Combining Mechanical Tuneability with Function: Biomimetic Fibrous Hydrogels with Nanoparticle Crosslinkers

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Fibrous networks of biopolymers possess unique properties: mechanical stability at low concentrations, an extremely porous architecture, and strong stiffening at small deformations. An outstanding challenge is to find methods that allow to tailor the mechanical properties of these bionetworks or their synthetic equivalents without changing the polymer concentration, which simultaneously changes all other hydrogel properties. Here, networks of dilute (0.1 wt.%) fibrous hydrogels are prepared and crosslink them with functional rod-shaped nanoparticles. The crosslinking is observed to induce an architectural change that strongly affects the mechanical properties of the hydrogels with a 40-fold increase in stiffness. The effect is strongest at the lowest polymer and particle concentrations (99.8% water) and is tailorable through tuning the crosslink density. Moreover, the nanoparticle components in the composite offer the opportunity to introduce additional functions; gels with magnetic and conductive properties are reported. However, through the generic crosslinking approach of a fibrous network with decorated nanoparticle crosslinkers as presented in this work, virtually any functionality may be introduced in highly responsive hydrogels, providing a guide to design next generations of multi-functional soft materials.

1. Introduction

The mechanical properties of the cellular cytoskeleton and the extracellular matrix are governed by various fibrous biopolymer networks.\[1–3\] The stiffness and other mechanical properties of these networks can be precisely regulated through physical\[4–6\] or chemical (crosslinking) interactions.\[7–9\] The fibrous network architecture introduces strain-stiffening behavior, that is, in these gels, the stiffness sharply increases upon deformation.\[10–13\] Synthetic hydrogels are highly attractive materials to mimic biopolymer networks but the fibrous architecture and the associated mechanical stiffening response to stress or strain are rarely found in gels of synthetic polymers. So far, only a few groups have reported on the strain-stiffening properties of gels composed of synthetic semi-flexible polymers.\[14–17\]

To increase hydrogel functionality, tunability, and in situ adaptiveness, researchers have introduced nanomaterials into hydrogels.\[18–21\] These composites are readily optimized for their mechanical, optical, electric, magnetic, etc. properties. Moreover, the responsiveness of the nanoparticles towards externally applied stimuli, including light and electric and magnetic fields, often renders the entire hydrogel composite responsive.\[18,20,22,23\] The combination of excellent mechanical and physical properties has drawn unprecedented interest in the development of functional hydrogels towards biological applications.\[18,22,24\] To generate hydrogel-nanomaterials composites, one can distinguish two different approaches: i) In the conjugated composite approach, the nanoparticles are covalently attached to the polymer matrix, where they act as a crosslinker and directly change the mechanical properties of the gel,\[18,20,25\] and ii) in the physical mixture approach, the particles are simply added prior to the gelation process and remain dispersed in the gel. Earlier, we found that for fibrous hydrogels, the physical mixture approach has limited influence on the hydrogel stiffness but more on strain-stiffening and mechanical adaptiveness.\[25\] Despite the intrinsic attractiveness of nanomaterial-gel composites, in vitro studies that benefitting from these unique properties are still limited in number.

In this work, we exploit the functionality and crosslinking properties of nanoparticles in a fibrous gel and find that they are much more effective than in classical gels of flexible polymers. We study three analogous iron oxide nanorods (FeNRs) which differ in magnetic responsiveness and in surface chemistry, which proves an important parameter to the composite stiffness: paramagnetic FeOOH (F1), superparamagnetic Fe3O4 (F2), and ferromagnetic Fe3O4 (F3). As a biomimetic fibrous matrix, we use the fully synthetic, yet highly biocompatible polyisocyanide (PIC)-based hydrogel. By controlling crosslinking between the fibril, the stiffness of the hydrogels can spectacularly increase 44-fold, even at minimal PIC (0.1 wt.%).
FeNR (0.1 wt.%) concentrations, in comparison to the PIC gels with the particles physically mixed in. We study the effect of the concentration dependencies of the polymer, FeNRs and the linker that bridges the two on the mechanical properties in the linear and nonlinear regime. In addition, we straightforwardly demonstrate the advantage of functional nanoparticles (F3) by studying the magnetic and conductive properties of the hydrogel composites. Our composite approach that combines gels of broadly tunable stiffness, without changing polymer concentrations, together with multifunctionality of remotely magnetic control, electric conductivity is generic for any (bundled) hydrogel crosslinked with nanomaterials, and open new avenues for application in biology and soft robotics.

