

SUPPORTING INFORMATION

Enabling Gradient Biomineralization and Prestress with a Macroscopic Matrix: The Fish Swim Bladder for Bioprocessing-Inspired Fabrication

Bingyu Xue^{a,b}, Junming Wu^b, Jiangyu Yi^b, Jing Li^a, Ziyi Guo^a, Guohai Huang^a, and
Kun Wang*^{a,b}, Hang Ping*^{a,c}, Zhengyi Fu*^{a,c}

a. Hubei Longzhong Laboratory, Xiangyang, Hubei, P. R. China

*b. School of Materials Science and Engineering, Wuhan University of Technology,
Wuhan, Hubei, P. R. China*

*c. State Key Laboratory of Advanced Technology for Materials Synthesis and
Processing, Wuhan University of Technology, Wuhan, Hubei, P. R. China*

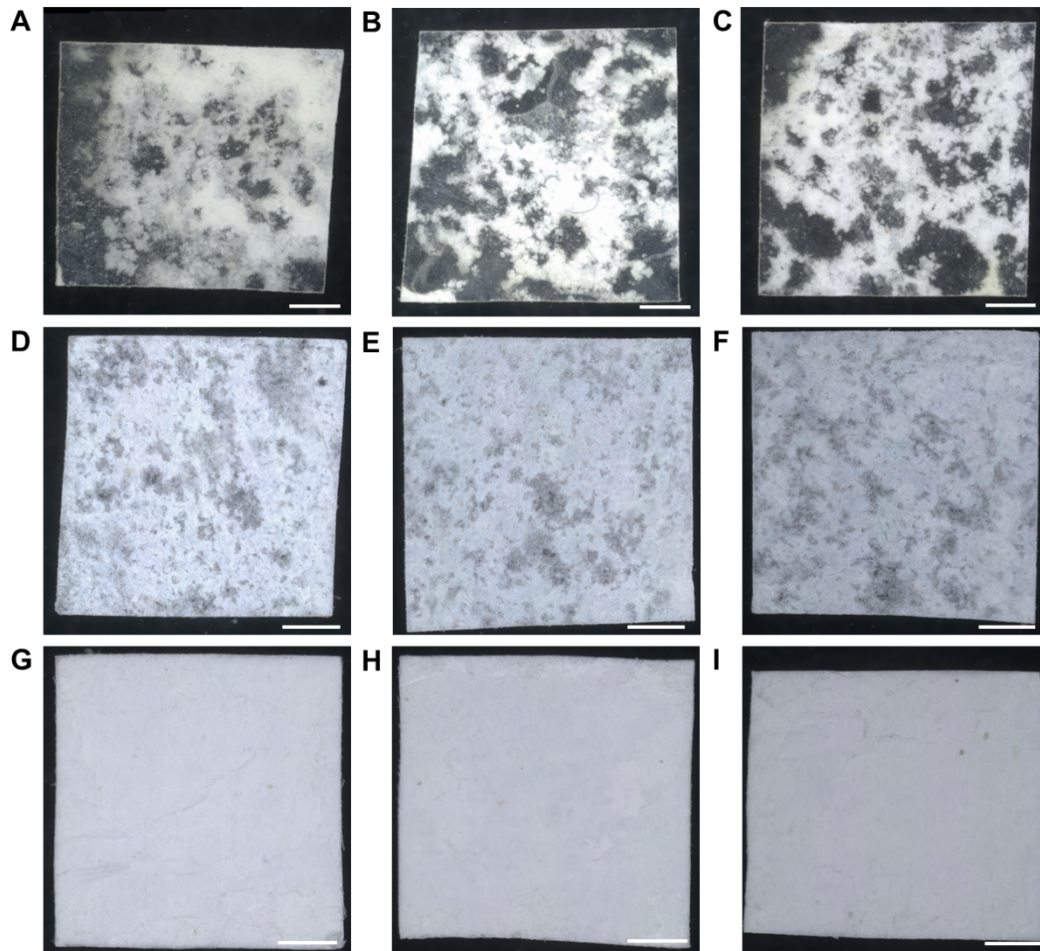


Figure S1. FSB mineralized for 3 days, 5 days, and 7 days, 3 replicates of FSB were mineralized in each experimental group. (A-C) 3 days. (D-F) 5 days. (G-I) 7 days. Scale bar: 1 mm. The relationship between the mineralization coverage rate and time was obtained from this.

