In Situ Bending Reveals Simultaneous Enhancements of Strength and Ductility of Cortical and Cancellous Layers Induced by the Cartilage Layer

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ABSTRACT: The energy absorption and toughening effect of cartilage could effectively protect bone from damage, and the enhancement mechanisms of cartilage on deformation resistance or strength need to be revealed. Using a self-developed in situ bending tester integrated with an optical microscope, in situ bending of the composite bone structure consisting of the cartilage layer and cortical and cancellous layers was carried out, accompanied by simultaneously obtained continuous morphological changes in diverse deformation layers. Although the bending resistance of pure cartilage layer was only 0.3 N, the significant enhancements of bone strength and ductility induced by the cartilage layer were experimentally revealed, as the peak loads and ultimate bending deflections of the composite structure increased by 1.49- to 2.14-fold and 1.43- to 2.12-fold, respectively. The scanning electron microscopy images of the composite bone structure at various locations with disparate stress conditions exhibited significant difference in crack sizes and degrees of tearing damage. The cartilage layer was verified to induce a layered tearing dimple feature to inhibit the crack propagation and further enhance the deformation resistance. The frequency shift comparison between the Raman spectroscopies of various microregions also indirectly verified the inhibition effect of the cartilage layer on the stress increment in the cortical layer.

1. INTRODUCTION

Composite bone structures consisting of cartilage layer and cortical and cancellous layers utilize the layered gradient structures and diverse compositions to realize the rigid-flexible coupling performances and excellent deformation resistances, which could effectively bear multidirectional static and dynamic loads.1 In actual, attributed to the hierarchical and ordered mineralized collagen fibrils and embedded hydroxyapatite structure, the cortical layer exhibit favorable resistance to deformation and high strength against fracture.2 Meanwhile, the articular cartilage is considered as a special connective tissue as no blood vessel, nerve, and lymphatic vessel exist in cartilage. Once injured, the cartilage is difficult to self-heal.3 Due to the composite structure consisting of chondrocyte, intercellular substance, and collagen fiber, in general, the most important role of cartilage is considered to be lubrication, energy absorption, and toughening.4 Up to now, a series of artificial cartilages have been developed to replace the natural damaged cartilage. Considering the complexity of the cartilage structure, the effects of natural cartilage on the strengthening and flexibility on composite bone structures are difficult to be replaced by artificial cartilage.1,5

The existing investigations on the mechanical properties of cartilage and cortical layer mainly focused on the independent performances, including the toughness, friction, and wear properties of cartilage,6 crack propagation path,7 distribution of surface hardness and Young’s modulus,8 and strength and toughness of the cortical layer at different scales.9,10 For instance, Fan et al. studied the deviation rate between natural knee cartilage and polyvinyl alcohol hydrogel artificial cartilage under different compression conditions,11 Tertuliano et al. prepared several pillars with various diameters to investigate the size effect-induced brittle-to-ductile transition of the cortical layer.12 Our previous studies also proposed a prediction approach of cross section fracture path of the cortical layer through establishment of nanoindentation array8 and investigated the mechanical properties of the cortical layer related to temperature and orientation of Haversian canals.2

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The independent tests after peeling the cartilage layer or cortical layer are unable to quantitatively obtain the effects of the cartilage layer on the deformation resistance and mechanical properties of the composite bone structure. Considering the toughness of cartilage and the interface binding force with the cortical layer, the protection of the cartilage layer might affect the capability of the composite bone structure to resist deformation, which needs to be taken into account.

In addition, in situ biomechanical testing could simultaneously obtain the morphological changes and the mechanical responses of composite bone structures, which could be considered as effective approach to reveal the real-time interface behaviors. Meanwhile, Raman spectrum analysis of stress condition could also be used to obtain the stress difference at various micro regions of composite bone structure through the calculation of scattering frequency shift, which would provide direct evidence to further reveal the effect of cartilage on bone mechanics. In this paper, on the basis of a novel horizontal bending tester, the in situ three-point bending tests of the cartilage layer, cortical and cancellous layers, and composite bone structure directly revealed the cartilage-induced simultaneous enhancements of bone strength and ductility. Continuous morphological changes, residual morphologies, and Raman spectroscopy analysis of the composite bone structure verified the contribution of the cartilage layer on the deformation resistance.

