Nanofiber Networks

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Tough Supersoft Sponge Fibers with Tunable Stiffness from a DNA Self-Assembly Technique**

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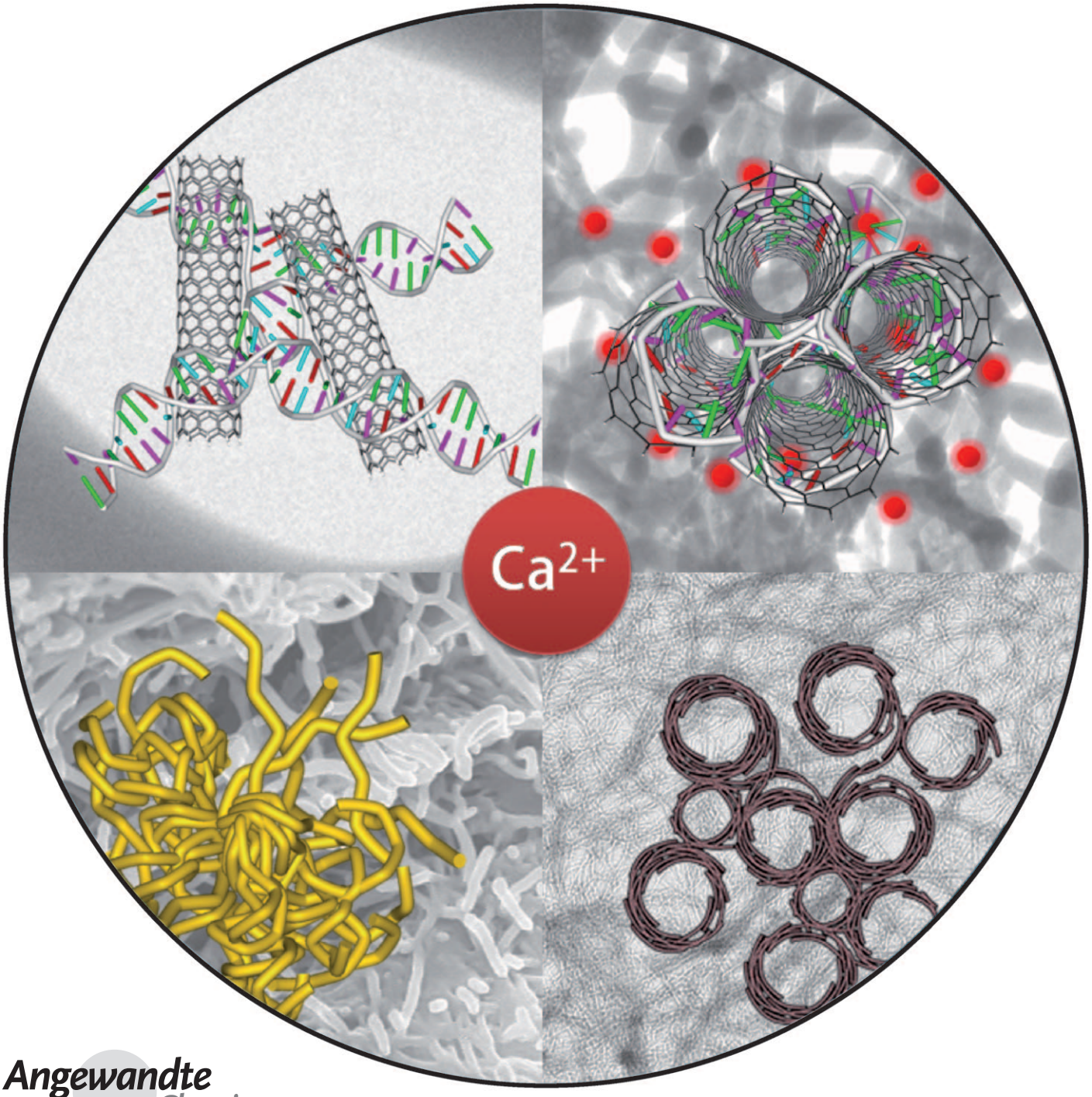