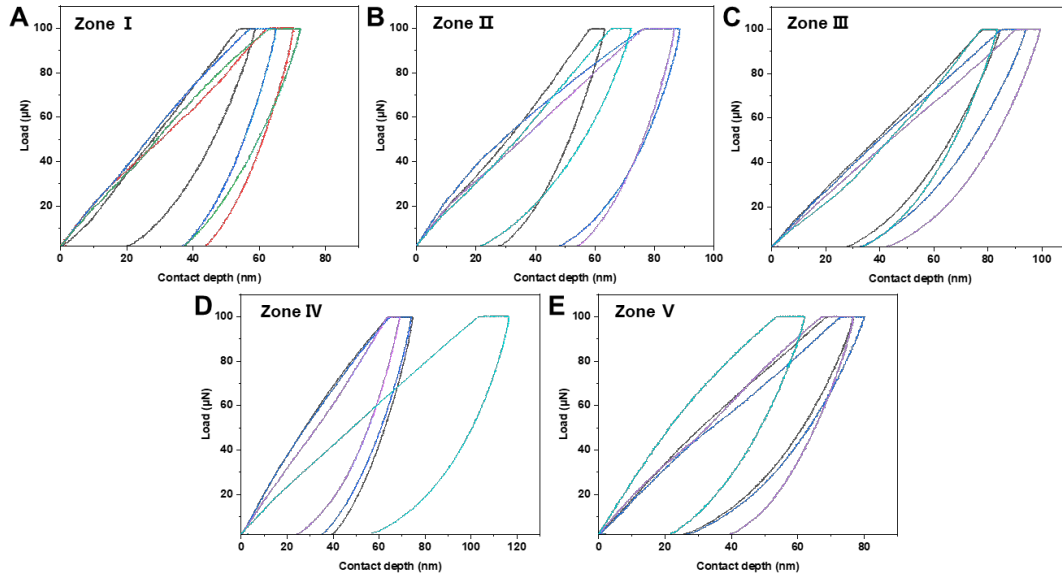


Figure S2. Nanoindentation load-depth curves of the mineralized FSB across thickness regions from top (Region I) to bottom (Region V). (A-E) Region I, Region II, Region III, Region IV, and Region V, respectively. The testing area was shown in Figure 5E.

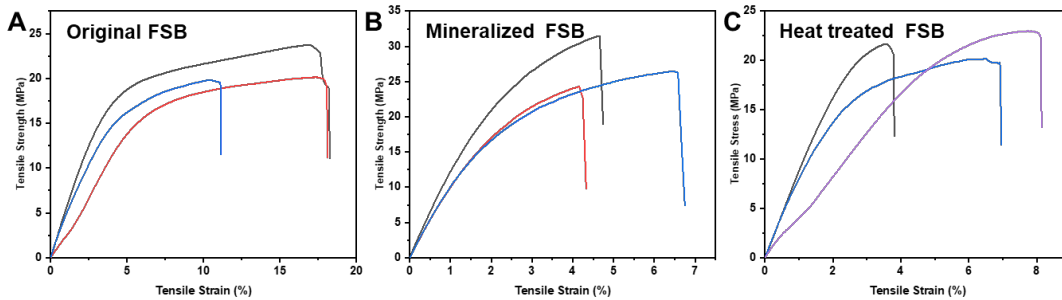


Figure S3. Tensile stress-strain curves of the FSB. (A) Original FSB (B) Mineralized FSB (C) Mineralized FSB with heat treatment.