2. MATERIALS AND METHODS

In order to accurately apply controllable bending load and meet the requirements of real-time monitoring of microstructure evolution of the composite bone structure under a microscope, an in situ horizontal bending tester integrating with an optical microscope was developed through appropriate modification of a developed three-point bending instrument. As shown in Figure 1a, a planetary reducer, two-stage worm gears, and two ball-screws with left- and right-hand constituted a reduction mechanism with a large reduction ratio, which facilitated to realize the quasi-static bending at a low speed (<10 nm/s) and timely image acquisition with high-resolution. Especially, the ball-screws with left- and right-hand could ensure that the monitoring micro region was throughout inside the imaging field of view and did not move during the loading process. The space between a pair of parallel ball-screws facilitated the critical contact between the microscope lens and bone specimen surface to obtain the maximum magnification. Meanwhile, a pair of symmetrical back-up plates was pasted on the arc-shaped supports. With regard to the bone specimens appropriate for the three-point bending tests, rectangular composite bone structures consisting of the cartilage layer and cortical and cancellous layers were sliced from a fresh porcine femoral joint and prepared using a precise saw blade. Considering that the interface between the cartilage layer and cortical layer could be clearly identified, and the interface between the cortical and cancellous layers was difficult to be distinguished, the pure cartilage layer and the cortical and cancellous layers were prepared, respectively. Regarding the cartilage layer, cortical and cancellous layers, and composite bone structures, the contact points of the bending indenter were on the cartilage, cortical, and cartilage layers, respectively. Seen from the working principle of in situ three-point bending tests of cortical and cancellous layers or composite bone structures, as shown in Figure 1b, the used optical microscope directly observed the interface deformation behavior between the cartilage layer and cortical layer at the bending contact micro regions rather than the lateral cartilage layer. As the bending load gradually increased, the nucleation and propagation of micro crack could be real-timely captured accompanied with the bending deflection. During the in situ bending tests, the bending indenter maintained contact with the concave cartilage or cortical layer until ultimate bending failure.

3. RESULTS AND DISCUSSION

3.1. Mechanical Properties. During the three-point bending tests, rectangular bone specimens with consistent lengths of 30 mm and width of 15 mm were symmetrically placed on a pair of arc-shaped supports with spacing of 20 mm. The composite bone structures exhibited three kinds of cortical and cancellous layer thicknesses ranging from 5 to 10 mm, and the thicknesses of cartilage layers were constant 2 mm. On the basis of a bending speed of 50 nm/s, the bending load–displacement responses of pure cartilage layers, cortical and cancellous and composite bone structures were obtained, as
shown in Figure 2. In order to verify the feasibility of the cartilage layer on the bone strength enhancement, the cortical and cancellous layers were sliced with thicknesses of 5 mm ($t_1$), 8 mm ($t_2$), and 10 mm ($t_3$), respectively, which represented various thickness ratios of the cortical layer to cancellous layer. The cancellous layer was partially sliced, and the cortical layer was reserved. On the basis of a thickness of cortical and cancellous layer of 5 mm, the cortical layer was dominant. Accordingly, with regard to the specimens with thickness of 10 mm, the cancellous layer was more dominant compared with the cortical layer. The established various thickness ratios of the cortical layer to cancellous layer would facilitate the further investigation of the quantitative effects of cartilage layers on the strengthening and flexibility of bone structures. In order to reduce the measurement error of bending-displacement curves, the number of specimens with the same thickness was 12. Specifically, on the basis of thicknesses of 5, 8, and 10 mm, the cortical and cancellous layers and composite bone structures exhibited the same length of 30 mm and width of 15 mm.

increased thickness ratio of the cancellous layer to cortical layer indicated a predominant bearing capacity of the cortical layer to realize the excellent deformation resistance. Meanwhile, the composite bone structures exhibited enhanced loads and ultimate bending deflections as both the peak loads and deflections were significantly greater than the corresponding values of cortical and cancellous layers without cartilage layers. Specifically, as seen from Figure 2b, compared with the peak loads of cortical and cancellous layers, the peak loads of composite bone structures consisting of 5, 8, and 10 mm thickness cortical and cancellous layers and 2 mm thickness cartilage layer increased by 1.49-fold (70.4 ± 6.3 N), 1.90-fold (131.2 ± 9.7 N), and 2.14-fold (165.1 ± 13.2 N), respectively. Meanwhile, the corresponding ultimate bending deflections of composite bone structures increased by 1.43- to 2.12-fold, respectively. Nevertheless, the pure cartilage layer with thickness of 2 mm exhibited rather weak peak loads lower than 0.3 N but substantial ultimate bending deflections over 6 mm (Figure 2c). The low strength and high strain characteristics of cartilage have been reported by Schinagl et al. 17 Therefore, the simultaneous enhancements of bone strength and ductility of cortical and cancellous layers induced by the

