When hydrogels are designed for biological applications, the mechanical properties are carefully chosen to match their precise application. However, traditional methodologies of mechanical characterization (simple shear or compression/extension) commonly ignore the multiaxiality of in vivo deformations. A recent study highlights that biopolymers and tissue indeed show a complex response to combined uniaxial and shear strains. In this study a synthetic yet biomimetic fibrous hydrogel is used, which is based on polyisocyanides and forms a self-assembled network of branched semiflexible chains, similar in architecture networks of structural biopolymers like actin, collagen, and fibrin. Its synthetic nature allows to decouple key parameters of these networks and individually understand their impact on the mechanical response under multiaxial deformation. Experimentally, it is found that the persistence length is a key parameter of biological networks, which tunes softening of gels under compression: The stiffer the polymer, the more the network softens in compression. This study provides insights into tissue behavior that likely is only obtainable from synthetic model systems and is able to direct further the design of new synthetic biomimetic soft materials that are in high demand as tunable bio-free extracellular matrix materials.

1. Introduction

Cells are the building blocks of life. Isolating and studying them outside the body enables us to understand complex biological processes and cellular pathways. Although historically in vitro cell cultures are performed on 2D models, researchers increasingly adopt 3D cultures that better mimic the 3D environment in our bodies.[1,2] The mechanical properties of such 3D cell culture matrices have to be chosen with care, since the stiffness, stiffening, and relaxation behavior are important parameters that can drive biological responses.[3–9] As cell culturing slowly shifts to the third dimension, the mechanical characterization of the matrices lags behind. Mechanical properties are nearly exclusively recorded in simple geometries (in shear or in compression/extension), which is not in line with the complex deformations in vivo, or even with cell-induced matrix deformations within in vitro cell cultures. A key question is: How important is it to study the mechanical response of soft materials under multiaxial deformation?

For many hydrogels, including those based on networks of flexible random-coil polymers, such as polyacrylamide and poly(ethylene glycol), it is not very important at all. Because these materials exhibit linear elastic responses, their properties are not altered by multiaxial deformation[10,11] and 1D mechanical characterization in shear, for example, is adequate to predict their response in compression or elongation. For gels based on fibrous networks that make up the intracellular and extracellular matrix of soft tissues, the mechanics are much more complex. For these gels, typically, the shear modulus decreases when a sample is compressed and increases under shear[12] or

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extension.\textsuperscript{[10,13]} Oppositely, both cancerous and healthy tissue stiffen under compression and soften when extended.\textsuperscript{[14–16]} Recently published work\textsuperscript{[17,18]} showed that this response requires a combination of cells or cell-like soft particles and a fibrous, semi-flexible network. The amplitude of the response is material dependent, and although theoretical work provides interesting insights,\textsuperscript{[13,19]} it remains very difficult to analyze experimentally which molecular properties are responsible for the effect.

In the present paper, we use a synthetic, but biomimetic hydrogel\textsuperscript{[20]} based on tri(ethylene glycol)-substituted polysiocyanides (PICs) to experimentally evaluate how the mechanical response under multiaxial deformation differs from the standard shear experiment. The use of a synthetic gel offers the advantage that we can manipulate key parameters independently.\textsuperscript{[21,22]} Besides the polymer concentration $c$, we will study the role of the polymer contour length $L_C$ and persistence length $l_p$, which are challenging to control in biological networks.\textsuperscript{[10,23]} The persistence length is directly related to the bending rigidity $\kappa$, which is often used in theoretical work, and is one of the most important parameters that defines the unique mechanical properties of networks composed of semiflexible chains, or in short: Of semiflexible networks.

Analogous to semi-flexible biogels, the biomimetic PIC gels were previously shown to be shear strain-stiffening.\textsuperscript{[20]} Here, we use multiaxial deformation studies that are based on axial extension and compression combined with small amplitude shear deformations with or without a pre-stress in the shear direction. We show that PIC gels, analogously to the biogels, show clear compression softening and extension stiffening. In contrast to current literature, we find that these effects are independent of the polymer concentration, but are affected strongly by the polymers’ contour and persistence lengths.