2. Results

2.1. Preparation of Nanocomposite Hydrogels

For the fibrous hydrogel, we employed the fully synthetic semi-flexible ethylene glycol-substituted PIC polymers. The thermal-responsive hydrogels are reversibly formed when an aqueous PIC solution is heated above the gelation temperature ($T_{gel}$). The gel is unique as it forms fibrous networks in a non-covalent bundled architecture that closely mimics the architecture of biopolymer networks, such as those based on collagen, fibrin, and actin. In addition, PIC gels display the same characteristic strain-stiffening response as the biopolymer hydrogels. The porous architecture makes PIC an ideal xenofree, lean matrix for biomedical applications, ranging from in vitro cell culture material to in vivo wound and dental applications. Through a copolymerization strategy, the PIC polymer chains were equipped with azide groups (3.3, 5, 10, or 20 mol% of the monomer) for crosslinking, analogous to earlier reports (details in the Experimental Section). We stress that, as a synthetic polymer, the mechanical properties of PIC gel can be easily tailored by changing polymer length, concentration, and conditions, like temperature, ionic strength of the solution, and, then reacting the functionalized particles with the polymer solution. Indeed, PIC gels display the same characteristic strain-stiffening response as the biopolymer hydrogels.

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As the nanomaterial in the functional nanocomposite network, three different types of FeNRs (F1, F2, and F3) were selected. To optimally benefit from the efficient crosslinking properties, which allow very low particle concentrations, we selected 1D nanorods that display much lower percolation concentrations than the corresponding spherical particles. The FeNRs were synthesized through optimized literature procedures that yield well-dispersed particles with similar aspect ratios (length/diameter = ~10, Figures S1 and S2, Supporting Information).

To covalently graft the FeNRs to the polymer network, we designed a bifunctional spacer with on one terminus the dopamine mussel adhesive motif that can bind to the nanorod surfaces, and on the other terminus a dibenzocyclooctyne (DBCO) group that efficiently reacts with the azide groups on the PIC backbone. Preliminary experiments showed that a two-step procedure of first grafting the spacer to the nanoparticles and, then reacting the functionalized particles with the polymer gel gives the best crosslinking results.

In the first step, the linker was grafted to the FeNRs by mixing both components in solution. By washing away and measuring the excess linker through UV–vis spectroscopy, we were able to quantify that the surface density of DBCO groups per (average) FeNR amounts to $10^7$ groups per particle (details in the Experimental Section, Equations (1,3,4) and Figure S5, Supporting Information), which makes them highly efficient multi-functional crosslinkers. Additionally, FeNR grafting was confirmed with IR spectroscopy (Figure S4, Supporting Information), albeit qualitatively. Subsequently, nano-composite hydrogels were prepared by the addition of a pre-cooled aqueous solution of functionalized FeNRs to a cold PIC solution, followed by rapidly heating the mixture to the desired crosslinking temperature (Figure 1). When the temperature surpasses the PIC $T_{gel} = 18 ^\circ C$, a bundled network is formed, and, due to the bundling-induced increased effective concentration, the click reaction between azide groups on the polymers and DBCO groups on the FeNRs accelerates (Figures S9 and S10, Supporting Information). The procedure yields the crosslinked hydrogels cF1, cF2, and cF3. As a control that allows us to study the role of crosslinking, we include the composites of the unfunctionalized FeNRs (no DBCO groups) with PIC, which forms the physical mixtures pF1, pF2, and pF3. Note that the preparation procedures for the crosslinked and the physically mixed samples were identical.

2.2. Mechanical Properties of Hybrid Networks

Considering that PIC gels have bundled network architectures with average pore sizes of the order of the dimensions of the FeNRs, we hypothesized that the nanorods predominantly act as crosslinkers between the bundles. For such architecture, we would expect a much higher stiffness than a gel that is only crosslinked inside the bundles, which were shown to have a limited effect on the mechanical properties and primarily affect the thermo-responsive behavior of the gels. The FeNRs in physical mixtures are not expected to significantly contribute to the stiffness due to the lack of interaction with the load-carrying PIC network.