Figure 2. Bending load—displacement responses of (a) cortical and cancellous layers, (b) composite bone structure, and (c) pure cartilage layers on the basis of consistent cartilage thicknesses of 2 mm and various thicknesses of cortical and cancellous layers ranging from 5 to 10 mm, the cortical and cancellous layers and composite bone structures exhibited the same length of 30 mm and width of 15 mm.

Figure 3. Statistical analyses of maximum bending loads of the cortical and cancellous layers and composite bone structures on the basis of various thicknesses ranging from 5 to 10 mm.
cartilage layer were preliminarily verified. In addition, as seen from the fracture morphologies, the cortical and cancellous layers without and with pure cartilage layers exhibited partial and entire tearing fractures. Although the peak load increased to 165.1 ± 13.2 N, no obvious bending failure was observed in the cartilage layer, and a complete cartilage structure without obvious crack tearing propagation was also confirmed.

3.2. Statistical Analyses. On the basis of the number of specimens with the same thickness of 12, the statistical analyses of maximum bending loads of cortical and cancellous layers and composite bone structures were obtained, as shown in Figure 3. With regard to the cortical and cancellous layers, the 5 mm thickness specimens exhibited an approximate Gaussian distribution with mathematical expectation of maximum bending loads $E(F_{\text{max}})$ of 47.0 N. Similarly, the $E(F_{\text{max}})$ of 8 and 10 mm thickness specimens gradually increased to 69.0 and 78.0 N, respectively, attributed to the significantly increased thicknesses. Although the maximum bending loads showed discreteness with different degrees, the discrepancy rate of $F_{\text{max}}$ defined as the ratio of minimum $F_{\text{max}}$ to maximum $F_{\text{max}}$ of specimens with constant thickness, fluctuated from 88.2 to 94.9%, which indicated the thickness-independent central tendency of $F_{\text{max}}$. Meanwhile, with regard to the composite bone structures, analogous peak distribution characteristics were obtained. The mathematical expectation $E(F_{\text{max}})$ of 5, 8, and 10 mm thickness specimens was calculated to be 69.0, 130.5, and 163.5 N, respectively. Accordingly, the discrepancy rate of $F_{\text{max}}$ exhibited a range from 84.2 to 95.6%. Therefore, the enhancement effects induced by the cartilage layer on the peak loads were verified, which was applicable for the cortical and cancellous layers with various thicknesses.

3.3. Micro Deformation Behaviors. In order to further investigate the microscopic deformation behaviors of composite bone structure and explain the strengthening and toughening mechanism of cortical and cancellous layers induced by the cartilage layer, continuous morphological changes of composite bone structure were obtained through real-time in situ characterization. As seen from Figure 4a, with regard to the cortical and cancellous layers without cartilage layer, the deflection ratio $\delta$ was defined as the ratio of current deflection to the bending deflection. On the basis of a $\delta$ of 0.7, the corresponding bending load was 54 N, and obvious micro cracks and micro voids intersected with the local tearing morphology were observed. As $\delta$ gradually increased to 0.8 and 0.9, respectively, the corresponding bending loads increased to 62 and 69 N. Evidently, the micro cracks exhibited a convergence tendency at an angle of approximate 45° to the bending axis. Meanwhile, the micro void gradually propagated to form a striped void with length of 50μm and width of 20μm. The void further extended to the main crack, the extension orientation was approximately perpendicular to the crack, which indicated a local tearing fracture mode when subjected to the bending load. Furthermore, when $\delta$ achieved the deflection of critical bending failure (0.95), the corresponding bending load increased to 73 N, and small piece was observed to separate from the cortical substrate. Regarding the interface between the cartilage layer and cortical and cancellous layers, on the basis of identical bending loads in a range from 54 to 73 N, the interface-induced deformation resistance was directly observed, as illustrated in Figure 4b. Specifically, when subjected to a bending load of 54 N, the interface was clearly visible, and no obvious cracks or voids could be identified through the optical image. When the load increased to 62 N, obvious stroma was overflowed from the cartilage layer attributed to the extrusion effect of the bending indenter, and a micro crack approximately parallel to the bending axis was also visible. As the bending load gradually increased to 69 and 73 N, significant stroma flow from the cartilage layer to the cortical layer was confirmed as the granular stroma covered on the cortical layer was visible. Meanwhile, slight cracks also converged into a main crack with an oblique orientation to the bending axis. Compared with the microscopic deformation behaviors between the cortical and cancellous layers without and with cartilage layer on the basis of identical loading conditions, also combined with the obvious shrinkage of the cartilage layer, under the protection of the cartilage layer, the damage failure of cortical and cancellous layers was effectively inhibited, and the cartilage layer-induced strengthening and toughening mechanisms of the composite bone structure were experimentally verified.