2. Results and Discussion

The PIC hydrogel composed of semiflexible polymers that bundle at low concentrations in water to form a 3D network. Just like biopolymer networks based on collagen or fibrin,\textsuperscript{[20]} PIC gels become much stiffer when a shear strain is applied, increasing the modulus by an order of magnitude of more, a process that is termed strain-stiffening. Strain-stiffening is associated with a variety of biological functions,\textsuperscript{[3,4]} for instance, it protects against tissue rupture,\textsuperscript{[5]} assists in intercellular communication,\textsuperscript{[5,6]} and is considered to be a factor in the regulation of stem-cell differentiation.\textsuperscript{[24]} PIC gels emerged as an opportune model system that is well described by theory, even near and below the isotropic point.\textsuperscript{[25]} Because of its synthetic nature, we can control the molecular parameters that, together, define the macroscopic mechanical properties.\textsuperscript{[26]} Moreover, chemical functionalization strategies allow us to introduce virtually anything on the polymer backbone, such as cell adhesive peptides,\textsuperscript{[24,27]} radiolabels,\textsuperscript{[28]} crosslinkers,\textsuperscript{[29,30]} nanoswitches,\textsuperscript{[31]} and (fluorescent) dyes.\textsuperscript{[32]} The mechanical and chemical versatility in combination with the excellent biocompatibility make PIC hydrogels promising materials for a broad variety of applications in the biomedical field.\textsuperscript{[33–40]}

We analyzed the properties of PIC hydrogels in a complex mechanical environment, using a stress-controlled rheometer. PIC gel formation is thermally induced (and reversible), which makes gelation between the rheometer plates reliable and reproducible. Typically, a cold PIC solution in deionized water was loaded between the rheometer plates and equilibrated at $T = 37 \degree C$ for 10 min to form a mechanically stable hydrogel. Subsequently, deionized water was placed around the edges of the hydrogel (Figure 1a; Figure S1, Supporting Information) and the system was equilibrated for 50 more minutes until all stresses completely relaxed. Note that PIC hydrogels (like other semi-flexible networks) do not swell in water.\textsuperscript{[41]} Unlike shear, compression and extension are not volume conserving, so the in and out flow of water across the edges allows volume change during axial deformations (Figure S1, Supporting Information). After equilibration (constant storage modulus), the gel was axially deformed at a rate of 2 $\mu m$ s$^{-1}$. Before subsequent measurements, the axially strained gel was equilibrated once more for 8–10 min (Figure S2, Supporting Information) to avoid contributions from poroelasticity. For extension experiments, the

![Figure 1](image_url). Axial strain causes architectural and mechanical variations in PIC networks. a) Representation of the experimental setup: The hydrogel is formed between the rheometer plates and surrounded by water to allow solvent flow. An axial strain $\varepsilon_N$ is applied, followed by a shear strain $\gamma$ to assess the mechanical response. b) Storage modulus $G'$ of PIC hydrogel (1 mg mL$^{-1}$ in deionized water) as a function of oscillatory shear strain for different $\varepsilon_N$. By convention, negative values of axial strain are used for compression, and positive values for extension. $G'$ is constant in the linear viscoelastic (LVE) regime and increases at higher strains. c) When the network is compressed or extended, network segments bend/buckle (blue) or stretch (red), which changes the linear and nonlinear mechanical properties.
axial deformation is limited by the eventual detachment of the gel from the plates. In compression, we measure until a plateau in the shear modulus is reached. Further compression results in stiffening (Figure S3, Supporting Information), which originates from the increased concentration due to the deceased compressed volume of the gel[42] as well as to increased fiber stretching at large compressions.[13]