We studied the mechanical properties of the composites using oscillatory rheology. In a typical experiment (Figure 2a), a freshly prepared cold solution of functionalized or non-functionalized FeNRs and azide-PIC was transferred to the rheometer at 5 °C and immediately heated (50 °C, 10 h) where a gel is formed and the crosslinking reaction is allowed to complete (stage I), which is monitored by observing the increase in the storage modulus to level off. Both the reaction temperature (Figures S9 and S10, Supporting Information) and time (Figure S11, Supporting Information) were optimized to reach the maximum stiffness of the gels. Note that at temperatures below $T_{gel}$, crosslinking is virtually nonexistent (Figure S10, Supporting Information). To study the effect of crosslinking, we exploit the thermo-reversible gelation of the PIC gels. On cooling below $T_{gel}$ (Figure 1d,e; Figure S8a,c, Supporting Information) to $T = 5 ^\circ C$ (stage II), the non-crosslinked gels will follow the transition back to the polymer solution. Indeed, the elastic gel is lost for the physically mixed composites. Crosslinking, however, prevents large conformational changes of the polymer chains and the network stays intact. As such, the modulus of a crosslinked gel, however, remains high throughout the cooling ramp (Figure 1f, Figure S8b,d, Supporting Information). To quantitatively
compare the mechanical properties of the physically mixed and crosslinked hydrogels, all samples were reheated to $T = 37^\circ C$ (stage III, Figure 2b and Figure S12, Supporting Information). We note that the data shown from 5 to $37^\circ C$ has been collected from stage III, unless mentioned otherwise. For the physical mixture pF1–pF3 (open symbols) and the PIC gel control (blue symbols), the hydrogels are reformed at 15–20 $^\circ C$ with characteristic shear moduli of $G' \approx 20$ Pa at $T = 37^\circ C$. The results confirm that in the absence of direct interactions, the FeNRs do not contribute significantly to the mechanical properties, even at high FeNR concentrations (Figure S13, Supporting Information). Crosslinked gels cF1–cF3 (solid symbols) show nearly athermal behavior, indicating efficient crosslinking of the network. In addition, the shear moduli at $T = 37^\circ C$ are $\approx 200$ Pa, which is a factor 10 higher than the corresponding physical mixtures or the PIC control. Despite the low FeNR concentrations (1 mg mL$^{-1}$), the observed increase in stiffness is large, much larger than that for PIC gels which are crosslinked inside the bundles with simple bifunctional linkers.[41] Furthermore, we believe that our approach of crosslinkers from nanoparticles and a linker with one coordination moiety is a straightforward and highly versatile method to generate functional crosslinked composite hydrogels of many other inorganic nanomaterials.

### 2.3. The Stress-Responsive Mechanical Properties

After reheating, the nonlinear mechanical (strain-stiffening) properties of the gels were assessed through the commonly used pre-stress protocol[36] where a small oscillatory stress is applied on top of a much larger steady pre-stress (Figure 2c). The method yields the differential modulus $K' = \delta \sigma / \delta \gamma$, where $\sigma$ and $\gamma$ are the oscillatory stress and strain, respectively, which much more accurately describes the mechanical response of the strained gel. In the linear viscoelastic regime (at low external strain or stress), $K' = G'$. At higher stress, the stiffening regime is entered, which is described by two additional parameters: the critical stress $\sigma_c$ that marks the onset of the stiffening response and the stiffening index $m$, from $K' \propto \sigma^m$, which is a measure for the extent of the stiffening response in the network with the theoretical limit[36] $m \leq 3/2$. The parameters that describe the nonlinear stiffening regime strongly depend on the architecture of the network.[43]

In the gel control (blue symbols) and pF gels (open symbols), $m$ approaches the theoretical limit 3/2 (Figure 2c), which is also found for reconstituted gels of actin.[12] For these three crosslinked gels, however, the stiffening response is weaker $m \approx 1$, which is more in line with collagen networks.[32] This weaker response may result from a reduced responsiveness in stretching the fibers,[26,44] indicating that the particles change the responsiveness inside the crosslinked composite hydrogels. In other words, by crosslinking PIC gels with stiff components, we are able to manipulate the linear and nonlinear mechanical response of the biomimetic gels. Particularly in the nonlinear regime, this level of control is new, since earlier examples of PIC conjugated with semi-flexible (fibrin) or flexible (polyacrylamide) networks[26] always needed a compositional change.

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**Figure 1.** Schematic diagram of the preparation of gel hybrids. a) The azide-functionalized PIC polymer is dissolved at 5 $^\circ C$. b) DBCO-functionalized FeNRs (F1, F2, or F3) in suspension are added to the cold PIC solution in a 1:1 volume ratio. c) The multi-responsive assembled particle-polymer network with enhanced functionalities forms by heating to 50 $^\circ C$, which induces instantaneous PIC bundle formation and gelation followed by a slower crosslinking reaction between the azides on the polymer and DBCO groups on the FeNRs. d–f) Reversibly of the thermoresponsive properties of: pure PIC (d, fully reversible gelation); the physical mixture pF3 (e, fully reversible gelation); and crosslinked cF3 (f, after the crosslinking reaction, the gel is irreversibly formed). Conditions: concentrations PIC and FeNRs are all 1 mg mL$^{-1}$, the molar ratio of [linker]/[azide] is 6:1 for the cF3 gel.
For all gels (1 mg mL\(^{-1}\) PIC, 1 mg mL\(^{-1}\) FeNR, with or without functional group), we measured the storage modulus \(G'\) at low stress (Figure 2d), critical stress \(\sigma_c\) (Figure 2e) and the critical strain \(\gamma_c = \sigma_c/G'\) (Figure 2f) with colors matching those in the other panels. The crosslinking-induced increase in plateau modulus \(G_0\) and \(\sigma_c\) of the hydrogels show that the stiffer gels display a higher stress threshold to enter the stiffening regime. The decrease in \(\gamma_c\), however, indicates that deformations as small as 5% may be enough to stiffen the gel, which is an important factor in designing mechanically sensitive and response networks.