3.4. Residual Morphologies. In actual, the regions of bending-failure composite bone structure adjacent to and far away from the bending indenter were subjected to compressive and tensile stresses, respectively. The difference under stress conditions would definitely affect the microstructures and determined the potential failure mode. The SEM images of the
composite bone structure at various locations with disparate stress conditions were captured, as shown in Figure 5. As analyzed in Figure 2b, without the protection of the cartilage layer, the cortical and cancellous layers exhibited relatively smaller ultimate bending deflection $\Delta l_u$, which was defined as the distance between the maximum bending point before complete cracking and the centers of arc-shaped supports. In the compressive region (i) on the left side of the virtual neutral layer, the cracks and micro voids in the cortical layer exhibited an envelope type, and the arc center of the virtual envelope was approximately on the bending axis (Figure 5a). Meanwhile, the selected region (ii) adjacent to the curved notch along the bending axis showed a partial tearing tendency, the arc centers of tearing dimples were also located on the bending axis.19 On the basis of relatively larger ultimate bending deflection $\Delta l_{ucb}$, as shown in Figure 5b, the tearing crack propagated from the cancellous layer edge to the interface between the cortical and cartilage layers. The observation micro regions (i and ii) selected from both sides of the virtual neutral layer were both adjacent to the tearing crack. With regard to the micro region (i) with compressive stress, plenty of micro cracks with diverse sizes and a clear interface adjacent to the crack were visible.20,21 Compared with the relatively smooth and flat cortical surface, the multi-scale cracks were mainly attributed to the stress concentration nearby the crack edge caused by the transversal crack propagation from the cortical layer to the cartilage layer during the tearing deformation process. Meanwhile, the layered tearing dimple structures indicated that the dimple sizes gradually decreased as the depth increased, and the crack also propagated along the depth direction. Therefore, based on the protection of the cartilage layer, the crack propagated along both the bending direction and the depth direction simultaneously. Without cartilage layer, the crack mainly propagated along the bending direction. The crack propagation along the depth direction increased the resistance of the crack propagation along the bending direction, thus enhanced the deformation resistance. In addition, as shown in Figure 5c, with regard to the cartilage layer, the micro regions (iii and iv) denoted the compressive stress and unstressed regions, respectively. The unstressed morphology showed a uniform non-smooth surface as the “cobblestone shape” microstructures exhibited consistent aspect ratios and regular array. Nevertheless, the prismatic feature structures with relatively consistent orientations to the bending axis were observed in the compressed region. The difference in morphologies accorded with the analysis of the

Figure 5. SEM images of the composite bone structure at various locations with disparate stress conditions. Specifically, (a) illustrates the microstructures of cortical and cancellous layers at respective micro regions parallel to the bending direction with compressive and tensile stress, (b) illustrates the corresponding microstructures at similar micro regions with the cartilage layer, and (c) shows the microstructure comparison between the micro regions of cartilage layer with and without bending load.

