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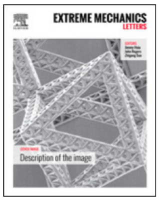

Nonlinear elastic behaviors and deformation mechanisms of nano-structured crosslinked biopolymer networks

Xiaobo Wang [a,b], Hanxing Zhu [a,*], Bo Song [b,*], Xindong Chen [a,c], David Kennedy [a], Yusheng Shi [b]

[a] School of Engineering, Cardiff University, Cardiff, CF24 3AA, UK
[b] School of Materials Science and Engineering, Huazhong University of Science and Technology, Wuhan, 430074, China
[c] Institute of Biomechanics and Medical Engineering, Department of Engineering Mechanics, Tsinghua University, Beijing, 100084, China

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ABSTRACT

Cells always undergo large deformation in response to external stimulations or internal stresses in many cell functions (e.g., cell migration, cell division and cell growth). When undergoing large deformation, crosslinked actin filament networks (CAFNs) always show strong nonlinear elasticity to maintain the cell shape and integrity, known as strain stiffening, which plays a crucial role in many cell functions. To investigate the nonlinear elastic behaviors of CAFNs, a three-dimensional representative volume element model is used to perform finite element method simulations. Simulation results show that actin filament volume fraction, crosslinking density and components' Young's moduli show significant influences on the nonlinear elastic behaviors of CAFNs. In addition, the shear stress–strain curves of CAFNs highly depend on the bending stiffness and tensile stiffness of filamins as well as the bending stiffness of actin filaments, however, they are almost insensitive to the tensile stiffness of actin filaments. The present work not only sheds light on the nonlinear elastic behaviors of CAFNs but also provides a valuable reference for developing advanced artificial composite structures that can be used as semi-flexible biomedical scaffolds and wearable electronics.

1. Introduction

In living cells, both internal and external stresses could cause large deformations of the cytoskeleton, which has been proved to play important roles in many cell functions (e.g., cell migration, cell division and cell growth) [1–3]. The cell cytoskeleton is a complex composite network structure consisting of filaments and tubules existing in all cells [4], and it is crucial in maintaining cell shape, signal transduction and molecule transportation. The cell cytoskeleton mainly consists of three types of proteins known as microtubules [5], intermediate filaments [6] and actin filaments [7]. Actin filaments can be connected into different structures (e.g., crosslinked network [8], branched network [9], actin bundle [10] and stress fiber [11]) by distinct actin binding proteins (e.g., filamin, Arp2/3, α-Actinin and myosin). These substructures of cell cytoskeleton extend throughout the cytoplasm and interweave with each other to form the whole cytoskeleton. As a main substructure of the cytoskeleton, crosslinked actin filament networks (CAFNs) have attracted much attention in recent years [12–15]. The CAFNs are nano-structured composite biopolymer structures consisting of actin filaments crosslinked by filamins, and they appear to be three-dimensional (3D) porous structures just beneath the plasma membrane. They have been confirmed to play crucial roles in maintaining the cell shape and determining the mechanical properties of living cells [16–18]. Due to the unique architecture of CAFNs, they are found to possess nonlinear elastic behaviors under large deformation, which is known as strain stiffening. And the strain-stiffening behavior of CAFNs is proved to play important roles in many cell functions [19]. In addition, recent works revealed that intermediate filaments are also critical for the nonlinear elasticity of living cells [6], indicating that the crosstalk of CAFNs with other cytoskeletal substructures (e.g., intermediate filaments, microtubules and stress fibers) has potential influences on the mechanical behaviors of living cells. Thus, investigating the nonlinear elastic behaviors of CAFNs becomes more and more important for understanding the mechanical behaviors of cytoskeletons and living cells.

In recent years, both *in vivo* and *in vitro* experiments have been conducted to study the nonlinear elastic behaviors of actin filament networks [16,20–24]. Though *in vivo* experiments could precisely capture the mechanical responses of actin filament networks, it is always difficult to recognize the components' contents. As *in vitro* experiments are conducted on reconstituted

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[*] Corresponding authors.
E-mail addresses: zhuh3@cardiff.ac.uk (H. Zhu), bosong@hust.edu.cn (B. Song).

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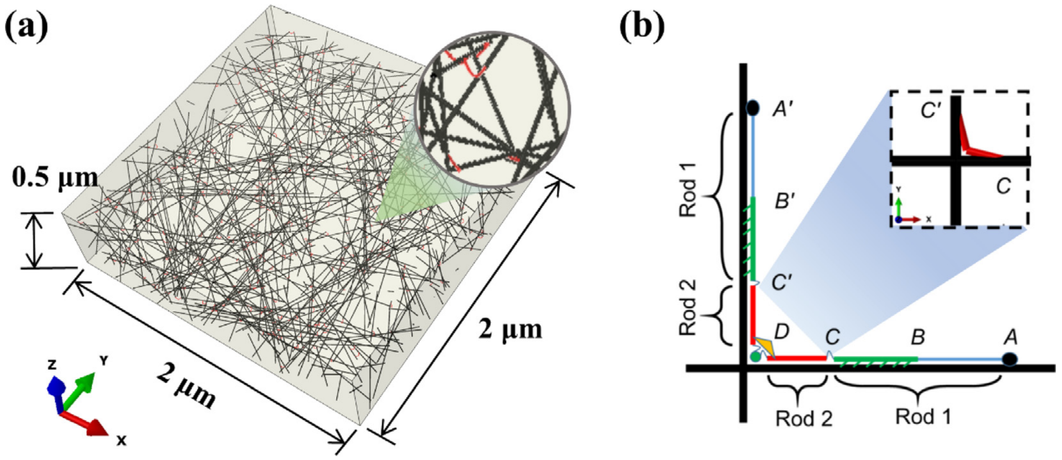