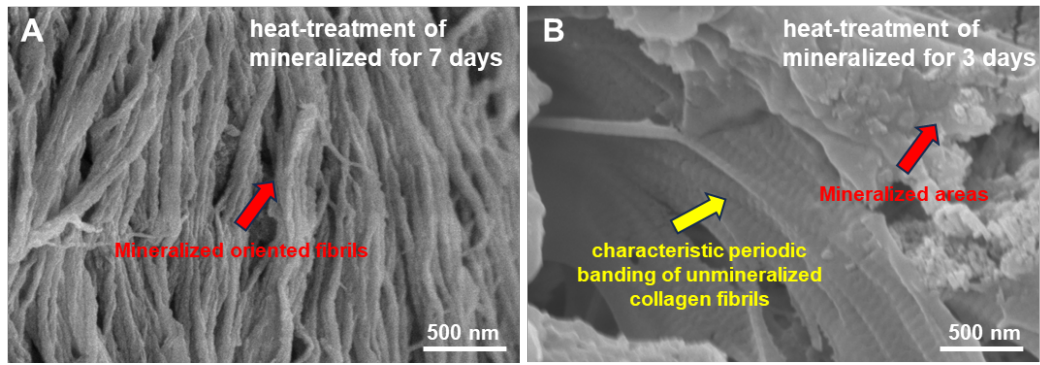


Figure S4. Morphology of the fish swim bladder after heat treatment following mineralization for 7 days (A) and 3 days (B). (A) Mineralized fibrils within the fish swim bladder after complete mineralization for 7 days. (B) Fibrils within the fish swim bladder after partial mineralization for 3 days, where the unmineralized collagen fibrils still exhibit the characteristic periodic banding pattern of collagen.

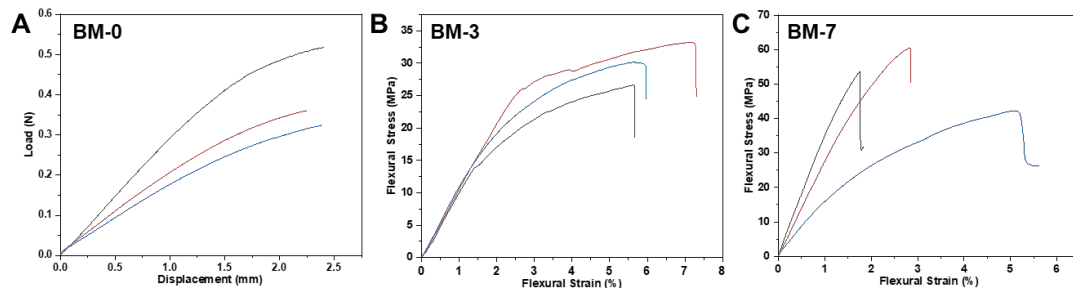


Figure S5. Stress-displacement curve and flexural stress-strain curves of the bulk materials. (A) BM-0 (B) BM-3 (C) BM-7.