There is great interest in the production of materials that match the mechanical properties of biological soft tissue, such as tendons, muscles, arteries, skin, and other organs. Mechanical support for biological tissue is provided by the extracellular matrix (ECM) in the form of a network of protein-based nanofibers. The morphology of the nanofiber network produces soft tissue with a wide range of stiffnesses:[1] tendon has a Young's modulus of 350 MPa,[2] cartilage 5–25 MPa,[3] and heart muscle 0.02–0.5 MPa.[4] These naturally occurring materials are both soft and tough, a property which has been challenging to reproduce in synthetic materials.[5] Toughened hydrogels have been reported,[6] but highly porous soft materials are typically very fragile. Porous materials are desirable as implants or tissue scaffolds in which the porosity maximizes the interfacial contact area (e.g. for neural prosthetics) or facilitates cell growth (e.g. for tissue scaffolds). Since many biological tissues are subject to regular, large mechanical strains, it is important that the implant material matches the tissue modulus (to avoid strain mismatch inflammation), whilst exhibiting high toughness (to avoid failure).

We describe herein a novel self-assembly method for preparing a tough and porous nanofiber network, in which the toughness, modulus, and swellability can all be tuned by controlling the density and strength of interfiber junctions. We have used a hydrophilic polymer (DNA) to form the matrix of the nanofibers and carbon nanotubes (CNTs) as the scaffold from which to build the nanofiber networks (Figure 1a). DNA is known to interact strongly with CNTs;[7] we found that CNTs dispersed in solutions of double-stranded DNA were stable without any observable aggregation for at least one month (Figure 1b and the Supporting Information). As in our previous work,[8] we used a hydrophilic ionic liquid (IL) to condense the DNA and form an insoluble hydrogel. Hydrophilic ILs are known to efficiently remove bound water from polymers[9] and to interact strongly with CNTs,[10] and have been used as coagulating agents for the wet spinning of DNA fibers. The fibers were rendered water-insoluble as the IL induced the DNA strands to form intertwined toroids.[8] In the present study, we introduced CNTs into the ds-DNA solution before addition of 1-ethyl-3-methyl imidazolium bromide ([emim]Br) IL. The DNA-wrapped CNTs in solution formed intertwined multitoroids (Figure 1c). Fibers were formed by injecting this solution into a coagulation bath of [emim]Br and ethanol. After washing, no trace of [emim]Br could be detected by using X-ray photoelectron spectroscopy. Once dried, the fibers showed a porous sponge structure consisting of a network of entangled nanofibers with a diameter of approximately 50 nm (Figure 2a).

Further refinement of the nanofiber networks could be achieved by soaking the dried fibers in deionized water and then in aqueous $CaCl_2$ solutions. Cryo-TEM images of the reswollen CNTs–DNA fiber that was not treated with Ca^{2+} ions show a loose CNT network (Figure 1d). Treatment with Ca^{2+} ions, however, drives the self-assembly of CNTs through ionic crosslinking of the DNA to form much denser nanofibers (Figure 1e and the Supporting Information), because of the tighter packing of DNA and CNTs. Depending on the concentration of Ca^{2+} ions in the posttreatment solution, the nanofiber diameters could be reduced to as low as 25 nm (Figure 2b). Figure 2c shows the surface of the microfiber, while Figure 2d is an elemental map taken of the sponge fiber by using electron energy loss spectroscopy. The latter image shows that the calcium distribution closely matches the phosphorous distribution, which supports the concept that the Ca^{2+}–PO_2^- ion pairing is responsible for the DNA aggregation. The sponge fibers are sufficiently robust to be knotted, braided, and woven into fabric structures (Figure 2e), so that porosity can be controlled at both the nano- and macroscales, which is important in tissue engineering applications.[11]

To verify the aggregation of DNA-wrapped CNTs induced by Ca^{2+} ions, we also treated CNTs–DNA solutions with $CaCl_2$. Dense CNT nanofiber assemblies of the solutions after treatment with Ca^{2+} ions were observed by using cryo-TEM. The nanofiber diameter was again found to decrease with increasing concentration of Ca^{2+} ions, which suggests that the Ca^{2+} ions screen the PO_2^- charges and induce a greater density of ionic crosslinks, which thereby leads to a tight aggregation of the DNA-wrapped CNTs.