Fig. 1. (a) The RVE model of CAFNs for FEM simulations. The width and length of the RVE are 2 μm, and the height of the RVE is 0.5 μm. The black fibers are actin filaments, and the curved beams in red are filamins. (b) The crosslinking mechanism between actin filaments and filamins: *A* and *A'* refer to the actin-binding domains (black solid points), *AB* and *A'B'* refer to the Ig1-8 of filamin dimer (blue segments), *BC* and *B'C'* refer to the Ig9-15 of filamin dimer (green segments), *C* and *C'* are Hinge-1 of filamin dimer, *CD* and *C'D'* refer to the Ig16-23 of filamin dimer which are also known as rod 2 segment (red segments), *D* refers to the dimerization (green solid point), the yellow solid triangle is FilGAP: an FLNA-binding RhoGTPase-activating protein. The inset indicates one of the crosslinkers in the FEM model.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

actin filament networks [16,20], it is easy to control the contents of actin filaments and actin binding proteins (e.g., filamins). This could help us to reveal the dependences of nonlinear elastic behaviors of CAFNs on the contents of actin filaments or actin binding proteins [23]. But the effects of Young's moduli, shear moduli and stiffnesses of actin filaments and filamins on the nonlinear elastic behaviors of CAFNs are difficult to be revealed by conducting *in vivo* or *in vitro* experiments as they are almost constant in experimental conditions. Numerical simulation is proved to be an effective way to investigate the mechanical behaviors of complex structures by applying reasonable assumptions and proper boundary conditions, and both two-dimensional (2D) and 3D network models have been developed according to the practical architecture of CAFNs [25–34]. However, most of them ignore the compliant nature of some actin binding proteins with large contour lengths (e.g., filamins and α-actinin). To precisely mimic the mechanical behaviors of compliant actin binding proteins, network models with semi-flexible and deformable crosslinkers have been proposed [28,35].

Though many works have been carried out to study the mechanical properties of CAFNs based on 3D network models, they mainly focus on the mechanical responses of CAFNs with specific actin filament volume fraction, crosslinking density and material properties. The effects of actin filament volume fraction, crosslinking density and material properties on the nonlinear elastic behaviors of CAFNs are not clear. In addition, there is little research concerning the deformation mechanisms of CAFNs in large strain regimes. In the present work, a 3D representative volume element (RVE) model is used to mimic actin filament networks crosslinked by filamins, and simulations are performed via the finite element method to capture the nonlinear elastic behaviors and deformation mechanisms of CAFNs. Simulation results show good agreement with experimental measurements, indicating the validity of our model in mimicking the large deformation behaviors of CAFNs. Simulation results demonstrate that the nonlinear elastic behaviors of CAFNs highly depend on actin filament volume fraction, crosslinking density and components' Young's moduli. Results obtained in the present work not only provide an insight into the mechanical responses of CAFNs but also help to develop advanced artificial composite structures that can be used as semi-flexible biomedical scaffolds and wearable electronics.

2. Methods

2.1. Geometry and materials

Numerical simulation is proved to be an effective way to study the mechanical behaviors of crosslinked biopolymer networks. In the present work, a 3D finite element model is used to mimic the mechanical responses of CAFNs according to their architecture in living cells. The details of developing the model have been elaborated in our previous work [35]. The model is presented as a square plate-like representative volume element (RVE), and the dimension of the RVE is 2 μm × 2 μm × 0.5 μm. Actin filaments are randomly generated by determining their lengths, orientations and central points. As the contour lengths of actin filaments are much smaller than their persistence lengths, actin filaments are modeled by straight elastic rods with the length obeying the exponential distribution law. Curved elastic beams are used to model filamins that prefer to crosslink actin filaments into orthogonal networks. The crosslinking angles are assumed to range from 60 degrees to 120 degrees, with an average value being 90 degrees. In living cells, unbinding and rebinding between actin filaments and filamins are dynamic processes and occur all the time. In the present work, as we focus on investigating the effects of volume fraction and material properties of actin filament and filamin on the mechanical behaviors of crosslinked actin filament networks under larger shear strain by performing static mechanics analysis, the unbinding and rebinding between actin filaments and filamins have not been considered. But it is a striving direction deserving our further attention to consider those mechanisms when constructing models. For simplicity, the binding points between actin filaments and filamins are regarded as permanent rigid points with all of the six degrees of freedom being constrained. The actin filament volume fraction, V_f, and crosslinking density, ρ_c, are defined as:

$$\begin{cases} V_f = \dfrac{\sum_{i=1}^{N} l_i \times A_f}{W \times L \times H} \\ \rho_c = n_c / L_t \end{cases}, \tag{1}$$

where W, L, and H are the width, length and height of the RVE model, N is the number of actin filaments, l_i refers to the length of actin filament i, A_f indicates the cross-sectional area of the actin filament, n_c and L_t are the total number of filamins and total length of actin filaments in the RVE model, respectively. Fig. 1

Table 1
The geometrical and material parameters of actin filament and filamin used in simulations.

Material	Mean length (μm)	Young's modulus (Pa)	Poisson's ratio	Cross-sectional type	Equivalent cross-sectional radius (nm)	Cross-sectional area (nm^2)
Actin filament	1	23E+08	0.35	circle	2.52	19.95
Filamin	0.04	12E+06	0.35	circle	1.80	10.18

shows the representative volume element model of CAFNs developed in this work for performing finite element method (FEM) simulations. According to the crosslinking mechanism shown in Fig. 1(b), the filamin dimer is composed of two arms, and each arm includes Ig 1–23. For one of the arms, point *A* and segment *BC* correspond to the binding domains between filamin and actin filament. Because it is a very complicated process to consider the unbinding behavior during the FEM simulations, for simplicity, we simply assume that point *A* and segment *BC* bind with actin filament permanently without unbinding. Therefore, *C* and *C'* are regarded as the binding points which are represented by shared nodes in the FEM model. That is to say, segment *CDC'* (i.e., the curved beam in red) is used to model the filamins in FEM simulations.