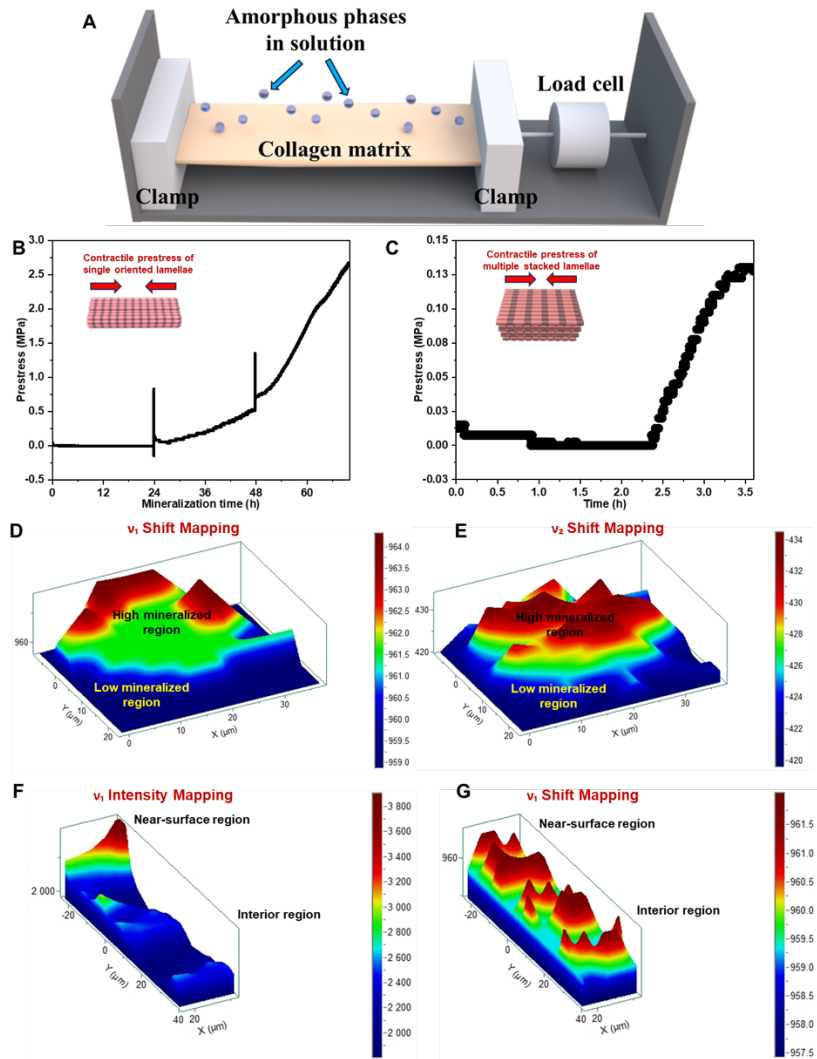


Figure S6. In-situ monitoring of prestress during mineralization process. (A) Schematic of the custom-built prestress monitoring setup. (B) Time-dependent evolution of prestress during mineralization of oriented collagen fibrils of tendon. (C) Time-dependent evolution of prestress during mineralization of the FSB, composed of stacked collagen lamellae. (D–E) Raman mapping of the ν_1 (D) and ν_2 (E) peaks shifts on the surface mineralized region of the FSB. (F–G) Spatial distribution of HAP intensity mapping (F) and Raman ν_1 peak shift mapping (G) across a longitudinal section of the FSB.