We measured the storage modulus as a function of shear strain $\gamma$ under different axial strains $\varepsilon_N = (h - h_0)/h_0$, where $h$ and $h_0$ are the gap sizes and the gap size of the rheometer at zero axial strain. Throughout the manuscript, we will use the storage modulus $G''$ for shear deformations beyond the linear viscoelastic (LVE) regime and we use the notation $G_c$ for the shear modulus at axial strain $\varepsilon_N$ probed at small shear strains (1%). We define the sign of the axial strain positive for extension, and negative for compression. Without compression (Figure 1b, $\varepsilon_N = 0$, black data points), we observe the characteristic strain-stiffening behavior associated with semi-flexible networks: Already at low shear strains of $\gamma = 0.1$, the modulus rapidly increases, accompanied by an increase of a (contracting) normal stress (Figure S4, Supporting Information).

When compressed (blue data points), PIC hydrogels soften; the modulus in the LVE regime halves under a compression of $\varepsilon_N \approx -10\%$ and softening levels off after increasing compression to ~20%. The observed compression-softening of the network cannot be attributed to network damage or irreversible fiber adhesions (a common origin of plasticity),[43] because $G_c$ fully recovers to the original value after removal of axial strain (Figure S5, Supporting Information). Under axial extension (red data points), the storage modulus increases. The profiles of compression-softening and stretch-stiffening are qualitatively close to the response of biopolymer hydrogels,[10] and we believe that this is the first report of this effect in a synthetic hydrogel. Quantitatively, however, we do observe some differences. In extension, the stiffening response, which seems associated with the presence of internal stress is slightly lower, and in compression, the degree of softening is lower and stiffening saturates at lower axial strains. In the next paragraphs, we first discuss how axial strain impacts hydrogel mechanics in and beyond the LVE regime; next we discuss the parameters that dominate the multiaxial response.

A closer look at Figure 1b shows that axial strain also impacts the strain-stiffening response that is characteristic for semiflexible networks.[44,45] To quantitatively describe strain-stiffening, we assess three parameters from the shear sweep: $G_c$, the shear modulus in the LVE regime under axial strain $\varepsilon_N$, the critical strain $\chi$ defined as the onset strain for stiffening and loosely interpretable as the sensitivity of the material toward strain, and, lastly, the stiffening index $m$, a value for the responsiveness of the hydrogel toward strain. The last two parameters are calculated from a power law fit to the differential modulus $K \equiv \partial \sigma/\partial \gamma$ (see Supporting Information for details). Besides an increased stiffness, we find for gels in extension (red data), an increased sensitivity (decrease of $\chi$) and a decreased responsiveness (decrease of $m$) toward shear strain. The compression data (blue data) show the opposite effect; the hydrogel softens, and the onset of strain-stiffening delays, while the material becomes more responsive.

The underlying mechanism for stiffening and softening under axial stress is well described in the literature.[10,13] When a semiflexible network is compressed, the fibers that bend and buckle do not contribute any longer to the mechanical stability of the gel, resulting in a softer material. This axial strain increases the so-called excess length, which is defined as the difference between the contour length $L_c$ and the end-to-end distance of fibers. Such increase implies that larger shear strains are required to elongate these fibers, which delays the strain-stiffening response. Simultaneously, the alignment of the fibers in the shear direction is more favorable, which results in an increase of $m$. For collagen, fibrin, and microtubules gels, microscopy images confirmed this buckling mechanism.[11,46-48] Under extension, the excess length decreases, and the removal of thermal undulations results in stiffening.[10,13] This pre-stress in the network causes stiffening at lower strains, but alignment is less favorable, resulting in lower responsiveness $m$. The striking similarities with biological gels[10,13] highlight the biomimetic character of PIC hydrogels.