In the present model, actin filaments and filamins are modeled by straight and curved elastic beams with circular cross-sections, respectively. And it is assumed that the effective radius of the cross-sections of actin filaments and filamins are 2.5 nm and 1.8 nm [35], respectively. Actin filaments are generated according to an exponential probability density function, and only actin filaments with lengths between 0.05 μm to 2 μm have been generated [35]. The total lengths of curved beams used for modeling filamin dimers are 0.04 μm, and the curved angles depend on the included angles between actin filaments. In living cells, both actin filaments and filamins are biopolymers with hyperelastic and viscoelastic properties. As we mainly focus on the effects of stiffnesses of actin filament and filamin on the mechanical behaviors of CAFNs and the geometrical nonlinearity of CAFNs, the materials are simply assumed to be isotropic linear elastic in the present work. Both actin filaments and filamins are regarded as homogeneous isotropic elastic materials whose properties can be simply determined by Young's modulus and Poisson's ratio. According to the mechanical responses of a single actin filament and filamin [36,37], Young's modulus of actin filament and filamin is 2.3 GPa and 12 MPa, respectively, and the Poisson's ratios of actin filament and filamin are 0.35. The material parameters used in FEM simulations are listed in Table 1. Though actin filaments and filamins are simply assumed to be homogeneous isotropic elastic materials in this work, the effects of material nonlinearity on the mechanical behaviors of CAFNs deserve more attention in further study.

2.2. Meshing and boundary conditions

To perform FEM simulations, actin filaments are meshed by Timoshenko beam elements (B32) with an element size of 40 nm, and filamins are discretized into 8 equal-size Timoshenko beam elements (B32). For RVE models, periodic boundary conditions (PBCs) are more suitable than other boundary conditions when performing FEM simulations [35,38,39]. When applying periodic boundary conditions, corresponding nodes on opposite faces are constrained to have the same displacement and rotation angle. As CAFNs often possess a larger in-plane dimension compared with the out-of-plane dimension, and mainly bear in-plane stress, PBCs are applied to the in-plane directions. The equations of PBCs can be written as:

$$\begin{cases} \mu_{a'} - \mu_a = \mu_{ref'} - \mu_{ref} \\ v_{a'} - v_a = v_{ref'} - v_{ref} \\ w_{a'} - w_a = w_{ref'} - w_{ref} \\ \theta_{a'} = \theta_a \end{cases}, \tag{2}$$

where *a'* and *a* refer to the two corresponding nodes on a pair of opposite faces, $\mu_{a'}$ and μ_a denote the deformation of nodes *a'* and *a* in *x* direction, $\mu_{ref'}$ and μ_{ref} denote the displacement of the two corresponding reference points in *x* direction, $\theta_{a'}$ and θ_a denote the rotation angles of nodes *a'* and *a*, *v* and *w* refer to the displacement in *y* and *z* directions, respectively. For out-of-plane direction, free boundary conditions are applied by simply ignoring the interactions between the cell cortex and plasm membrane.

3. Nonlinear elastic behaviors

3.1. Stress–strain relationships of CAFNs

To study the nonlinear elasticity of CAFNs, a relatively large shear strain is incrementally applied to the RVE model when performing FEM simulations. The shear stress, τ, and shear strain, γ, are determined by:

$$\begin{cases} \gamma = \alpha + \beta \approx \tan\alpha + \tan\beta \\ \tau = F/A \end{cases}, \tag{3}$$

where α and β are the changes of the included angle between two neighboring edges (i.e., edges along the *x* and *y* axes) of the RVE model caused by applying displacement boundary conditions, *F* denotes the reaction force of the reference point, *A* is the area of loading surface. The shear stress–strain curves and tangent moduli of CAFNs are obtained and displayed in Fig. 2, and they show good agreement with experimental results reported in the literature [16], indicating the validity of our model in mimicking the mechanical responses of CAFNs. In the small strain stage, the shear stress increases almost linearly with the applied strain, which results in a constant tangent modulus. In the large strain stage, the shear stress tends to increase nonlinearly with the applied strain, and consequently results in a nonlinear increase of tangent modulus. It can be also found that the tangent modulus could be enhanced by more than one order of magnitude when the applied strain reaches 0.5.

3.2. Effects of components' contents on nonlinear elastic behaviors of CAFNs

As CAFNs are composite structures consisting of two distinct components, the contents of components can no doubt influence the mechanical responses of CAFNs. To reveal the effects of actin filament volume fraction and crosslinking density on the nonlinear elastic behaviors of CAFNs, different actin filament volume fractions or crosslinking densities are applied in FEM simulations with the other being fixed at a constant level. The actin filament volume fraction is changed by controlling the number of fibers generated in the model via Python scripting. By setting up a target actin filament volume fraction, the fiber-generating process will be terminated once the actin filament volume fraction reached our preset target. The crosslinking density is also regulated by a similar program. When fixing crosslinking density at 1.2, simulation results show that the larger the V_f, the stiffer the CAFNs, and the earlier the transition from the linear elastic stage to the nonlinear elastic stage (Fig. 3). When actin filament volume