While the structures formed in the sponge fibers were built around the CNT scaffold, the properties of the sponge were also strongly influenced by the DNA. The dried fibers rapidly absorbed water, which resulted in a 15–30-fold swelling based on the weight gain, depending on the concentration of Ca^{2+} ions used in the posttreatment bath (Figure 3a). The use of higher concentrations of Ca^{2+} ions resulted in less swelling and correlated with thinner nanofibers with small pore sizes. These nanofiber sponges behave like crosslinked hydrogels in which the swelling pressure is offset by the elastic stretching of the polymer chains in which the degree of swelling is inversely proportional to the crosslink density. The nanofibers act like polymer chains

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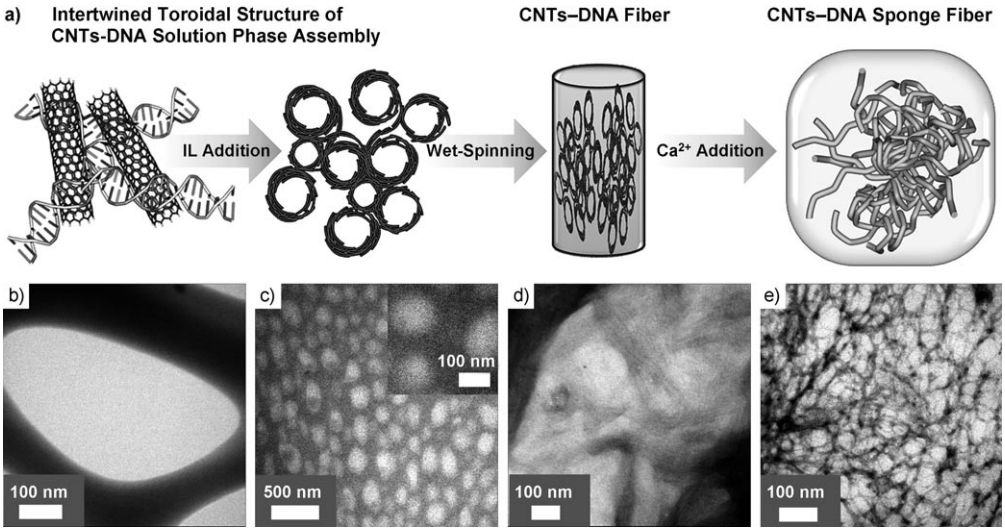

Figure 1. a) Self-assembly of DNA-wrapped CNTs into a nanofiber network. Cryo-TEM images of the spinning solutions and sections of the swollen CNTs–DNA sponge fibers: b) solution without IL treatment (dark regions are the supporting carbon holey grid used for supporting the thin section of frozen solution); c) intertwined toroidal structure of CNTs–DNA solution arising from IL treatment; d) fiber without $CaCl_2$ treatment; e) fiber after $CaCl_2$ treatment (100 mM). A video showing the rotation of the sponge fiber shown in part (e) is also available in the Supporting Information.