We highlight that this nanoparticle-based crosslinking approach provides a unique opportunity to manipulate hydrogel mechanics where other common strategies are less effective. For instance, changing the polymer concentration changes \(G'\) but does not affect the \(\gamma_c\) and while changing the temperature does affect \(\gamma_c\), it is not always compatible with (biological) applications. \(^{[36]}\)

The effects of extra-bundle crosslinking on the mechanical properties (and network architecture) will depend on the efficiency of the crosslinking reaction, which will depend on the concentrations of azide, linker, and FeNRs. To systematically study the crosslinking effect, we introduce a phenomenological parameter called the crosslinking efficiency \(S\) of the gels, which is defined as the ratio of the shear moduli of the crosslinked composites at \(T = 5\) and 37 °C, \(S = G'_c/G'_e\). \(S\) ranges from \(\approx 0\) (not crosslinked) to 1 (highest crosslinking efficiency) and is used throughout the manuscript.

### 2.4. Network Morphologies of the Composites

The change in stiffness and nonlinear mechanics is expected to be associated with a change in the architecture of the composites. We use cryo-scanning electron microscopy (cryo-SEM) to investigate the impact of the introduction of (functionalized or non-functionalized) FeNRs on the morphology of the network. The control PIC-only network (Figure 3a, b) shows a highly porous network structure. At low densities of non-functionalized FeNRs (Figure 3c, d, FeNRs marked in the red ovals), the particles are randomly dispersed in the network. As expected, non-interacting FeNRs do not change the architecture of PIC network. When functionalized-FeNRs are introduced into the PIC gel and the material is chemically crosslinked (Figure 3g, h), the pores in the structure open up, leading to the formation of a bicontinuous network structure at the micron length scale, that is, similar to the length of the FeNRs, which is beneficial for nutrient exchange in the biological experiments. The images also indicate the formation of thicker fibers, which contributes to the

![Figure 2. Mechanical properties of the composites. a) Crosslinking protocol. A freshly prepared solution of PIC with or without (functionalized) FeNRs was placed on the rheometer and heated instantly to 50 °C where the gel is formed. This temperature was maintained for 10 h (stage I). Then the sample was cooled to 5 °C (stage II, cooling rate 1 °C min\(^{-1}\)), shortly equilibrated, and heated to 37 °C (stage III, heating rate 1 °C min\(^{-1}\)). Panels (b) and (c) show the stage III the temperature ramp. b) Linear viscoelastic regime: storage modulus as a function of the temperature of crosslinked composites (solid squares; orange: FeOOH; red: Fe\(_2\)O\(_3\); black: Fe\(_3\)O\(_4\)), physical mixtures (open squares), and the PIC control (blue data). Data from stage III. Loss modulus \(G''\) is provided in Figure S12a, Supporting Information. c) Nonlinear mechanics: differential modulus \(K'\) as a function of applied shear stress at \(T = 37\) °C. d–f) Shear modulus \(G'\) in the linear viscoelastic regime (d), critical stress \(\sigma_c\) (e) and critical strain \(\gamma_c = \sigma_c/G'\) of the composites and the PIC control gels. For all samples: concentration PIC = 1 mg mL\(^{-1}\); concentration FeNRs = 1 mg mL\(^{-1}\), the molar ratio of [linker]/[azide] is 6:1. Error bars represent the standard deviation of \(n = 2\) or 3 measurements.](image)
increased stiffness. We anticipate that the crosslinking reaction between PIC bundles pulls the bundles together, inducing the architectural change of thicker bundles (and consequently, larger pores), which implies that the density of linkers between the particles and the polymer chains is a key parameter that determines the architecture and, associated, the mechanical properties of the composites, vide infra. This hypothesis is supported by the cryo-SEM images of partially crosslinked cF3, prepared...
by adding less linker in the particle functionalization step (Figure 3e, f). We find that in the less crosslinked sample, the morphology is less interconnected than in the fully crosslinked network. Closer examination of the cryo-SEM images shows that the FeNRs interconnect with each other in the PIC network (yellow arrow) to form a continuous assembled structure.