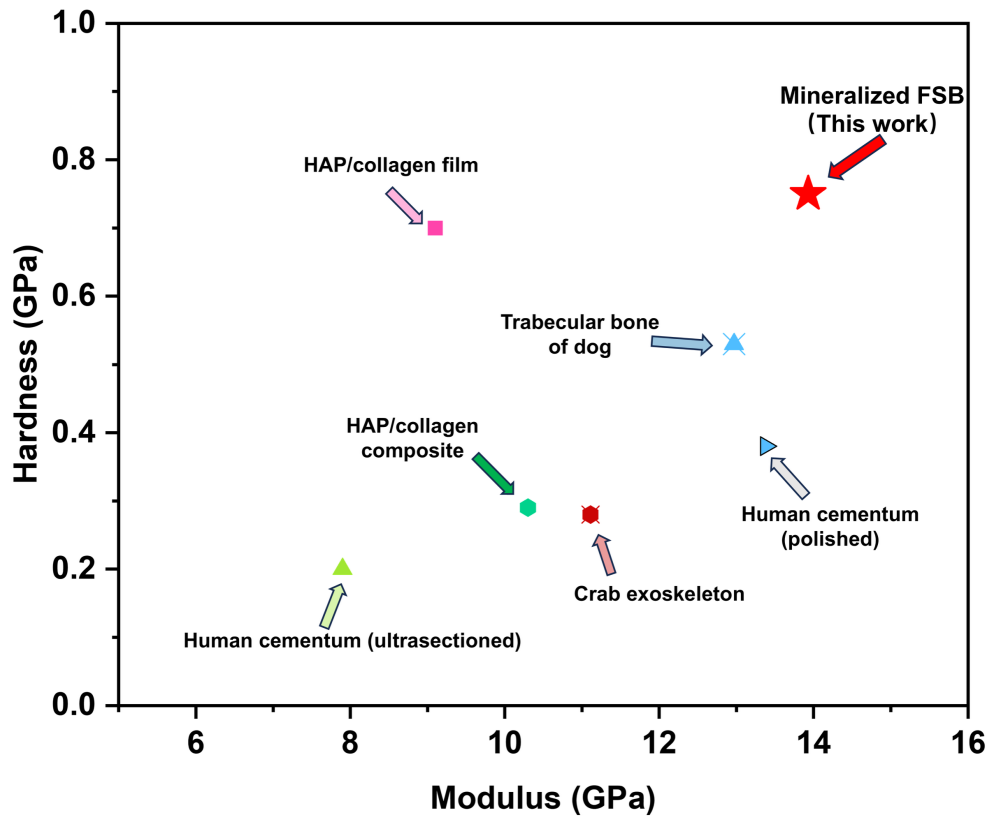


Figure S7. Comparison of modulus and hardness among natural or artificial collagen-based composites

Table S1 Mechanical properties of the reported natural or artificial collagen-based materials using various testing methods

Mineralized Collagen Materials	Minerals	Testing methods	Mechanical properties	Refs.
Human trabecular	HAP	Nanoindentation	E:15.0 ± 2.5 GPa H:0.515 ± 0.082 GPa	1
Trabecular bone of dog	HAP	Nanoindentation	E:12.97 ± 4.15 GPa H:0.53 ± 0.24 GPa	2
Human cementum (ultrasectioned, wet specimens)	HAP	Nanoindentation	E:7.9 ± 1.3 GPa H:0.20 ± 0.04 GPa	3
Human Cementum (polished, wet specimens)	HAP	Nanoindentation	E:13.38 ± 0.91 GPa H:0.38 ± 0.06 GPa	3
Proximal end (L) of Carp rib bone	HAP	Nanoindentation	E:8.25 ± 0.27 GPa	4
Distal end (T) of Carp rib bone	HAP	Nanoindentation	E:9.64 ± 0.39 GPa	4
Crab exoskeleton	HAP	Nanoindentation	E:11.11 ± 3.56 GPa H:0.28 ± 0.16 GPa	5
Healthy mouse bone	HAP	Nanoindentation	E:13.9 GPa H: 0.28 GPa	6
PILP-recovery bone	HAP	Nanoindentation	E:14.3 GPa H: 0.37 GPa	6
Collagen-based composite	HAP	Nanoindentation	E:10.3 ± 2.5 GPa H:0.29 ± 0.07 GPa	7
Collagen films	HAP	Nanoindentation	H:0.7 ± 0.1 GPa (dry) E:9.1 ± 1.4 GPa (dry) H: 177 ± 31 kPa (hydrated) E: 8 ± 3 kPa (hydrated)	8
Collagen scaffolds	HAP	Tensile	Modulus of 1.0 MPa (dry)	9
Collagen gel	Carbonated HAP	Tensile	Strength of 1.4 MPa (dry) Modulus of 4.7 MPa (dry)	10

Mineralized FSB	HAP	Nanoindentation	E: 13.93 ± 4.02 GPa H: 0.75 ± 0.52 GPa	This work
Mineralized FSB	HAP	Tensile	Ultimate tensile strength 27.4 ± 3.7 MPa Elastic modulus 1213.4 ± 140.1 MPa	This work

Table S2 Mechanical properties of the reported bioinspired collagen/HAP composites using flexural or compression tests.