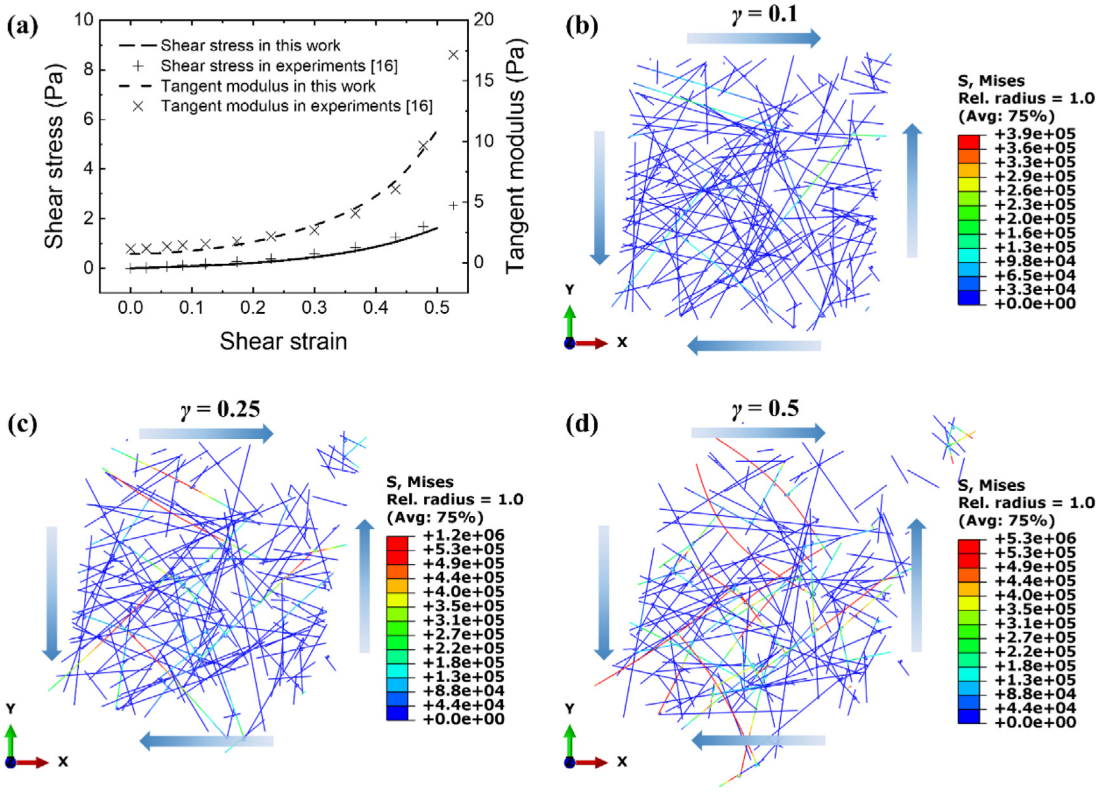

Fig. 2. (a) The stress–strain relationship and tangent modulus of CAFNs obtained from FEM simulations compared with experimental measurements [16]. (b-d) Stress distributions of CAFNs under shear strains of 0.1, 0.25 and 0.5. The arrows indicate the shear direction, and the color axes refer to the magnitude of von Mises stress. The volume fraction of actin filament, V_f, is 0.2%, and the crosslinking density, ρ_c,is 3.0.

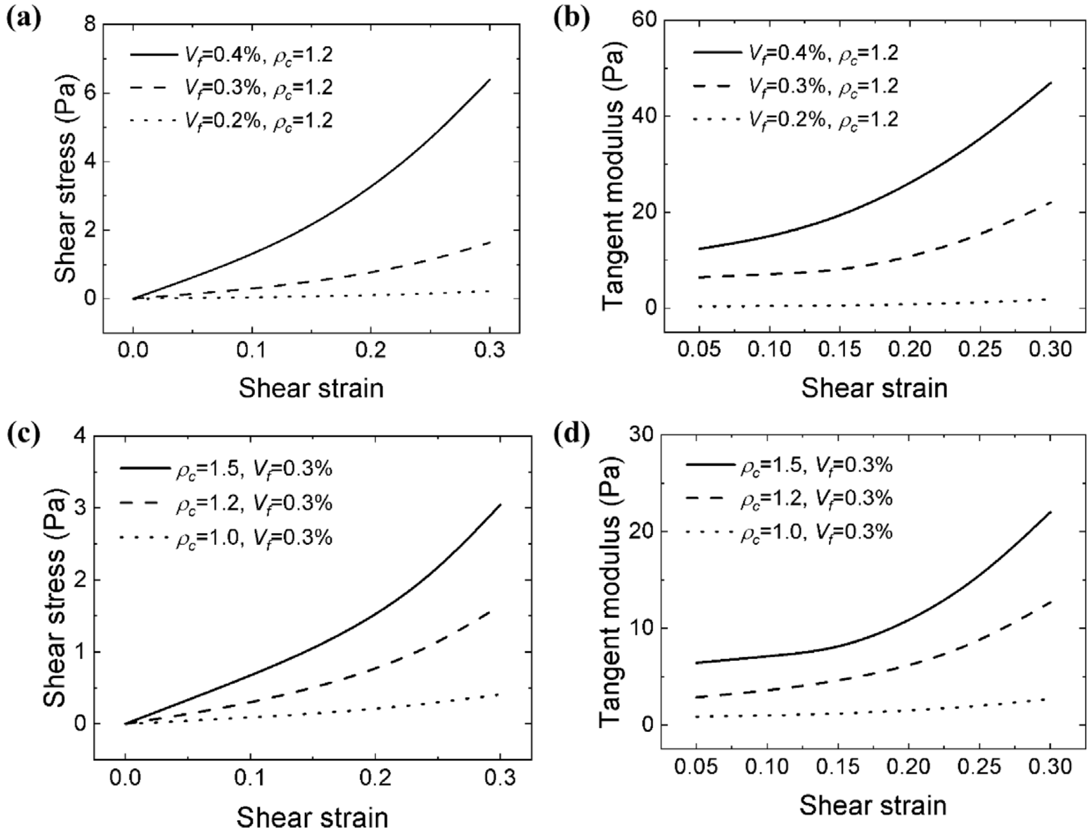

Fig. 3. Influences of actin filament volume fraction (a-b) and crosslinking density (c-d) on the shear stress–strain relationship and tangent modulus of CAFNs.

