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Hierarchical levels of the ginkgo seed shell

Jing Sun[a], Zuankai Wang[b,*]

[a] *Department of Mechanical Engineering, City University of Hong Kong, Hong Kong 999077, China*

[b] *Department of Mechanical Engineering, The Hong Kong Polytechnic University, Hong Kong 999077, China*

* Corresponding author. E-mail address: zk.wang@polyu.edu.hk

One of the innate features of many natural structural materials is excellent damage tolerance enabled by hierarchical anisotropic architectures. Highly anisotropic structural materials usually demonstrate high performance in one direction, but become weak in other directions. For example, the osteon of compact bone is longitudinally aligned with high anisotropy, making the bone difficult to break but still easily to be split [1-3]. Similarly, abalone shell shows superior crack resistance owing to its hierarchical structure, including macrostructure with layers of approximately 0.2-0.4 mm, mesostructure consisting of “bricks-like layers” of 0.5-1 μm, and microstructure with single crystals of aragonite polymorph [4]. However, if the crack propagates in a direction parallel to the surface of the lamella in the abalone shell, the relative sliding between the lamellas will be greatly limited, and as a result, the crack will become easier to propagate [5]. Researchers have continued to search for materials with weak anisotropic structures that exhibit high toughness in all directions.

Many natural organisms have evolved elegant structures to process and manage information, matters and energy for survival, which gives a lot of inspirations to the design and fabrication of bionic materials [6, 7]. For example, the plant seed shells also have hierarchical structures to protect their embryo from external damage. These hierarchically organized structures, combined with the gradient motif, yield impressive fracture tolerance in the endocarp of the seed shells. Schmier et al. have identified six hierarchical levels (H1-H6) of the coconut endocarp [8], scaling from the entire spheroidal shell (H1) to the molecular levels of the cellulose (H6). At the tissue level (H2), the endocarp can be determined as packed sclereid embedded in a dense matrix of vascular bundles. The preceding-mentioned matrix composed of individual sclereids (H3) as well as thick cell walls (H4) accounting for main parts of the cross-sectional area of the cells. The cell walls consist of more than 30 individual layers (H5) and symmetrically connected with each other via pit pairs. Although researchers are aware of the hierarchical structures of various plant seed shells, such as walnut, pistachio [9], and Brazil nut shells [10], the before research only analyzed the relationship between the structure and properties of shells on a single level (such as cell level, fiber level, etc.), while the relationship between the multi-scale three-dimensional microstructure and the properties of shells remains unexplored. A recent work published in PNAS from Cheng's group at Beihang University, reported a weakly anisotropic structure of *Ginkgo biloba* (ginkgo) seed shell exhibiting superior crack resistance in different directions [11].

The structure of ginkgo seed shell displays hierarchical levels: spheroidal shells, packed sclereids, individual sclereids, several layers of sclereids, pits in the layers, and helicoid cellulose microfibers. Careful inspection shows that the ginkgo seed shell (Figure. 1a) is the aggregation of massive polygonal sclereids tightly bonded with each other (Figure. 1b and c). Distinct from the 3D puzzle sclereids of pistachios and walnuts [12], isodiametric sclereids of pines [9], wavy sclereids of pecans [9], and fiber bundles of coconuts [13], these sclereids in ginkgo seed shell exhibit elongated shape in the outer surface and equiaxed shape in the middle and inner parts. The sclereids of ginkgo seed shell show a multi-shelled architecture, with inside and interfaces between them being "interlocked" and "strengthened" by pits (Figure. 1d and e). The pit structure also exists in other seed shells, such as endocarps of *Argania spinosa* [14] and *Pinus koraiensis* [9]. The pit density they observed in the shell of ginkgo seed was about 3–5 per 100 μm^2 in the middle part and 1–3 per 100 μm^2 in the outer and inner parts, which is similar to that of *Argania spinosa* and *Juglans regia*, and less than that of *Pinus koraiensis* (7–11 per 100 μm^2). For each sclereid, the inner cell walls are composed of cellulose microfibrils, which are woven in a manner similar to the twisted plywood (Figure. 1f). The cross-section of a sclereid reveals that the cell wall displays periodically layered pattern through repeating helical structure. To constitute the helical structure, the cellulose microfibrils are first parallelly aligned in layers, and these layers are further piled up and rotated about the normal direction. Cheng and co-workers found that the orientation of cellulose microfibrils at different layers was also different [11].

