as shown in Figure 3. It was found that the trabecular bone was plate-like structure for the Great Spotted Woodpecker.

The SMI values do not provide accurate information on spongy bone which needs to be complemented with measurement of the thickness, number, and spacing of the spongy bone (Tb.Th, Tb.N, and Tb.Sp, resp.). Trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) of Eurasian hoopoe, Mongolian skylark, and Great Tit were lower than those of the Great Spotted Woodpecker ($P < 0.05$). And there was no significant difference between Great Spotted Woodpecker and Grey Headed Woodpecker. Tb.Th, Tb.N, and Tb.Sp of spongy bone on the forehead, the occiput, and the temporomandibular were the highest for Great Spotted Woodpecker and Grey Headed Woodpecker, respectively. But there was no consistent trend for Eurasian hoopoe, Mongolian skylark, and Great Tit. Tb.N on the occiput was higher than that of the forehead and temporomandibular for the two kinds of woodpecker, Eurasian hoopoe, and Great Tit. Tb.Th on the forehead was higher than other parts for two kinds of woodpecker; but it was the highest on the temporomandibular for the Mongolian skylark and Great Tit. For bone mineral density (BMD), there was no difference among the five birds. It was suggested that the microstructure of spongy bone on woodpecker's skull has achieved the optimized mechanical properties of resisting the impact. These features were combined to confer a better ability of resistance impact for woodpeckers.

4. Conclusions

In this study, we examined the microstructure of spongy bone in different parts (including the forehead, temporomandibular, occiput) of Great Spotted Woodpecker, Grey Headed Woodpecker, Eurasian hoopoe, Mongolian skylark, and Great Tit's skull based on the analysis of microparameters and observation of scanning electron microscope. It was shown that the microparameters of woodpeckers have a significant difference compared with the other three birds. There was no significant difference between Great Spotted