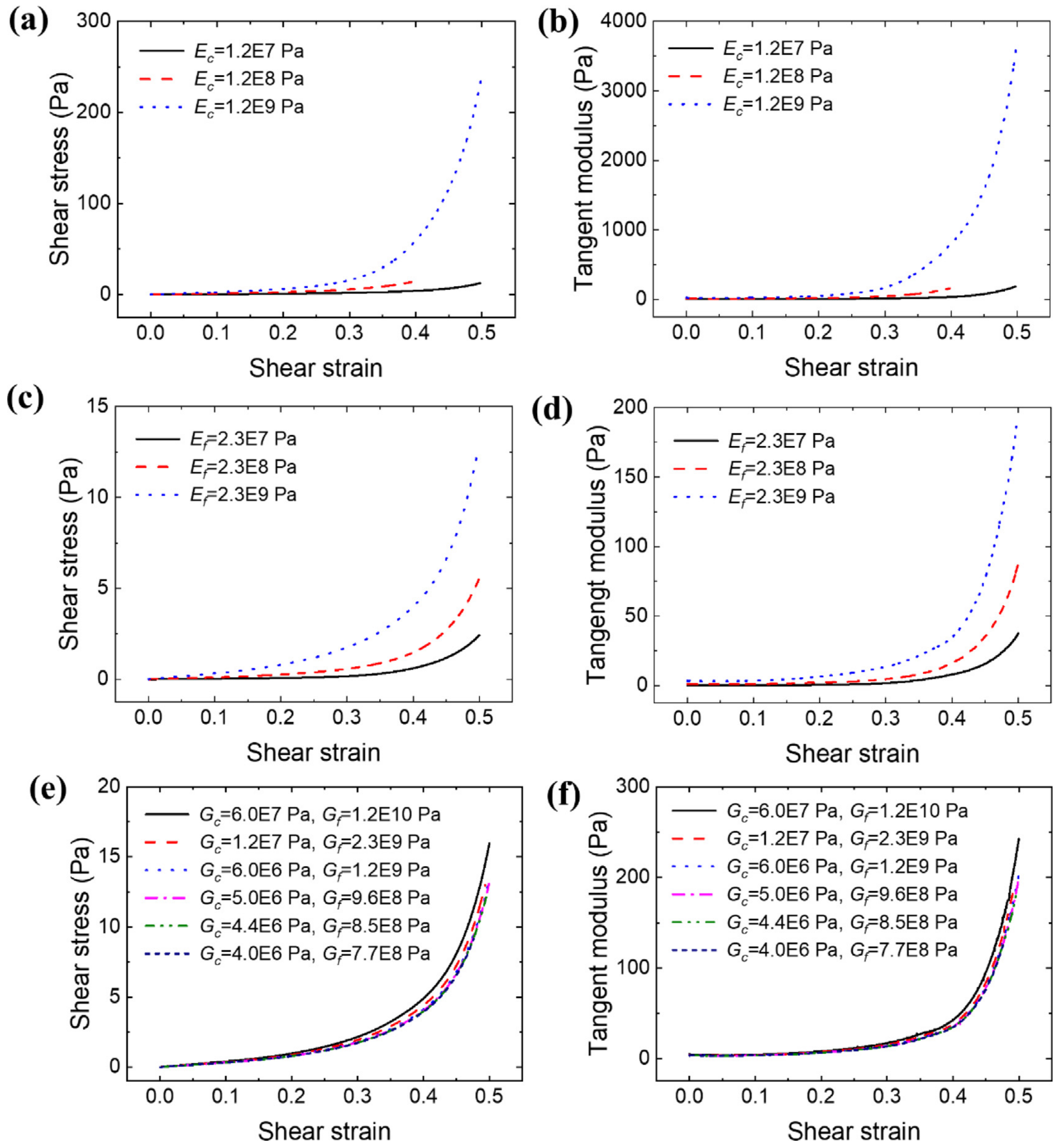

Fig. 4. Dependences of the shear stress–strain relationships and tangent moduli of CAFNs on the components' Young's moduli (a-d) and shear moduli (e-f), where V_f =0.3% and $\rho_c = 1.2$.

fraction is increased from 0.2% to 0.3%, the tangent modulus of CAFNs increases by about one order of magnitude. If the volume fraction of actin filaments is improved from 0.3% to 0.4%, the tangent modulus of the network increases by more than 100%. This indicates that the influence of actin filament volume fraction on the nonlinear elasticity of CAFNs is more crucial when the volume fraction of actin filaments is small. To investigate the influence of crosslinking density on the nonlinear elastic behaviors of CAFNs, three different levels of crosslinking density ($\rho_c = 1.0$, 1.2, 1.5) are applied with actin filament volume fraction being fixed at 0.3%. According to FEM simulations, it can be found that CAFNs with larger crosslinking density possess larger stress. In addition, larger crosslinking density could result in an earlier onset of strain stiffening. When increasing the crosslinking density, ρ_c, from 1.0 to 1.2, the shear stress and tangent modulus increase by more than 200%, and they increase by 100% when ρ_c is increased from 1.2 to 1.5.

3.3. Effects of components' Young's moduli and shear moduli on nonlinear elastic behaviors of CAFNs

In the present work, both actin filaments and filamins are assumed to be homogeneous isotropic elastic materials with only two independent elastic constants including Young's modulus E and Poisson's ration ν. For isotropic elastic materials, the shear modulus G could be determined by the Young's modulus and Poisson's ratio of material as $G = E/2(1+\nu)$. The components' material properties (e.g., Young's modulus and shear modulus) could affect the mechanical behaviors of CAFNs but their effects on the mechanical behaviors of CAFNs have not been revealed. To probe the effects of Young's moduli and shear moduli of actin filaments and filamins on the nonlinear elastic behaviors of CAFNs, the values of Young's moduli and Poisson's ratios of actin filaments and filamins are changed in FEM simulations. FEM simulation results show that components' Young's moduli can greatly affect the stress–strain relationships of CAFNs (Fig. 4(a)–(d)). The larger