2.1. Concentration Dependence

The default approach to tune the stiffness of virtually any gel is to change the concentration $c$. In line with earlier results,[20,21] we find for PIC gels $G' \propto c^{2.3}$ and, since the bundle dimensions are independent of the concentration, a decreasing pore size $\xi$ with increasing $c$ that follows $\xi \propto c^{-0.5}$. The latter is directly related to the excess length, which affects the linear and nonlinear shear mechanical properties of axially deformed networks. Figure 2a shows $G_c$ (at small shear strains) as a function of $\varepsilon_N$ for PIC concentrations $c = 0.7-4.0$ mg mL$^{-1}$. Despite the narrow concentration range dictated by the experimental considerations,[49] the effect of polymer concentration on the mechanical properties is clear: Similar compression-softening and stretch-stiffening is observed for any $c$. When we correct the increase in $G_c$ at any axial strain for concentration effects, all the data collapse to a single curve (Figure 2b), indicating that concentration variations do not affect compression softening or extension stiffening. With the excess length model in mind, it would be wise to compare how the buckling length scale $\lambda$ and the pore size of the bundled network $\xi$ scale with the polymer concentration. Earlier, we found that $\xi \propto c^{-0.5}$. For rods in an elastic environment,[50] we know that $\lambda \propto (kT/Gc)^{1/4}$ where the bending rigidity $k$ is independent of the polymer concentration because of the constant bundle dimensions. The square dependence of $G'$ with $c$ gives $\lambda \propto c^{-0.5}$, similar to the pore size. In this respect, the experimentally observed independence of $c$ may have been expected, but only for gels where the bundled architecture does not change with the polymer concentration.

2.2. Shear Stiffening under Axial Deformations

The most striking mechanical feature of semiflexible networks is shear strain-stiffening; networks become many times stiffer with imposed shear stress[12,21] or internal stress,[41] yielding extremely responsive matter. We quantify how the parameters that describe strain-stiffening, that is, the stiffening index ($m$) and the critical strain ($\chi$) are impacted by axial strain.

With increasing extension, the gels become less responsive and less sensitive to applied shear stress (monotonic decrease in $m$ and $\chi$, Figure 2c,d). We measure quantitatively the same values for different polymer concentrations (0.7–1.5 mg mL$^{-1}$), which indicates that the pre-stress that builds up in the network as a
result of extension spreads similarly in the network, irrespective of the higher polymer density, at least in the range of 10% extension.

In compression, \( m \) initially increases, then levels off at axial strains larger than \( \approx 5\% \) (Figure 2c). Further compression does not provide additional flexibility to the network. At fixed \( \varepsilon_N \), \( m \) does not depend on \( c \). We interpret the result in terms of architectural changes; in the non-linear regime, the enthalpic stretching of the network dominates and is independent of pore sizes or buckled fibers.

The second parameter, \( \gamma_c \) (Figure 2d) continuously increases with compression, that is, the gels become less responsive to shear stress under increasing axial stress. Mechanistically, as the excess length in the network increases, higher shear strains are necessary to enter the strain-stiffening regime. Only at high compression, we observe a difference in critical strain with different concentrations: At higher \( c \), gels are even less sensitive to shear deformations (in contrast to \( m \), which was fully independent of \( c \)). The results fit well to the mechanistic model: The concentration-dependent excess length is directly related to the presence of thermal undulations of the bundles, which determines the onset of strain stiffening. In the non-linear regime, where we determine \( m \), all thermal undulations are removed and network stiffening is due to enthalpic stretching.

Uniaxial deformations have a double effect on the shear stiffening response of semiflexible networks. Uniaxial stretch causes a pre-stress in the network, which makes the gel stiffer and lowers \( \gamma_c \), but the responsiveness toward shear strain also decreases. Uniaxial compression, causes the opposite effect.

Considering how difficult it is to tune the nonlinear properties of gels in vitro, understanding (uni)axial deformations and transposing them into organized networks with internal or oriented deformations are the key to design truly adaptive gels with dynamic mechanical properties.