2.5. Conductivity of FeNR Gel Hybrids

The crosslinking-induced change in network architecture forces both the polymer chains and the FeNRs into a confined volume, increasing particle-particle interactions up to the percolation point. To illustrate the possibilities that this morphological change offers, we performed conductivity measurements on composite gels with the ferromagnetic F3 particles (Figure S16 and Table S1, Supporting Information). Although iron oxide particles are known to be relatively poor conductors—much less than for instance silver nanorods or wires—the combination with their magnetic properties (Figure 3i) makes them interesting components in multi-response soft matter.[45,46] By changing the linker concentration in the composite, we are able to tailor the extent of the architectural change (Figure 3j) and, simultaneously, the interparticle distance and the macroscopic conductivity of the material. The concentrations of PIC and FeNRs are in all experiments the same. Figure 3k shows that the conductivity $\kappa$ (Equation (6)) of fully crosslinked hybrid gels ($\kappa = 0.037 \text{ S m}^{-1}$) increased up to 18-fold compared to pure PIC (no FeNRs, $\kappa = 0.002 \text{ S m}^{-1}$), and sevenfold compared with that of physical mixture ($\kappa = 0.005 \text{ S m}^{-1}$). The value of the crosslinked gel is comparable to that of previously reported conductive hydrogels.[47,48] An increasing degree of crosslinking $\Delta S$ directly results in an increase in conductivity. We believe that the low percolation density, $C_{\text{FeNR}} = 0.1 \text{ wt.\%}$, is a combination of the anisotropy of the particles with the condensation of the particles in the fibrous structure of the crosslinked composite. To confirm this idea, we performed conductivity experiments on composites with nanospheres at a similar weight fraction, crosslinking density, and preparation procedure as the nanorod composites (Figure S17a, Supporting Information). The results show that the conductivity of the nanosphere composite is much lower than that of nanorods equivalent (Figure S17b, Supporting Information), demonstrating that the high aspect ratio of the particles increases particle-particle interactions and drives functional particle concentrations down.

The difference in conductance of the aqueous gels is further illustrated by a complete electrical circuit (Figure 3i) containing the gel and a light-emitting diode (LED), with one electric wire placed in the middle of gels and the other one attached to the diode. Following the conductivity of the samples, the fully crosslinked hybrids show the brightest LED, while the emission from the control PIC, the physical mixture pF3, and partially crosslinked cF3 are much lower.

2.6. Tailoring the Stiffening Response

Both the cryo-SEM and the conductance results indicate that the network morphology is readily tunable with the crosslink density. In addition, key composite hydrogel properties are expected to scale with polymer and FeNR concentrations. In the next paragraphs, we will systematically study how these factors contribute to the hydrogel stiffness. For the particles, we select the superparamagnetic F2 particles ($\text{Fe}_2\text{O}_3$), which lack both the surface $-\text{OH}$ groups of F1 (competition with catechol binding will underestimate the crosslinking density) and the dipolar magnetic interactions of the ferromagnetic F3 nanorods[49] (additional interaction will overestimate the crosslinking density). Comparisons are given in Figure S15, Supporting Information. For the experiments, we used oscillatory rheology of in situ prepared hydrogels with different compositions.

Again, we exploit the thermoreversible characteristics of PIC gels to phenomenologically quantify crosslinking efficiency of the gels. For uncrosslinked composites that cross $T_{\text{gel}}$ on cooling, the difference in moduli will be large but for ideally crosslinked samples, the network architecture will barely change upon cooling and the ratio, or crosslinking efficiency, will approach unity. In addition, we quantify the relative degree of stiffening as a result of crosslinking from the ratio between shear moduli of the composite at $T = 37 \degree\text{C}$ with (cF2) and without (pF2) crosslinking $G'_{cF2}/G'_{pF2}$.

First, we changed the concentration of F2, while keeping the polymer concentration and the density of linkers on the FeNRs constant (Figure 4a, Table 1 entries 1–4). Compared to the composite with 1 mg mL$^{-1}$ of F2, both the relative stiffening and the crosslinking efficiency marginally decreases for the composite with increased particle density (3 mg mL$^{-1}$) of FeNRs. When the concentration of the FeNRs is increased further (12 and 24 mg mL$^{-1}$), however, the effects become much more prominent and the composites behave more like the physical mixture than like a crosslinked gel, characterized by the clearly observed gelation transition (and, associated, low $G'_{cF2}/G'_{pF2}$) and the low degree of crosslinking-induced stiffening. The mechanical results are supported by additional cryo-SEM experiments that show a reduction in the formation of open network morphology with increasing FeNR concentration (Figure S18a–c, Supporting Information).

The most interesting approaches to tune the mechanical properties are the ones that do not considerably change the hydrogel composition, that is, without changing the polymer and FeNR concentrations. Here, we vary the linker concentration on the particles to tune the functionality per particle and, correspondingly, the crosslink density (Figure 4b, Table 1 entries 4–6). The grafting density of the linker was determined by quantitatively measuring the concentration of linker in the washings after the grafting reaction (see Methods Section and Equations (2–5)). To clearly visualize the effect, we started with a high particle density (24 mg mL$^{-1}$), which gave poor crosslinking results at lower linker grafting densities (Figure 4a). Increasing the number of linkers on the FeNRs induces more crosslinking opportunities, leading to much more stable hydrogels. Both the crosslinking efficiency and the stiffening increase considerably.