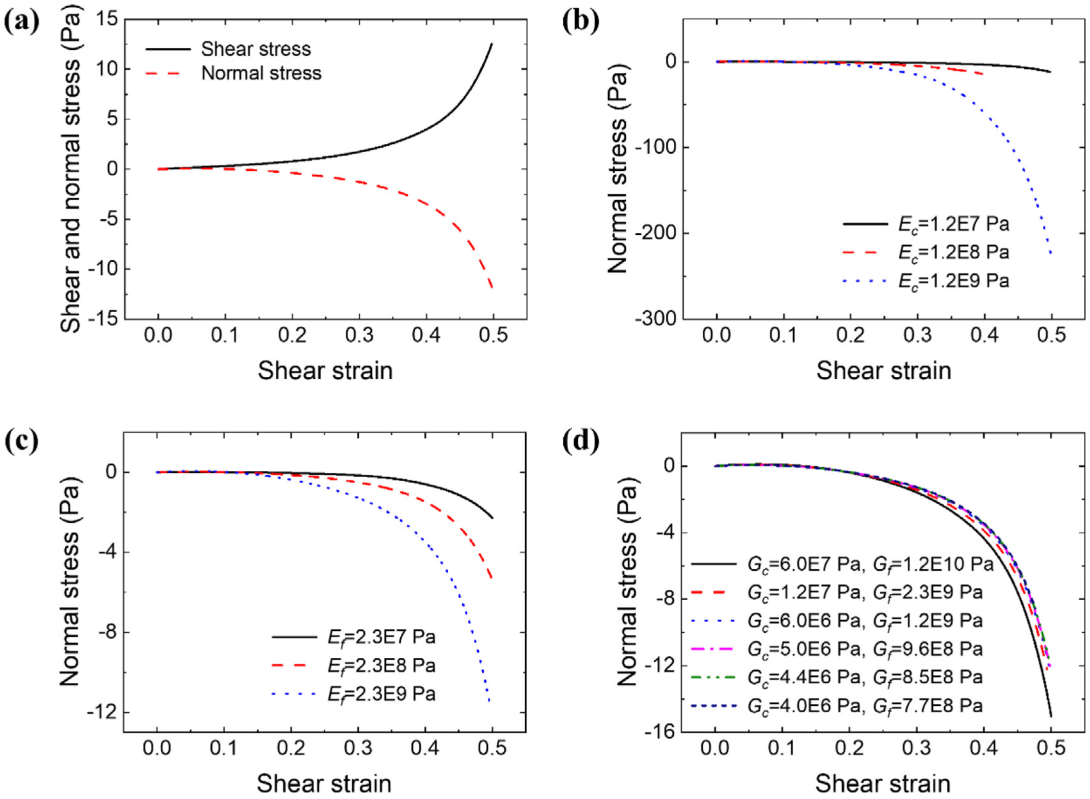

Fig. 5. (a) Comparison between the normal stress and shear stress of CAFNs. (b-d) Dependences of the normal stress of CAFNs on Young's moduli (b-c) and shear moduli (d) of actin filament and filamin, where V_f =0.3% and $\rho_c = 1.2$.

components' Young's moduli, the larger the shear stresses and tangent moduli of CAFNs. When Young's modulus of filamin, E_c, is increased from 12 MPa to 120 MPa, the shear stresses and tangent moduli of CAFNs increase by more than 200%. When Young's modulus of filamin, E_c, is decreased by one order of magnitude to 1.2 MPa, the shear stresses and tangent moduli of CAFNs decrease by 75%. When decreasing Young's modulus of the actin filament, E_f, by one order of magnitude to 230 MPa, the shear stresses and tangent moduli of CAFNs reduce by more than 50%. By reducing E_f from 230 MPa to 23 MPa, the shear stresses and tangent moduli of CAFNs reduce about 75%. These results suggest that both Young's moduli of filamins and actin filaments can greatly affect the nonlinear elastic behaviors of CAFNs, and filamins play a more significant role than actin filaments.

The effects of shear moduli of filamins and actin filaments on the nonlinear elastic behaviors of CAFNs are studied by changing their Poisson's ratios when performing FEM simulations (Fig. 4(e)–(f)). For simplicity, Poisson's ratios of filamins and actin filaments are assumed to be the same in simulations. When the Poisson's ratio is increased from −0.9 to 0.49, the shear modulus of filamins, G_c, is accordingly changed from 6.0E7 Pa to 4.0E6 Pa, and the shear modulus of actin filaments, G_f, is accordingly changed from 1.15E10 Pa to 7.7E8 Pa, however, the shear stresses and tangent moduli of CAFNs just change slightly. This indicates that components' shear moduli just have slight influences on the nonlinear elastic behaviors of CAFNs.

3.4. Negative normal stress of CAFNs

Most of natural materials tend to expand in the normal direction under shear strain, which could result in positive normal stress. However, crosslinked biopolymer networks are reported to exhibit negative normal stresses [40]. Due to the strain-stiffening behaviors of the crosslinked biopolymer networks, the normal stresses of crosslinked biopolymer networks, σ_n, could be as large as the shear stresses. Thus, investigating the relationships between negative normal stresses and applied shear strains is of major significance to probe the nonlinear elastic behaviors of CAFNs. To obtain the negative normal stresses of CAFNs, FEM simulations are performed on the RVE model ($V_f = 0.3\%$, $\rho_c = 1.2$). According to the simulation results shown in Fig. 5(a), the normal stresses of CAFNs are negative, and their absolute values increase nonlinearly with the applied shear strain. In addition, the absolute values of normal stresses are comparable with the shear stresses, implying that the normal stresses also play important roles in the nonlinear elastic behaviors of CAFNs.

The effects of Young's moduli and shear moduli of actin filaments and filamins on the normal stresses of CAFNs are also studied by changing material parameters (i.e., Young's modulus and Poisson's ratio) in FEM simulations, and simulation results are shown in Fig. 5(b)–(d). When increasing Young's moduli of actin filaments or filamins, the absolute value of normal stress increases, and Young's modulus of filamin presents more significant influences on the normal stresses of CAFNs. When varying the components' shear moduli by changing Poisson's ratio from −0.9 to 0.49, the normal stresses of CAFNs just change slightly, and the absolute values of the normal stresses of CAFNs decrease slightly. For isotropic materials, a smaller Poisson's ratio results in a larger shear modulus, which further brings higher torsional stiffness. The increases in components' torsional stiffnesses tend to make stiffer CAFNs, which could well explain the effects of components' shear moduli on normal stresses of CAFNs.