2.3. The Influence of Polymer Rigidity

An alternative parameter to manipulate the mechanical properties of semiflexible hydrogels is the polymer persistence length or, alternatively, the persistence length of the polymer bundle \( l_{p,B} \). For PIC gels, the shear modulus scales with the persistence length of the bundle \( l_{p,B} \) as: \( G' \propto l_{p,B}^2 \). For bionetworks, \( l_{p,B} \) usually increases with the incorporation of more fibrils or monomers into the bundle.\(^{[51]}\) The forces that control and limit lateral bundle or fibril dimensions, however, are often unclear and difficult to regulate, which makes this approach experimentally challenging. In the case of our synthetic PIC gel, an easy and reliable way to tune \( l_{p,B} \) is through addition of salts. At the same polymer concentration, kosmotropic anions yield stiffer hydrogels, while chaotropic anions soften the network.\(^{[52]}\) The salt effects are a combination of specific electrostatic interactions and changes in hydrophobicity, which effectively result in an increase or decrease of \( l_{p,B} \) while other architectural network parameters like bundle thickness \( N \) and pore size \( \xi \) remain constant.\(^{[22]}\) Analysis of PIC hydrogels in the presence of different salts allows us to directly measure the effect of \( l_{p,B} \) on their multiaxial mechanical response.

Figure 2. Storage moduli of PIC hydrogels under axial strain as a function of concentration. a) Plateau modulus \( (G_\varepsilon) \) as a function of axial strain \( (\varepsilon_N) \). For all concentrations, PIC hydrogels show compression-softening and stretching-stiffening. b) By normalizing \( G_\varepsilon \) with \( c^2.3 \) all data collapse to a single curve. c,d) For concentrations 0.7–1.5 mg mL\(^{-1} \), the nonlinear mechanical parameters stiffening index \( m \) (c); and critical strain \( \gamma_c \) (d) vary with extension and increase with compression. Strain-stiffening experiments under axial compression of the stiffest hydrogel were difficult to record. The data in all diagrams show the mean of 2 samples with \( \pm SD \).
Figure 3. Storage modulus of PIC hydrogels under axial deformations as a function of persistence length and contour length. 

a) Relative stiffening or softening of PIC gels with modified persistence length \( l_p \) as a result of the addition of kosmotropic (NaH\( _2 \)PO\( _4 \)) or chaotropic (NaI) salts. Particularly, the compression response is affected by \( l_p \).

b) Relative stiffening or softening of PIC gels as function of the contour length of the polymers forming the bundles \( L_C \). Again, only in compression, differences between the samples are observed. The data shown are the mean of 2 or 3 samples with \( \pm SD \).

Data before normalization are provided in Figure S6, Supporting Information.

We prepared PIC solutions in 0.25 m NaH\( _2 \)PO\( _4 \) (kosmotropic anion) and 0.25 m NaI (chaotropic anion). From the shear modulus at \( \varepsilon = 0 \) of the corresponding gels compared to that of the gel in pure water, we derived an increase in \( l_p \) of a factor 1.5 for NaH\( _2 \)PO\( _4 \) and a decrease of a factor 0.6 for NaI. To facilitate comparison of axial stiffening or softening, we normalize the storage modulus of the axially deformed gel \( G' \) with the initial storage modulus without axial strain \( G_{\varepsilon = 0} \) (Figure 3a; Figure S6, Supporting Information). Compression of the gel composed of stiffer bundles (in NaH\( _2 \)PO\( _4 \)) leads to increased softening (\( G_{\varepsilon = 0.2}/G_{\varepsilon = 0} = 0.51 \)) in comparison to PIC hydrogels in pure water (\( G_{\varepsilon = 0.2}/G_{\varepsilon = 0} = 0.58 \)) and in aqueous NaI (\( G_{\varepsilon = 0.2}/G_{\varepsilon = 0} = 0.72 \)). When compressed, the stiffer networks bend and buckle more, and the relative softening is more pronounced. In extension, \( G_{\varepsilon = 0}/G_{\varepsilon = 0} \) supersedes for all \( \varepsilon \) and for samples in pure water and the NaH\( _2 \)PO\( _4 \) solution, indicating that increasing \( l_p \) does not change stiffening in extension. NB: For the NaI solution, the gel is rather soft and we observe anomalous behavior at very small extensional strains, after which we observe an increase with the same slope as for the other two samples. Interestingly, simulations of axially stressed semi-flexible networks\(^{[8]} \) show that a change in the persistence length causes different shear mechanics, particularly in extension. In compression, only small effects were found, which is quite opposite to our experimental observations.