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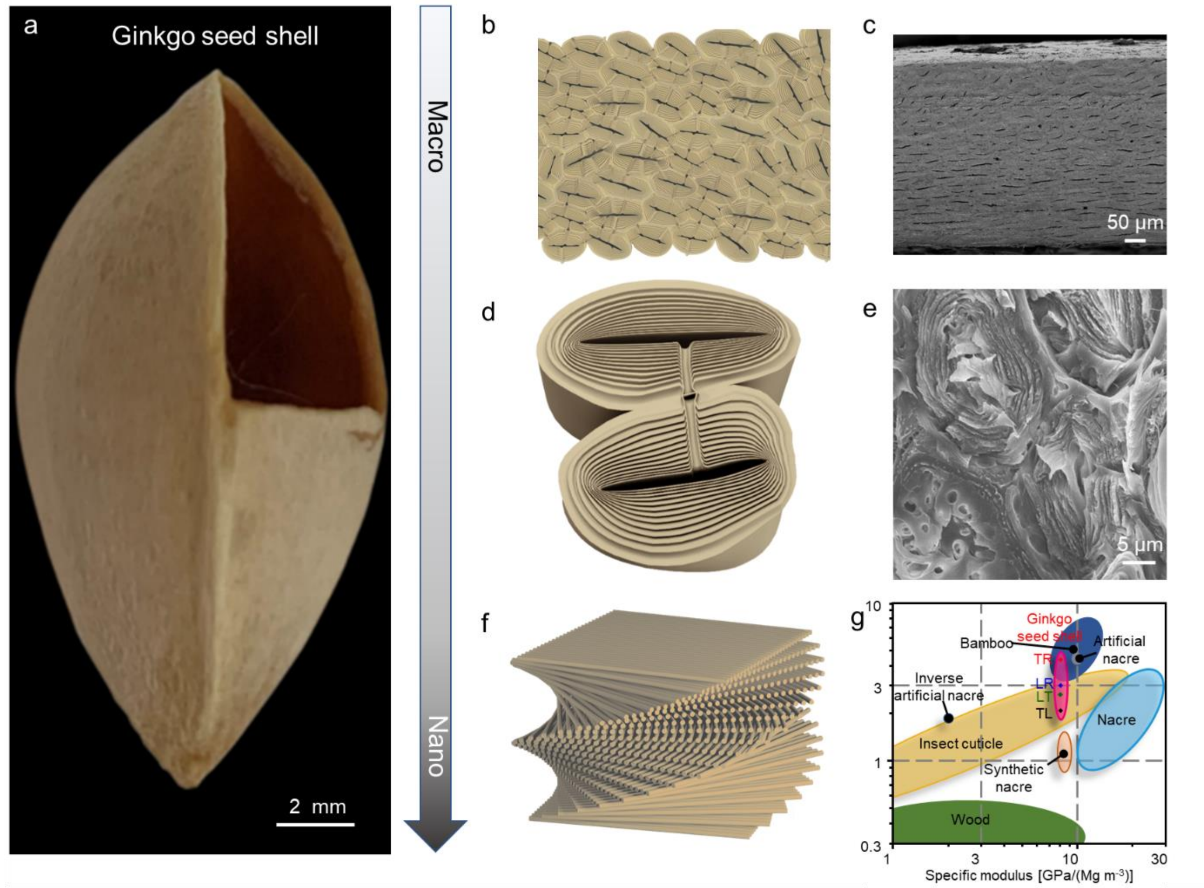

Figure 1. Hierarchical structure of Ginkgo seed shell [11]**.** (a) The digital image of the ginkgo seed shell. (b, d and f) The schematic illustration of the polished surface, two neighboring sclereids and the helicoidal pattern. (c and e) SEM image of the polished surface and two neighboring sclereids are "interlocked" and "strengthened" by a pit. (g) Comparison of the specific fracture toughness and modulus of the ginkgo seed shell with other natural and artificial materials.

The hierarchical design with a weak isotropy enables the ginkgo seed shell to yield excellent crack resistance in all four directions. The fracture toughness of ginkgo seed shell ranges between 3 and 5 $MPa{\cdot}m^{1/2}$, which is distinct from wood that is characterized by largely different fracture toughness in different directions. The specific fracture toughness of ginkgo seed shell can rival other highly anisotropic natural materials, such as nacre (Figure. 1g). Similar to other natural structures, the weak interfaces in the ginkgo seed shell facilitate damage tolerance. Crack first tends to propagate along the softer and weaker compound middle lamella that serves as the matrix for embedding sclereids, then the sclereid unit transfers deformation and induces small cracks ahead of the crack tip to promote crack bridging, which significantly reduces the stress around crack tip, thereby slowing down the propagation of crack.

The authors also demonstrated the mechanism for the superior crack resistance of ginkgo seed shell, which can be attributed to pit-guided crack propagation. Briefly, when forcing the adjacent sclereids to separate under external load, the embedded pits interlocked with the helicoidal cell wall can transfer the stress from the interfacial region to the interior of the sclereids, and decreases the stress concentration. The pit-guided crack propagation mechanism was also verified through finite element simulation.

This inspiring work by Cheng and co-workers reveals the hierarchical organization of ginkgo seed shells and uncovers the combined contribution of each level to the mechanical performance, which is distinct from previous papers that only focused on sclereids or microfibers that make up sclereids but neglected the role of pits. This work encourages researchers to discover the elements that are often overlooked in other natural materials, and to explore the relationship between cross-scale hierarchical structures and performance.

This landmark work opens up new horizons to understand the hierarchical levels of the organization of shells, and also shed light for designing high-performance bulk materials. These materials hold great potential in a variety of application areas ranging from our daily life such as shoe soles and safety helmets, biomedical field such as artificial intervertebral discs and bone materials, to automotive and aerospace industry, and even military field such as bulletproof vests. However, human engineering is hard to replicate these materials with multi-scale differences, more advanced manufacturing technology should be developed for mimicking natural hierarchical structures and expanding the capacity of material choices for real-world applications.

Conflict of interest

The authors declare that they have no conflict of interest.

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Jing Sun is currently a Postdoc in Department of Mechanical Engineering, City University of Hong Kong. She received her Bachelor's and Master's degrees from China University of Petroleum (East China) in 2013 and 2016, respectively, and PhD degree from City University of Hong Kong in 2020. Her current research interest lies in the design and fabrication of nature-inspired materials.

Zuankai Wang is currently Chair Professor of Nature-Inspired Engineering in the Department of Mechanical Engineering at The Hong Kong Polytechnic University. He received his Bachelor's degree from Jilin University in 2000, Master's degree from Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences in 2003, and PhD degree from Rensselaer Polytechnic Institute in 2008. His research is focused on fundamental understanding of the basic structure–function properties of natural materials as well as translating nature's inspiration for water-energy nexus and biomedical applications.