4. Deformation mechanisms

In physiological conditions, living cells could bear large deformations in response to external and internal mechanical stimulations. When applying large strains to CAFNs, they exhibit complex

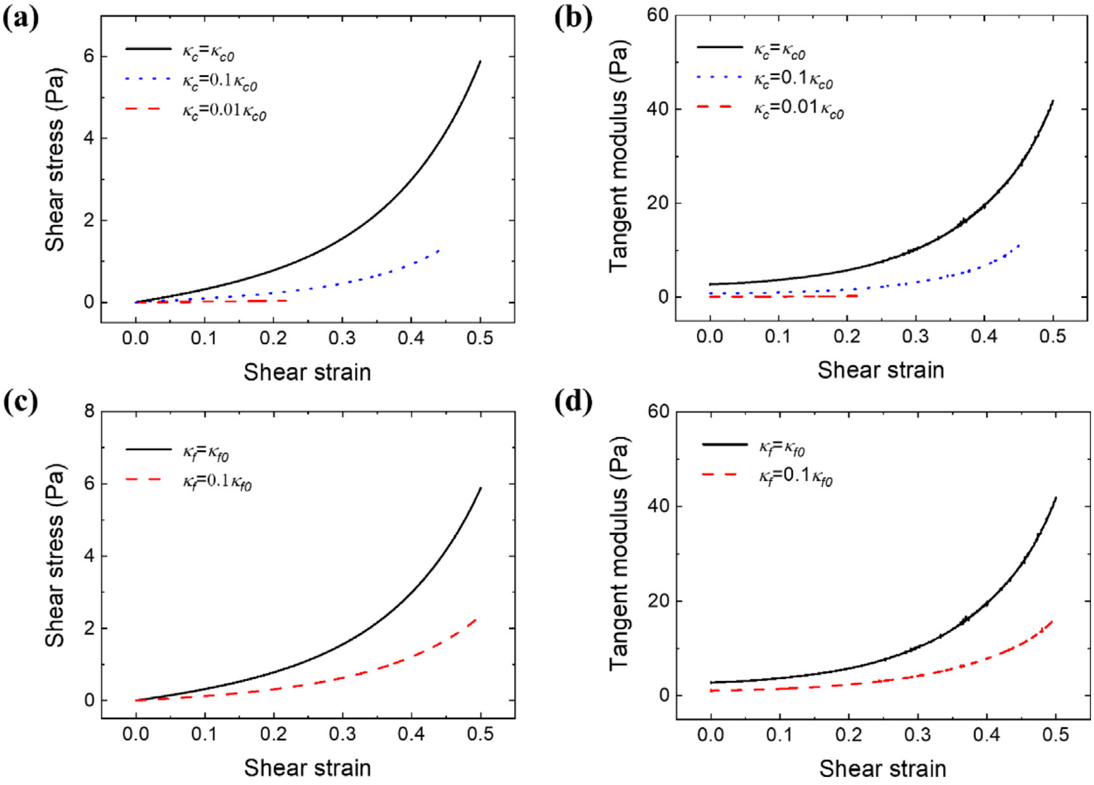

Fig. 6. (a)–(b) Effects of the bending stiffness of filamins on the stress–strain relationships and tangent moduli of CAFNs. (c)–(d) Effects of the bending stiffness of actin filaments on the stress–strain relationships and tangent moduli of CAFNs. Where V_f =0.3% and $\rho_c = 1.2$.

mechanical responses that play important roles in many cell functions. Therefore, it is essential to investigate the deformation mechanisms of CAFNs in large strain regimes. The deformations of filamins and actin filaments could be split into three main parts (i.e., bending, torsion and tension), and the effects of components' cross-sectional bending stiffnesses, torsional stiffnesses and tensile stiffnesses on the nonlinear elastic behaviors of CAFNs are investigated by conducting FEM simulations. The cross-sectional bending, torsional and tensile stiffnesses of actin filaments and filamins can be determined by:

$$\begin{cases} \kappa = EI_z = E\pi d^4/64 \\ \lambda = GI_p = G\pi d^4/32 \\ \mu = EA = E\pi d^2/4 \end{cases}, \tag{4}$$

where κ, λ and μ are cross-sectional bending, torsional and tensile stiffnesses, E and G denote Young's modulus and shear modulus respectively, I_z refers to the area moment of inertia, I_p is the polar moment of inertia, and A is the cross-sectional area. When investigating the effects of cross-sectional bending, torsional and tensile stiffnesses of actin filaments and filamins on the nonlinear elastic behaviors of CAFNs, only one of the stiffnesses is changed with the other two stiffnesses being fixed. As the torsional stiffness could be changed by varying the value of Poisson's ratio, the effects of torsional stiffness on the nonlinear elastic behaviors of CAFNs are in accordance with the shear moduli discussion in Section 3.3. This indicates that the torsional stiffnesses of actin filaments and filamins only affect the nonlinear elastic behaviors of CAFNs slightly. Therefore, only the effects of cross-sectional bending and tensile stiffnesses of actin filaments and filamins on the nonlinear elastic behaviors of CAFNs are discussed in this part.

4.1. Effects of components' bending stiffnesses on nonlinear elastic behaviors of CAFNs

When conducting FEM simulations, both actin filaments and filamins are modeled by deformable elastic beams with a large aspect ratio. The bending of beams is proved to take a considerable part of the total deformation in small strain regimes, however, its roles in large strain regimes remain unclear. In the present work, κ_{c0} and κ_{f0} are defined as the initial bending stiffnesses of filamins and actin filaments, respectively. In FEM simulations, the bending stiffness of filamins, κ_c, or actin filaments, κ_f, is decreased by one order of magnitude with the torsional stiffnesses and tensional stiffnesses being fixed. The stress–strain curves and tangent moduli of CAFNs at different levels of κ_c and κ_f are obtained and displayed in Fig. 6. According to simulation results, both the bending stiffnesses of filamins and actin filaments could affect the nonlinear elastic behaviors of CAFNs. In addition, their influences on the nonlinear elastic behaviors of CAFNs are comparable, indicating that the bending of filamins and actin filaments contribute almost equally to the nonlinear deformation of CAFNs.

4.2. Effects of components' tensile stiffnesses on nonlinear elastic behaviors of CAFNs

In large strain regimes, the stretching of filamins and actin filaments may play important roles in the deformation of CAFNs. Thus, the effects of axial tensile stiffnesses of filamins, μ_c, and actin filaments, μ_f, on the nonlinear elastic behaviors of CAFNs are studied by performing FEM simulations. In the present work, μ_{c0} and μ_{f0} refer to the axial tensional stiffnesses of filamins and actin filaments, respectively.