The alternative route to increase the polymer-particle interaction in the composite is to increase the density of complementary functional groups in the polymers, that is, the concentration azide groups, which was realized by increasing the azide monomer feed ratio in the polymerization reaction to 5%, 10%, and 20%. Subsequent crosslinking of the polymers (0.1 wt.%)
with FeNRs (0.1 wt.%) gives extremely high crosslinking and stiffening efficiencies with storage modulus increasing about 50 times $G'$ of the corresponding pF2 gel or the pure PIC gel (Figure 4c, Table 1 entries 7–11). The near temperature independence of the modulus, indeed indicates a high crosslinking efficiency.

It is interesting to compare the effects, in particular the crosslinking-induced stiffening, of the FeNR-fibrous PIC composite with that of other composites, presented in the literature. A comparative plot (Figure 4d) shows the stiffness change of the crosslinked hydrogel hybrid relative to its stiffness in the absence of the nanoparticle crosslinker. Compared with hydrogels of polyacrylamide/alginate,[50,51] protein,[52,53] poly(ethylene glycol) (PEG),[54] hyaluronic acid,[20] polysaccharide,[25] poly(acrylic acid) (PAA),[55,56] and poly(vinyl alcohol) (PVA),[57] the PIC gel composites stand out with a minimal concentration of polymer and nanoparticles to realize the highest increases, most likely due to the combination of an enhanced nanoparticle-supported fibrous architecture of the matrix. It is remarkable that the highest increase is observed in the sample with a FeNR concentration of only 0.1 wt.%, that is, much lower than in other composites in Figure 4d. Our results demonstrate that the concentrations of nanoparticles and the crosslinking sites are critical parameters in the crosslinking process and that the largest mechanical effects are the result of the changed morphology that can be tuned by tuning the crosslinking parameters. These insights not only provide a route to easily manipulate the mechanical properties of bundled hydrogels

Figure 4. The mechanical properties of F2 gels with different compositions. a) Shear modulus $G'$ as a function temperature $T$ for different F2 nanorod concentrations (see schematic inset). Values for the crosslinking efficiency and the crosslinking-induced stiffening are given in the diagram. The schematic inset highlights the varied parameter as the solid object in the network. Concentrations are given in Table 1. b) Shear modulus as a function of $T$ for composites with FeNRs with different densities of linkers per particle. c) Shear modulus as a function of $T$ for composites with PIC with different azide densities. Note that within (b) and (c), the concentrations PIC and FeNRs remain unchanged. Loss modulus $G''$ is provided in Figure S19a–c, Supporting Information, respectively. d) The comparative chart of crosslinked-induced stiffening of different hydrogel composites. Key: HA = hyaluronic acid; PVA = poly(vinyl alcohol); PAAm = polyacrylamide; PAA = poly(acrylic acid); PEG = poly(ethylene glycol), CNC = cellulose nanocrystals.
without affecting the material composition but, more generally, also guides the development of stiffer nanocomposite hydrogels at very low concentrations polymer (and nanoparticle) concentrations.

### 3. Discussion

In summary, we prepared mechanically tunable biomimetic composite networks composed of functional rod-like nanoparticles and synthetic semi-flexible polymers. The combination of a fibrous matrix and submicron rod-shaped particles gives rise to crosslinking between polymer bundles, where the functionalized FeNRs act as bridges inside network. As a consequence, the well-connected polymer-particle network forms a very open porous network architecture with thicker bundles. To our best knowledge, such architectural change has not been reported in other crosslinked fibrous (biopolymer) networks. As a result, the crosslinked composites show strongly improved mechanical properties in comparison to non-crosslinked gels or to gels that are crosslinked specifically within the bundles. The increase in shear modulus can be as high as 50 times, which results in relatively stiff hydrogels ($G' \approx 1 \text{kPa}$) at minimal polymer/nanoparticle concentration (fraction water 0.998). The stiffening is much higher than what is commonly observed in other composites.

The physical and chemical designability of the PIC gel makes it an attractive synthetic matrix for 3D cell and culture applications (including spheroids and organoids). Although in this work, we drive the crosslinking process to completion at 37°C, we can also carry out the reaction at 37°C where it takes a bit longer and is completely biocompatible (Figure S20, Supporting Information). It is key that the crosslinking takes place at temperatures above the gelation temperature where the bundled architecture is established. Moreover, we stress that, since the semiflexible fibrous architecture is maintained, the stress responsiveness of the gels, which is considered to play a unique biological role,\(^{[58]}\) is also preserved.

The well-interconnected porous network offers the opportunity to generate a percolated conduction pathway at low concentrations. In this work, we demonstrate the formation of such a path at a volume fraction FeO$_4$ nanorods of =0.002, which is well below the percolation threshold for (nano)rods with an aspect ratio of 10. Other nanoparticles, such as (multiwalled) carbon nanotubes may further enhance the effect.\(^{[59]}\) The architecture induced by the crosslinking process offers a convenient strategy to design low-weight fraction conductive gels.