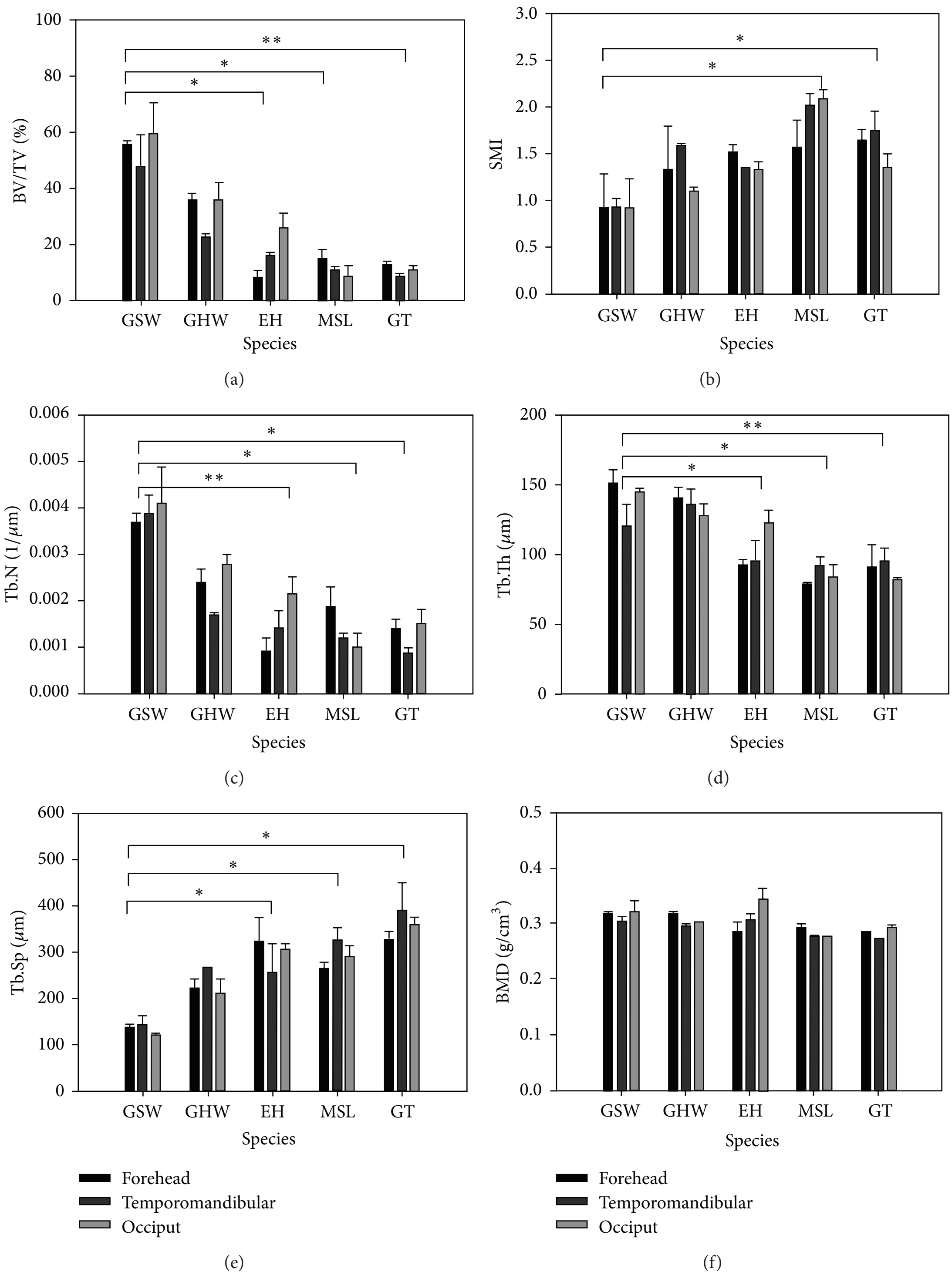
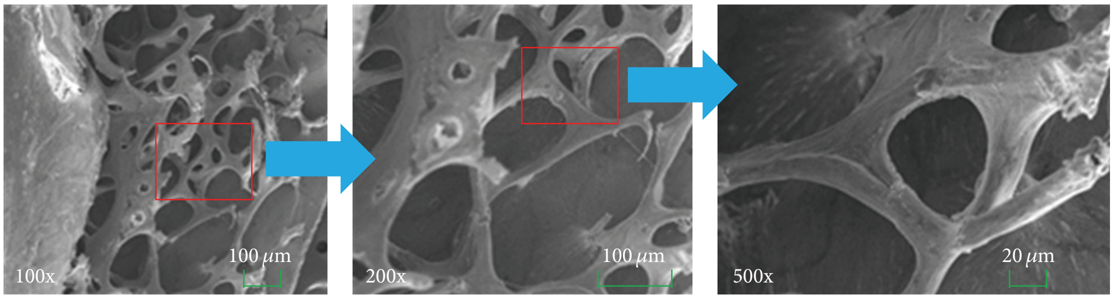


Figure 2: The microstructural parameters of spongy bone on the forehead, temporomandibular, and occiput for Great Spotted Woodpecker (GSW), Grey Headed Woodpecker (GHW), Eurasian hoopoe (EH), Mongolian skylark (MSL), and Great Tit (GT). Significance was assigned as $^{*}P < 0.05$; $^{**}P < 0.01$. (a) Bone volume fraction (BV/TV); (b) structural model index (SMI); (c) trabecular number (Tb.N); (d) trabecular thickness (Tb.Th); (e) trabecular separation (Tb.Sp); (f) bone mineral density (BMD).



(a) (b) (c)

Figure 3: Microstructure of spongy bone of Great spotted woodpecker.

Woodpecker and Grey Headed Woodpecker. For woodpeckers. Tb.Th, Tb.N, and Tb.Sp were different on the forehead, temporomandibular, and occiput significantly. Tb.Th was higher on the part with lower level of Tb.N. The smaller Tb.Sp in woodpecker means it has a tight microstructure of spongy bone, which may contribute to impact prevention of woodpecker together with the higher Tb.Th and BV/TV. So Tb.Sp was a tool to adjust the level of Tb.Th and Tb.N. Then, it made the mechanical properties on the different parts reach the average values, which is the good design of natural optimization on biomaterials. In this study, the effect of microstructure of spongy bone in different parts of woodpecker's skull was studied by comparison of two kinds of woodpeckers and the other three kinds of birds. The unique coordinate ability of microparameters including Tb.Th, Tb.N, and Tb.Sp in different parts of woodpecker's skull could be one of the most important roles of its resistance to impact injury.

Authors' Contribution

Lizhen Wang and Xufeng Niu contributed equally to this work.

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