Bulk HAP mineralized collagen composites	Component	Testing methods	Mechanical properties	Refs
Collagen-calcium phosphate bone cement	Collagen and Calcium phosphate	Flexural Test	Flexural strength from 13.9 ± 2.3 MPa to 9.0 ± 2.5 MPa	11
Collagen composite membranes	Collagen chitosan and β -tricalcium phosphate	Flexural Test	Flexural strength of 13.6 MPa	12
HAP/Collagen composites	HAP/collagen	Flexural Test	Flexural modulus of 2.46 ± 0.48 and Flexural strength of 0.651 ± 0.103 MPa	13
Biomimetic Collagen/HAP Composite Scaffolds	HAP/collagen	Compression Test	Compressive modulus of about 3 MPa	14
BM-3	HAP/collagen	Flexural Test	Flexural strength 29.93 ± 3.3 MPa and Elastic modulus 0.90 ± 0.19 GPa	This work
BM-7	HAP/collagen	Flexural Test	Flexural strength 51.9 ± 9.5 MPa and Elastic modulus 3.0 ± 1.2 GPa	This work

References:

1. J. Y. Rho, M. E. Roy, 2nd, T. Y. Tsui and G. M. Pharr, *J. Biomed. Mater. Res.*, 1999, 45, 48-54.
2. E. Mittra, S. Akella and Y. X. Qin, *J. Biomed. Mater. Res. Part A*, 2006, 79A, 86-93.
3. S. P. Ho, H. Goodis, M. Balooch, G. Nonomura, S. J. Marshall and G. Marshall, *Biomaterials*, 2004, 25, 4847-4857.
4. M. E. Roy, S. K. Nishimoto, J. Y. Rho, S. K. Bhattacharya and G. M. Pharr, *J. Mater. Sci.-Mater. Med.*, 2001, 12, 699-702.
5. T. Inoue, Q. Hai and K. Nakazato, *J. Mater. Res. Technol-JMRT*, 2024, 33, 6.
6. S. S. Yao, X. F. Lin, Y. F. Xu, Y. W. Chen, P. C. Qiu, C. Y. Shao, B. Jin, Z. Mu, N. Sommerdijk and R. K. Tang, *Adv. Sci.*, 2019, 6, 10.
7. A. Stanishevsky, S. Chowdhury, P. Chinoda and V. Thomas, *J. Biomed. Mater. Res. Part A*, 2008, 86A, 873-882.
8. Y. Liu, D. Luo, X. X. Kou, X. D. Wang, F. R. Tay, Y. L. Sha, Y. H. Gan and Y. H. Zhou, *Adv. Funct. Mater.*, 2013, 23, 1404-1411.
9. N. Saxena, M. A. Cremer, E. S. Dolling, H. Nurrohman, S. Habelitz, G. W. Marshall and L. B. Gower, *Dent. Mater.*, 2018, 34, 1378-1390.
10. N. Nassif, F. Gobeaux, J. Seto, E. Belamie, P. Davidson, P. Panine, G. Mosser, P. Fratzl and M. M. G. Guille, *Chem. Mat.*, 2010, 22, 3307-3309.
11. J. L. Moreau, M. D. Weir and H. H. K. Xu, *J. Biomed. Mater. Res. Part A*, 2009, 91A, 605-613.
12. S. B. Lee, J. S. Kwon, Y. K. Lee, K. M. Kim and K. N. Kim, *J. Biomed. Mater. Res. Part B*, 2012, 100B, 1935-1942.
13. S. Yunoki, H. Sugiura, T. Ikoma, E. Kondo, K. Yasuda and J. Tanaka, *Biomed. Mater.*, 2011, 6, 10.
14. J. C. Wang and C. Z. Liu, *J. Bionic Eng.*, 2014, 11, 600-609.