A third, independent approach to manipulate the mechanical properties of PIC gels is to tune the contour length of the polymers \( L_C \) that form the bundles.\(^{[3]} \) In the regime where \( L_C \) is of the same order of magnitude as pore size \( \xi \), we experimentally find \( G' \propto L_C^2 \) (\( \xi = 0 \)) in line with earlier results\(^{[21]} \) and with theory,\(^{[54]} \) which also predicts a regime for longer chains where \( G' \) becomes constant with \( L_C \). Note that for PIC gels, also the bundle dimension are not entirely constant with \( L_C \), which simultaneously impacts \( \xi \).\(^{[22]} \) Understanding the effect of \( L_C \) on the 2D mechanical response has a very practical benefits: \( L_C \) is an experimentally easily accessible parameter, which, in contrast to the addition of salts can be used in a biological context, for instance to tailor the mechanical properties of 3D cell growth matrices.\(^{[27]} \) Varying \( L_C \), however gives rise to a complex response, since multiple parameters change simultaneously;\(^{[22]} \) the persistence length \( l_p \), the number of polymer chains inside a bundle \( N \), and, consequently, also pore size \( \xi \). Polymers with different contour length \( L_C = 77, 160, \) and 250 nm were obtained by tuning the monomer:catalyst ratio during the polymerization reaction.\(^{[20]} \) We measure the shear moduli of the corresponding hydrogels under axial deformation (Figure S6, Supporting Information) and normalize the results to \( G_{\varepsilon = 0} \) (Figure 3b).

The shear moduli of gels of different length PIC polymers respond analogously to axial stresses as the gels with the different bundle persistence lengths: Gels of the longest polymers display (relatively) the strongest softening (lowest \( G_{\varepsilon = 0}/G_{\varepsilon = 0} \)) in compression. In extension, all polymer gels behave the same. Let us briefly analyze the expected complex response anticipated by a variation of \( L_C \), which impacts \( l_p, N, \) and \( \xi \). From the concentration measurements, discussed above, we concluded that the shear modulus response to axial deformation is independent of concentration and, for PIC gels, independent of pore size \( \xi \). For PIC gels, the bundle dimensions, expressed as the number of chains per bundle \( N \), cannot be varied independently. Looking, however, at the great similarities between the variation of \( l_p \) and \( L_C \) (Figure 3a,b), we confidently pose that the polymer length variation is dominated by persistence length effects. Comparisons between hydrogels of long and short PIC polymers \( (L_C = 250, 77 \text{ nm}) \) in the presence of 0.25 m NaI and NaH\( _2 \)PO\( _4 \), clearly illustrate this “interchangeability” (Figure S7, Supporting Information).

In brief, experimental polymer length and persistence length effects show the largest contribution to the mechanical properties in compression; in extension, the PIC gels behave similarly. This result contrasts to simulations that vary the bending rigidity of the filaments that comprise the networks.\(^{[31]} \) In a biological tissue engineering context, the results are important for in vitro cell cultures under a (periodic) deformation. Even at limited axial stresses, the mechanical properties of the tissue culture matrix can be significantly overestimated.

2.4. Axial Stress under Uniaxial Deformation

So far, we studied how axial deformation impacts the mechanical properties in shear, that is, perpendicular to the deformation.
direction. Obviously, axial deformation also generates stresses in the $z$-direction.\cite{55} Simple synthetic and flexible hydrogels, like polyacrylamide, present a linear and symmetric normal stress $\sigma_N$ response toward $\varepsilon_N$, where the slope is the Young’s modulus $E_{\varepsilon\varepsilon}$:

$$\sigma_N = E_{\varepsilon\varepsilon} \varepsilon_N.$$  

The response of biological semiflexible networks is asymmetric.\cite{10} Indeed, the experimental results of the PIC gels show a similarly asymmetric response (Figure 4a).