We anticipate that our findings are not restricted to the PIC gels or the FeNRs, but that the results can be extrapolated to other soft, fibrous gels, either synthetic or biological in nature. The crosslinking approach can make them stiffer, more conductive, and responsive. Moreover, the stiffness of PIC composite gels can be higher than 30 kPa by increasing polymer concentration (Figure S21, Supporting Information). Ultimately, the combination of functional nanomaterials in fibrous hydrogels makes an excellent design for the next generation of highly tunable soft materials for applications in or beyond biology.

### 4. Experimental Section

#### Materials

Details of materials preparation are given in the Supporting Information. Three different types of FeNRs (FeOOH, Fe$_2$O$_3$, and Fe$_3$O$_4$) were synthesized following previously reported methods. The size distributions were analyzed by transmission electron microscopy experiments (Figure S1, Supporting Information): FeOOH (diameter $d = 36 \text{nm}$, length $l = 420 \text{nm}$), Fe$_2$O$_3$ ($d = 54 \text{nm}$, $l = 520 \text{nm}$), Fe$_3$O$_4$ ($d = 48 \text{nm}$, $l = 420 \text{nm}$). The structures were confirmed by X-ray diffraction (Figure S2, Supporting Information). The preparation of Fe nanospheres is also detailed in the Supporting Information. The FeNRs were decorated with a bifunctional linker with a catechol at one terminus and a DBCO group at the other terminus (Supporting Information for details). Azide-functionalized PIC polymers were synthesized based on previously reported procedures with a minor modification: the monomer: Ni$_2$ catalyst ratio for all polymers was 1000:1 with the azide-functionalized monomer present in 3.3%, 5%, 10%, or 20%, based on viscometry, the viscosity averaged molecular weight was 354, 387, and 358 kg mol$^{-1}$, respectively.

#### Preparation of Hydrogel Hybrid Samples

To form physically mixed PIC/FeNRs composites, the PIC cold solution was mixed with a pre-cooled, sonicated non-functionalized FeNRs solution in a 1:1 ratio. The solution mixture was transferred into the rheometer at 5°C and heated to induce gel formation. For the crosslinked gel, a pre-cooled, sonicated functionalized FeNRs solution was added to a cold PIC solution in a 1:1 ratio on ice. Then, the briefly mixed solution was transferred to the

### Table 1. The concentrations of FeNRs, linker and azide, grafting linker density $\rho_l$, and stiffness of the investigated PIC composites.

| Entry | $[\text{F}2]$ [mg mL$^{-1}$] | [linker] [mm] | [N$_3$] [μM] | [linker]/[N$_3$] | $\rho_l$ [nm$^{-2}$] | $G'_1$ [Pa] | $G''_1$ [Pa] |
|-------|--------------------------|-------------|--------------|-----------------|-----------------|------------|------------|
| 1     | 1                        | 0.9         | 105          | 8.6             | 39              | 176        | 224        |
| 2     | 3                        | 0.9         | 105          | 8.6             | 13              | 142        | 185        |
| 3     | 12                       | 0.9         | 105          | 8.6             | 3.2             | 0.25       | 25         |
| 4     | 24                       | 0.9         | 105          | 8.6             | 1.6             | 3          | 40         |
| 5     | 24                       | 1.8         | 105          | 17.2            | 3.2             | 70         | 131        |
| 6     | 24                       | 3.6         | 105          | 25.8            | 6.4             | 101        | 144        |
| 7     | 1                        | 0.85        | 0            | –               | 35              | 0.3        | 16         |
| 8     | 1                        | 0.85        | 105          | 8.1             | 35              | 163        | 199        |
| 9     | 1                        | 0.85        | 175          | 4.9             | 35              | 241        | 379        |
| 10    | 1                        | 0.85        | 350          | 2.4             | 35              | 942        | 892        |
| 11    | 1                        | 0.85        | 750          | 1.2             | 35              | 1633       | 1031       |
rheometer (at 5 °C), which was immediately heating to 50 °C where an elastic hydrogel was obtained and the mixture was allowed to crosslink. Unless specified otherwise, the fraction azide-appended monomer of the PIC in the composites was 3.3 mol% and the concentration PIC in the composite was 1 mg mL⁻¹.