When investigating the influences of tensile stiffness of filamins on the nonlinear elastic behaviors of CAFNs, μ_c is increased or decreased by orders of magnitude with the bending stiffness

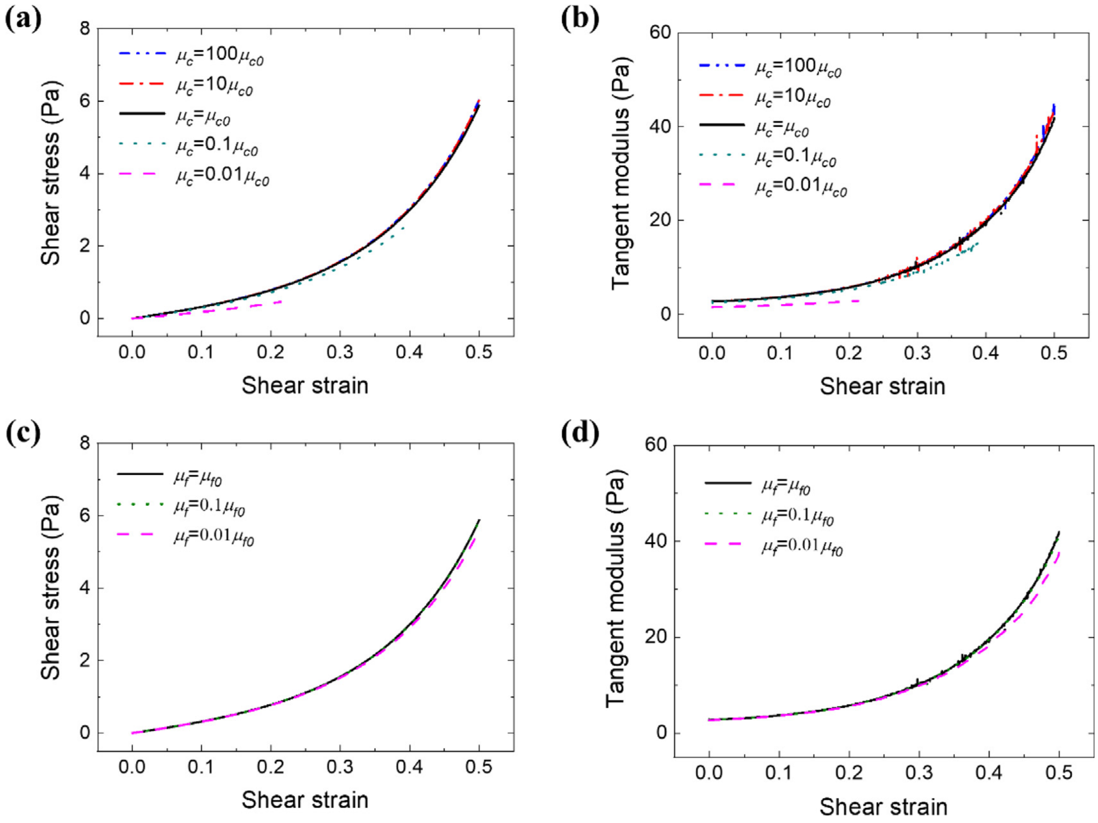

Fig. 7. (a)–(b) Effects of the tensile stiffness of filamins on the stress–strain relationships and tangent moduli of CAFNs. (c)–(d) Effects of the tensile stiffness of actin filaments on the stress–strain relationships and tangent moduli of CAFNs. Where V_f =0.3% and $\rho_c = 1.2$.

and torsional stiffness of filamins being fixed. Thus, the effects of the tensile stiffness of filamins on the nonlinear elastic behaviors of CAFNs could be split. According to the simulation results shown in Fig. 7, decreasing μ_c results in the decrease of shear stresses and tangent moduli of CAFNs. However, the shear stresses and tangent moduli of CAFNs almost do not change when μ_c is increased by one order or two orders of magnitude. Differently, when decreasing the tensile stiffness of actin filaments by one order or two orders of magnitude, the shear stresses and tangent moduli of CAFNs almost do not change. This indicates that the stretching of actin filaments almost does not contribute to the deformation of CAFNs as actin filaments are much stiffer than filamins.

In the range of applied strain studied in this research (i.e., 0 to 0.5), the bending and tension of filamins are proved to make considerable contributions to the deformation of CAFNs. However, for actin filaments, only bending make considerable contributions to the deformation of CAFNs. The nonlinear elastic behaviors of CAFNs are almost insensitive to the tensile stiffness of actin filaments at least in the range of applied strain studied in this research.

5. Conclusions

In the present work, nonlinear elastic behaviors and deformation mechanisms of CAFNs are investigated by performing FEM simulations. The main findings and conclusions drawn from this study are:

(1) Simulation results show that the nonlinear elastic behaviors of CAFNs highly depend on the volume fraction of actin filaments (V_f) and crosslinking density (ρ_c). Larger V_f and ρ_c result in earlier onset of strain stiffening and higher stiffness of CAFNs.
(2) Young's moduli of filamins and actin filaments could influence the nonlinear elastic behaviors of CAFNs, however, the components' shear moduli just have slight effects on the nonlinear elastic behaviors of CAFNs.
(3) The normal stresses of CAFNs are negative under shear strain, and Young's moduli of filamins and actin filaments could greatly influence the negative normal stress of CAFNs. The components' shear moduli are proved to have slight effects on the negative normal stresses of CAFNs.
(4) The shear stress–strain curves of CAFNs highly depend on the bending stiffness, and tensile stiffness of filamins as well as the bending stiffness of actin filaments, however, they are almost insensitive to the tensile stiffness of actin filaments.

The train of thought and methods introduced in this work could provide a valuable reference to investigate the mechanical behaviors of other biopolymer networks with different architectures and design advanced artificial composite structures. For example, by changing the filaments' volume fraction or crosslinking density, the mechanical performance of fibrous network structures could be optimized to meet practical application requirements. In addition, the deformation mechanisms of such composite structures could be easily regulated by altering the specific stiffness of the components.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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