In extension, we observe a linear increase of $\sigma_N$ with axial strain without any signatures of strain hardening in this regime. Note that in shear, PIC gels typically enter the strain stiffening regime at 10–20% shear strain depending on exact conditions. In compression, on the other hand, $\sigma_N$ slightly decreases until 5% strain (same slope as in extension) and then remains constant onward. The initial linear response is expected for any elastic solid at small strains, and has been reported for fibrin networks.\cite{56}

Also, for semiflexible networks, one can calculate the Young’s modulus $E_x$ from the slope of a stress–strain curve ($E_x = \sigma_N/\varepsilon_N$). As the shear modulus is known as well, we are able to estimate the incremental apparent Poisson’s ratio $\nu$ at any axial strain $\varepsilon_N$ from $E_x = 2G_{\varepsilon\varepsilon}(1 + \nu)$. Note that for flexible networks $\nu = 0.5$, which is the theoretical value for isotropic incompressible materials. The incremental apparent Poisson’s ratios of biomaterials\cite{57} and tissues\cite{58} are commonly larger as a result of a strong contraction perpendicular to the deformation direction, suggested by recent simulation results.\cite{59} The large $\nu$ in uniaxial extension in biomaterials is attributed to fiber densification, to a decrease of sample volume during deformation, and to a smaller energy required to buckle fiber segments.\cite{60}

We calculate the $E$ and $\nu$ from the data in Figure 4a, although one should consider that both will be dependent on the axial deformation for nonlinear stress–strain curves.\cite{13} In

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**Figure 4.** Mechanical response in two directions upon axial deformation. a) Storage modulus ($G_{\varepsilon\varepsilon}$) and axial stress $\sigma_N$ as function of axial strain. Both $G_{\varepsilon\varepsilon}$ and $\sigma_N$ linearly increases with extension, while a small change is seen for the compression. A positive $\sigma_N$ represents the network pulling down the upper plate. Each plot shown is averaged over three independent samples. b–d) Variation of storage modulus ($G_{\varepsilon\varepsilon} - G_{\varepsilon\varepsilon,0}$) versus the generated normal stress $\sigma_N$ (both normalized to $G_{\varepsilon\varepsilon,0}$) for different axial strains in extension and compression, determined for gels with different polymer length (b), polymer concentration (c), and polymer persistence length (d), as measured in three independent samples. Note that the soft materials give poor results. A linear dependence is seen independent of contour length (b), concentration (c), and persistence length (d). Linear fits show slopes of 1.2 in extension, and slope of $\approx 2$ in compression.
compression at $\varepsilon_N = -30\%$, we observe $E = 160$ Pa and $\nu = 0.5$, but in extension at $\varepsilon_N = +10\%$, $E = 1.6$ kPa, and $\nu = 2.7$. The large discrepancy between compression and extension, in particular for the calculated Young’s modulus, originates from the nonlinear axial stress response under compression, and from the asymmetric response in compression and extension. For extension of higher concentration PIC gels, 4 mg mL$^{-1}$, we find $\nu = 1.5$ (at $\varepsilon_N = +5\%$, Figure S8, Supporting Information). With increasing concentration, the pore size reduces, the shorter chains between crosslinks require larger forces to buckle, and $\nu$ decreases. This analysis is in line with simulations\cite{59} and earlier observations in collagen gels\cite{60}. Overall, the results highlight that any direct translation of shear to compression or extension experiments (and vice versa)\cite{13} should be handled with great care.