**UV–Vis Spectroscopy** The density of linking groups on the FeNR surface was determined through UV–vis spectroscopy during the functionalization reaction. UV–vis absorption spectra from 600–200 nm were collected on a JASCO V-630 UV–vis spectrophotometer. The background adsorption was subtracted before every sample measurement and a quartz cuvette with a path length of 0.2 cm was used to measure the samples. A linear fitting standard curve was obtained from absorbance at 280 nm by collecting spectra of linker samples with different concentrations ranging from 0.05 to 0.5 mg mL⁻¹. To quantitatively determine the conjugation amount of catechol groups in different kinds of iron oxide samples, certain amounts of linker solutions were added to iron oxide solutions and stirring overnight under Ar protection against catechol oxidation. After centrifugation at high speed, all of the iron oxide particles were suspended and the supernatant was collected. The residual amounts of linker were calculated from the absorbance at 280 nm using the standard curve.

**Estimation of Linker Density** The number of linkers per FeNR n and the density of linker groups per average FeNRs surface area ρ were calculated as below, respectively:

\[ n = \frac{N_{\text{cat}}V}{N_{\text{cat}}} \]  
\[ \rho = \frac{N_{\text{cat}}V}{N_{\text{cat}}S_{\text{NR}}} \]  

Here, \( N_{\text{A}} \) is Avogadro’s number, \( c_i \) is the concentration of linker that are attached to FeNRs, \( V \) is the volume of the solution, \( N_{\text{NR}} \) is the total number of FeNRs, and \( S_{\text{NR}} \) is the surface area of single FeNR, which are defined as:

\[ N_{\text{NR}} = \frac{m_{\text{cat}}}{M_{\text{cat}}} \]  

where \( m_{\text{cat}} \) is the total mass of the FeNRs, and \( M_{\text{cat}} \) is the mass of an average FeNR that can be calculated from:

\[ M_{\text{cat}} = \pi rl \rho \]  
\[ S_{\text{NR}} = 2\pi rl + 2\pi r^2 \]  

where r and l are the radius and length of the average FeNR, which were considered to be cylinders, \( \rho \) is the density of the FeNRs measured by Archimedes principle.

**Rheology Measurements** The mechanical properties were analyzed on a rheometer (Discovery HR-2, TA Instruments) using a steel parallel plate geometry with a plate diameter of 20 mm and a gap of 500 μm, fitted with a Peltier plate for temperature control. All samples were loaded into the rheometer at \( T = 5 °C \). The physical mixture samples were heated to \( T = 37 °C \) at a rate of 1 °C min⁻¹, during which the storage modulus \( G' \) was measured in oscillation in the linear viscoelastic regime (amplitude \( \gamma = 0.04 \), frequency \( \omega = 1.0 \) Hz). The crosslinked samples were immediately heated to \( T = 50 °C \), cooled down to \( T = 5 °C \) and equilibrated for 5 min, reheated to \( T = 37 °C \) at a rate of 1 °C min⁻¹ and equilibrated for 5 min. During the entire temperature program, the storage modulus \( G' \) was measured in oscillation (\( \gamma = 0.04 \), \( \omega = 1.0 \) Hz). The crosslinking efficiency was defined as the ratio of the moduli at 5 and 37 °C, \( G'_5/G'_37 \), of the final heating curves. The nonlinear regime of the physical mixtures and the crosslinked samples were evaluated by applying a constant pre-stress (\( \sigma = 0.5−150 \) Pa) with a small superposed oscillatory stress (\( \omega = 10−0.1 \) Hz) to determine the differential modulus \( K' \). Each sample was measured at least twice to ensure repeatability. Note, for samples with long measurement times, a layer of silica oil was added to seal the gap to prevent sample dehydration.

**Conductivity Measurements** The difference of sample preparation between the conductivity measurements and mechanical measurements was that polymers were dissolved in PBS buffer for conductivity measurements. The resistance of the gel composites was tested by a digital multimeter with two-probe setup. To prepare the samples for conductivity measurement, the gels were prepared in 8-well chamber slides with 1.07 cm in length, 0.94 cm in width, and 0.4 cm in height. The conductivity \( \kappa \) was calculated from:

\[ \kappa = \frac{1}{R} \left( \frac{L}{W \times D} \right) \]  

where \( R \) is the measured resistance, \( W \) is the width of the sample, \( D \) is the thickness of the sample, and \( L \) is the distance between the two probes of the multimeter.

**Supporting Information**

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

**Acknowledgements**

The authors acknowledge funding from the China Scholarship Council, grant number 201706260280 and the Institute of Molecules and Materials (IMM), and microscopy services from the department of General Instrument of the Radboud University.

**Conflict of Interest**

The authors declare no conflict of interest.

**Author Contributions**

W.C. synthesized the materials and did the mechanical studies. W.C. and P.H.J.K. wrote the manuscript. P.H.J.K. supervised the project.

**Data Availability Statement**

The data that supports the findings of this study are available in the supporting material of this article. Materials are available on reasonable request from the corresponding author.

**Keywords**

- conductive responsiveness
- iron oxide nanorods
- magnetic responsiveness
- mechanical properties
- nanocomposite hydrogels

Received: June 14, 2021
Revised: August 9, 2021
Published online: August 26, 2021

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