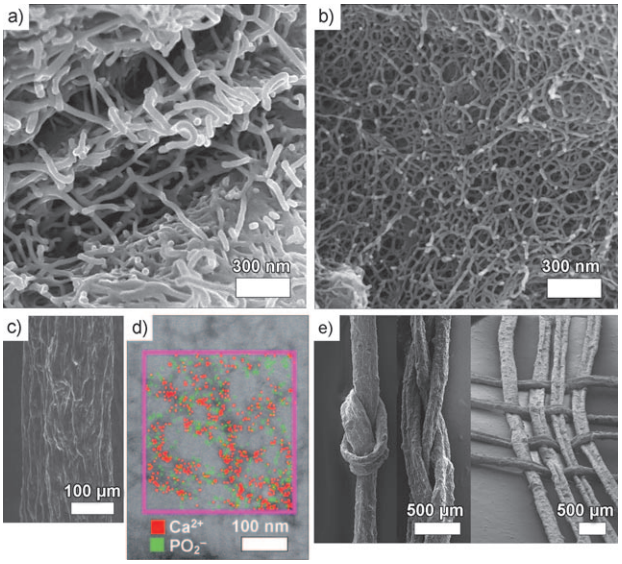

Figure 2. SEM images of continuous porous nanofiber networks a) before and b) after $CaCl_2$ treatment; c) SEM image of the surface of the CNTs–DNA sponge fiber; d) Ca and P mapping image using electron energy loss spectroscopy (EELS) to confirm the binding state of Ca^{2+} ions at PO_2^- sites; e) SEM micrographs of knotted, braided, and woven sponge fibers.

and the nanofiber junctions are the crosslink points. This analogy is further supported by the significant increase in the Young's modulus of the nanofiber sponges with increasing Ca^{2+} concentration of the posttreatment bath (Figure 3b). As with hydrogels, there is an inverse relationship between the modulus of the wet nanofiber sponges and the equilibrium swelling ratio.

A major advantage of these sponges is the use of DNA in their formation, which gives a high toughness compared to previously reported porous nanofiber networks. In the wet state, the elongation at break of the sponge fibers can be as high as 160% and the tensile strength as high as 355 kPa (Figure 3b). These values are similar to those reported for first-generation toughened hydrogels (75% and 680 kPa)[6] and are much higher than most other soft porous materials (Figure 3c). Conventional gels made from biopolymers, such as collagen (gelatin) and fibrinogen, show low toughnesses of 60 $kJ\,m^{-3}$ or less, which is why these materials are often regarded as inadequate for tissue implants.[12] Soft porous materials are fragile,[13,14] with toughnesses usually less than 50 $kJ\,m^{-3}$ when the modulus is less than 500 kPa (equivalent to heart muscle). A few examples of electrospun fabrics show that both low modulus and very high toughness is possible in systems characterized by strong interfiber junctions. Nanofibrous poly(L-lactic acid), with a structure similar to the CNTs–DNA sponges, showed a toughness of 5000 $kJ\,m^{-3}$ when strong junctions were formed.[15] In another study, a similar system gave a toughness of only 3 $kJ\,m^{-3}$ at a modulus of 4000 kPa,[16] presumably as a result of poor interfiber junctions. It is known from the study of microporous materials such as paper that their strength and modulus is dictated by the strength and density of interfiber junctions.[17,18] We regard the improved strength of the nanofiber sponges to arise from "ionic spot-welding" of the interfiber junctions. Divalent Ca^{2+} ions can ionically bind two DNA strands, so the presence of these ions at the nanofiber junctions serves to increase the local adhesion. As a result, the CNTs–DNA sponges give toughnesses of up to 320 $kJ\,m^{-3}$, which is more than three times higher than most biopolymer gels. Without CNTs, the DNA fibers showed a toughness that was 4–40 times smaller than the equivalent CNTs–DNA sponge fiber (see the Supporting Information). Previous work[19] showed that the addition of single-strand DNA to CNT-filtered sheets ("bucky paper") and CNT fibers[20] increased the tensile