Figure 6. (a) Raman spectroscopy analysis of the composite bone structure to indicate the enhancement of deformation resistance induced by the cartilage layer, (b) Raman spectroscopy peak analysis of the neutral layer of the composite bone structure to indicate the negligible effect of the local bending load on the stress condition change in the neutral layer.
3.5. Raman Spectroscopy Analysis. Moreover, Raman spectroscopy analysis of the composite bone structure subjected to the bending load was carried out to evaluate the stress conditions of various micro regions. As illustrated in Figure 6a, the intersection point (i) between the neutral layer and specimen edge and two points (ii and iii) along the bending axis distributed on both sides of the neutral layer were selected for the Raman scattering measurement. Theoretically, these measuring points showed obvious difference under stress conditions as the stress-free, compressive, and tensile conditions could be confirmed in corresponding micro regions. When subjected to external stress, the spectral bands sensitive to stress in the composite bone structure would produce a frequency shift of the Raman peak.22 Specifically, under compressive stress, the molecular bond distance would shorten. According to the relationship between the force constant and the bond distance, the increased distance would cause the increment of vibration frequency, the spectral bands would correspondingly move to the high frequency bands.23 With regard to the neutral layer, before applying the bending load, the Raman shift of the peak position was measured as 2930. Attributed to the stress-free condition of the neutral layer, the bending load did not induce significant Raman shift, as illustrated in Figure 6b, which indicated that the effect of the local bending load on the stress condition change in the neutral layer was negligible. Meanwhile, the regions (ii and iii) exhibited a slight spectral band shift with a frequency shift of 10 and apparently reverse frequency shift of 80, respectively. Obviously, compared with the region subjected to tensile stress with relatively larger frequency shift, the cartilage layer has restrained the reverse frequency shift and further inhibited the stress increment in the cortical layer, which facilitated to the enhancement of deformation resistance.

4. CONCLUSIONS

Cartilage layer-induced simultaneous enhancements of bone strength and ductility were experimentally verified through the obtained bending load−displacement responses, real-time morphological changes, residual morphologies at various locations with disparate stress conditions, and Raman spectroscopy analysis. Combined with the cortical and cancellous layers without cartilage layer, the peak loads and ultimate bending deflections of composite bone structures increased by 1.49- to 2.14-fold and 1.43- to 2.12-fold, respectively, in terms of thicknesses of cortical and cancellous layers ranging from 5 to 10 mm. Continuous morphological changes of composite bone structures revealed the stroma flow-dependent strengthening and toughening mechanisms as significant stroma flow from the cartilage layer to the cortical layer combined with the shrinkage of the cartilage layer was observed. With the protection of the cartilage layer, the residual morphologies indicated a layered tearing dipple feature in the cortical layer, which indicated that the crack propagated along both the bending direction and the depth direction simultaneously. The orthogonal propagation of crack increased the resistance of the crack propagation and further enhanced the deformation resistance. Furthermore, Raman spectroscopy analysis also revealed a relatively smaller frequency shift in the compressed cortical layer attributed to the cartilage layer, which indicated that the cartilage layer has restrained the reverse frequency shift and contributed to the stress decrement in the cortical layer.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding authors on reasonable request.

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Notes
The authors declare no competing financial interest.