Finally, we directly compare the axial and shear modulus responses to applied axial extensional and compressional strains by plotting the increase in shear modulus $\Delta G = G_e - G_c = 0$ against the normal stress $\sigma_N$, both normalized to $G_{s \rightarrow 0}$. We highlight again that both $G_e$ and $\sigma_N$ fully relax after $2$ min, which excludes any poroelastic from the results (Figure S9, Supporting Information). Soft incompressible biopolymer networks\cite{30, 13} and tissues\cite{14, 15} all display a linear dependence of $\Delta G$ with $\sigma_N$, with slopes between 1 and 5 in extension. The parameters that determine the slope are currently still unclear. Once again, we use the designability of a synthetic hydrogel to isolate structural effects of pore size (polymer concentration), persistence length (ion concentrations), and polymer contour length (Figure 4b–d). Although all these variables change the shear and axial mechanical properties, we find that the ratio between shear and axial response is identical for the materials in extension. In compression, a linear dependence is preserved, although the slope differs to the extensional ones. Based on the result and the variations found between different biological gels, it is tempting to consider that this ratio between shear and axial response is dictated by the material architecture and properties.

3. Conclusion and Outlook

Strains occurring in vivo are multiaxial, rather than the uniaxial deformations (shear, extension, and compression) that we often use to characterize materials. The response of biopolymer networks and tissues (i.e., biopolymer networks filled with cells) to multiaxial deformations are quite complex.\cite{37} To predict the behavior of biological tissues, one needs to combine the nonlinear mechanical properties of fibrous ECM gels with that of particles that interact or do not interact with the network. We present a synthetic and semiflexible network based on PIC, that similar to gels based on fibrin and collagen softens under compression and stiffens under extension. The high degree of tunability makes the PIC gels ideal model materials to learn more about the parameters that drive the complex multiaxial mechanics.

Simulation studies already provided insights into mechanisms\cite{13} of compression softening and extension stiffening by isolating individual parameters, like the bending rigidity of the networks. Experimental work is currently limited to changes in concentration and crosslink density\cite{30, 13} which not only changes the mechanical properties but also the network architecture. In fact, our work indicates that polymer concentration does not affect axial strain-induced softening and stiffening at all. On the other hand, the persistence length $\ell_p$ proves an excellent control parameter for softening/stiffening. For PIC gels, or synthetic semi-flexible gels more generally, the contour length $L_C$ of the polymers that form the filaments gives a similar effect, but is experimentally much easier to vary. Additionally, changes in $L_C$ are commonly easy to accommodate in cell culture studies.\cite{27, 33, 34}

Our work on synthetic model systems contributes to the limited understanding of the mechanical behavior of semiflexible networks undergoing multiaxial deformations. The results underscore that the mechanical properties of fibrous hydrogels already under physiologically relevant deformations cannot be extrapolated from simple shear experiments. At significant axial deformations, for instance as is commonly applied in (cyclic) strain bioreactors, the true mechanical properties of the matrix may be readily misinterpreted.

4. Experimental Section

Materials: PICs were synthesized as previously described,\cite{21} using a monomer:catalyst ratios from 500:1 to 4000:1. The polymer contour length ($L_C$) was determined from the viscosity average molecular weight. The gels were prepared by dissolving the polymers in cold, deionized water for at least 12 h, unless mentioned otherwise.

Mechanical Analysis: Mechanical tests were performed in two stress-controlled rheometers: A Kinexus (Malvern) or Discovery HR-2 (TA Instruments), using a 20 or 40 mm aluminium or steel parallel plate geometry with a 1.0 or 1.5 mm gap. The polymer solution was loaded onto the plate at $T = 5$ °C, the top plate was lowered to the experimental gap and the gel was formed by heating to $T = 37$ °C. After 10 min equilibration deionized water or a salt solution (preheated to 37 °C) was pipetted around the edges of the gel to prevent water evaporation and to allow in-and out-flow of water during extension and compression steps, respectively. The storage modulus was recorded using 1% strain at 1 Hz for 50 min. The gel was then axially deformed at a rate of 2 μm s$^{-1}$, and the nonlinear mechanical properties were measured using an amplitude sweep protocol at 0.16 Hz, from 0.1% strain until the gel failed.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.
Data Availability Statement

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

Keywords

biomimetic hydrogels, fibrous matrices, multiaxial deformation, polysiloxanes

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