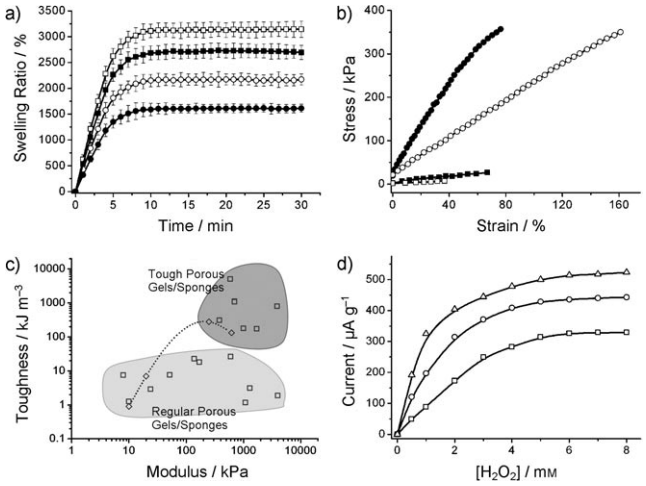

Figure 3. Effect of Ca^{2+} ions on CNTs–DNA sponge fiber properties. a) Water uptake of the dried CNTs–DNA sponge fibers, prepared by using different Ca^{2+} ion treatments (□ 0 mM, ■ 1 mM, ○ 10 mM, ● 100 mM), by soaking in deionized water; b) variation of the adjustable mechanical properties of the CNTs–DNA sponge fiber by treatment with Ca^{2+} ions (□ 0 mM; ■ 1 mM; ○ 10 mM; ● 100 mM); c) comparison of elastic modulus and toughness for various porous compliant materials (□)[15,26–36] and CNTs–DNA nanofiber networks (◇); d) steady-state current response of sponge fibers in presence of hydrogen peroxide at different concentrations and at different applied anodic potentials (△ 0.6 V, ○ 0.4 V, □ 0.2 V; versus Ag/AgCl reference electrode).

strength of the sheets by a factor of between two and four, again through an increase in the strength of interfiber junctions.

In addition to their tunable mechanical properties, the CNTs–DNA sponges were also electrically conductive and can be potentially used as electrodes for sensing, energy storage, and mechanical actuation. Sponge fibers were used as an electrochemical hydrogen peroxide sensor and achieved a steady-state current proportional to the peroxide concentration (Figure 3d and the Supporting Information). The use of CNTs as efficient catalysts for hydrogen peroxide oxidation has previously been reported.[21] Hydrogen peroxide is known to be involved in normal heart function, but has also been implicated in heart disease;[22] the availability of a tough sensor that matches the compliance of heart muscle may provide new insights in cardiovascular research.

In summary, we have demonstrated a method that uses DNA for the self-assembly of CNTs into three-dimensional nanoporous structures. The mechanical properties can be manipulated by altering ionic interactions within and between the DNA-wrapped CNTs. Importantly, the junctions between nanofibers are mechanically robust so that the nanofiber networks are tough. The structures closely resemble the collagen fiber networks that are present in the ECM of biological tissue.[23] Their electrically conductive network is useful for sensing, and is potentially useful for the controlled release of ionic species[24] and mechanical actuation.[25] The latter two functions may be utilized to stimulate specific biological interactions, for example, the control of tissue morphogenesis.[5]

Experimental Section

DNA from Salmon testes (ca. 20000 bp) comprising oriented fibers was purchased from Sigma–Aldrich (St Louis, MO, USA). The room-temperature ionic liquid, 1-ethyl-3-methyl imidazolium bromide ([emim]Br), was purchased from the Solvent-Innovation Co. (Köln, Germany). All other chemicals were used without further purification. The DNA was completely dissolved in deionized water (2% w/w) and the CNTs were added at a specific weight ratio. The CNTs–DNA solution formed a pre-gel state when [emim]Br was added dropwise to a final concentration of about 5% w/w. A narrow jet of the CNTs–DNA solution was injected though a needle (inner diameter = 1 mm) at 1.5 mL min^{-1} into a coagulation bath containing [emim]Br/ethanol (weight ratio 9:1) rotated at 15 rpm. The coagulation time was about 30 min, and the coagulated microfibers were then washed several times with ethanol and deionized water.

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