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REFERENCES

(1) Huey, D. J.; Hu, J. C.; Athanasiou, K. A. Unlike bone, cartilage regeneration remains elusive. Science 2012, 338, 917−921.
(2) Ma, Z.; Qiang, Z.; Zhao, H.; Piao, H.; Ren, L. Mechanical properties of cortical bones related to temperature and orientation of Haversian canals. Mater. Res. Express 2020, 7, 015408. DOI: 10.1088/2053-5073/ab6899.
(3) Shi, W.; Sun, M.; Hu, X.; Ren, B.; Cheng, J.; Li, C.; Duan, X.; Fu, X.; Zhang, J.; Chen, H.; Ao, Y. Structurally and functionally optimized silk-fibroin-gelatin scaffold using 3D printing to repair cartilage injury in vitro and in vivo. Adv. Mater. 2017, 29, 1701089.
(4) Moutous, F. T.; Freed, L. E.; Guilk, F. A biomimetic three-dimensional woven composite scaffold for functional tissue engineering of cartilage. Nat. Mater. 2007, 6, 162−167.
(5) Lin, W.; Klein, J. Recent progress in cartilage lubrication. Adv. Mater. 2021, 33, 2005513.
(6) Parkes, M.; Tallia, F.; Young, G. R.; Cann, P.; Jones, J. R.; Jeffers, J. R. T. Tribological evaluation of a novel hybrid for repair of articular cartilage defects. Mater. Sci. Eng., C 2021, 119, 111495−111500.
(7) Khor, F.; Cronin, D. S.; Watson, B.; Gierczycka, D.; Malcolm, S. Importance of asymmetry and anisotropy in predicting cortical bone response and fracture using human body model femur in three-point bending and axial rotation. J. Mech. Behav. Biomed. Mater. 2018, 87, 213−229.
(8) Ma, Z.; Qiang, Z.; Zeng, K.; Xiao, J.; Zhou, L.; Zu, L.; Zhao, H.; Ren, L. Prediction of cross section fracture path of cortical bone
through nanoindentation array. *J. Mech. Behav. Biomed.* 2021, 116, 104303–104340.
(9) Ma, Z.; Qiang, Z.; Guo, C.; Jiang, Y.; Zhao, H.; Wen, C.; Ren, L. Aggravated stress fluctuation and mechanical size effects of nanoscale lamellar bone pillars. *NPG Asia Mater.* 2021, 13, 61.
(10) Ma, Z.; Qiang, Z.; Guo, C.; Jiang, Y.; Zhao, H.; Wen, C.; Ren, L. Disparate micro-mechanical behaviors of adjacent bone lamellae through in situ SEM micropillar compression. *Mater. Sci. Eng., A* 2021, 825, 141903.
(11) Fan, Y.; Zhou, G.; Zhang, G.; Li, F. Comparative study on the mechanical behavior of the interface between natural cartilage and artificial cartilage. *Soft Mater.* 2021, 19, 400–419.
(12) Tertuliano, O. A.; Greer, J. R. The nanocomposite nature of bone drives its strength and damage resistance. *Nat. Mater.* 2016, 15, 1195–1202.
(13) Ma, Z.; Zhang, H.; Liu, D.; Zhao, H.; Feng, Y.; Ren, L. In situ observation on the failure behavior of ZrO2-resin-dentin bonding interface with prefabricated indentation defects. *Mater. Res. Express* 2020, 7, 085401.
(14) Shang, L.-W.; Fu, J.-J.; Ma, D.-Y.; Zhao, Y.; Huang, B.-K.; Yin, J.-H. Raman spectroscopic study and identification of multi-period osteoarthritis of canine knee joint. *Appl. Phys. B: Lasers Opt.* 2021, 127, 1–9.
(15) Ma, Z.; Zhao, H.; Hu, X.; Cheng, H.; Lu, S.; Zhang, L. Influences of tensile pre-strain and bending pre-deflection on bending and tensile behaviors of an extruded AZ31B magnesium alloy. *Mater. Des.* 2014, 64, 566–572.
(16) Ma, Z.; Zhao, H.; Huang, H.; Zhang, L.; Wang, K.; Zhou, X. A novel tensile device for in situ scanning electron microscope mechanical testing. *Exp. Tech.* 2015, 39, 3–11.
(17) Schinagl, R. M.; Gurskis, D.; Chen, A. C.; Sah, R. L. Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. *J. Orthop. Res.* 1997, 15, 499–506.
(18) Han, J.; Cuomo, R.; Zhao, Y.; Pan, B.; Yang, Q. The Morphology and Bending Behavior of Regenerated Costal Cartilage with Kawanabe-Nagata Method in Rabbits - the Short Term Result of an Experimental Study. *J. Invest. Surg.* 2020, 34, 1047–1051.
(19) Coen, M. J.; Caborn, D. N. M.; Johnson, D. L. The dimpling phenomenon: Articular cartilage injury overlying an occult osteochondral lesion at the time of anterior cruciate ligament reconstruction. *Arthroscopy* 1996, 12, 502–505.
(20) Vendra, B. B.; Roan, E.; Williams, J. L. Chondron curvature mapping in growth plate cartilage under compressive loading. *J. Mech. Behav. Biomed. Mater.* 2018, 84, 168–177.
(21) Sadeghi, H.; Espino, D. M.; Shepherd, D. E. T. Fatigue strength of bovine articular cartilage-on-bone under three-point bending: the effect of loading frequency. *BMC Musculoskel. Disord.* 2017, 18, 142.
(22) Srikar, V. T.; Swan, A. K.; Unlu, M. S.; Goldberg, B. B.; Spearin, S. M. Micro-Raman measurement of bending stresses in micromachined silicon flexures. *J. Microelectromech. Syst.* 2003, 12, 779–787.
(23) Shaikh, R.; Nippolainen, E.; Virtanen, V.; Torniainen, J.; Rieppo, L.; Saarakkala, S.; Afara, I. O.; Töyräs, J. Raman spectroscopy is sensitive to biochemical changes related to various cartilage injuries. *J. Raman Spectrosc.* 2021, 52, 796–804.