New class of crosslinker-free nanofiber biomaterials from Hydra nematocyst proteins

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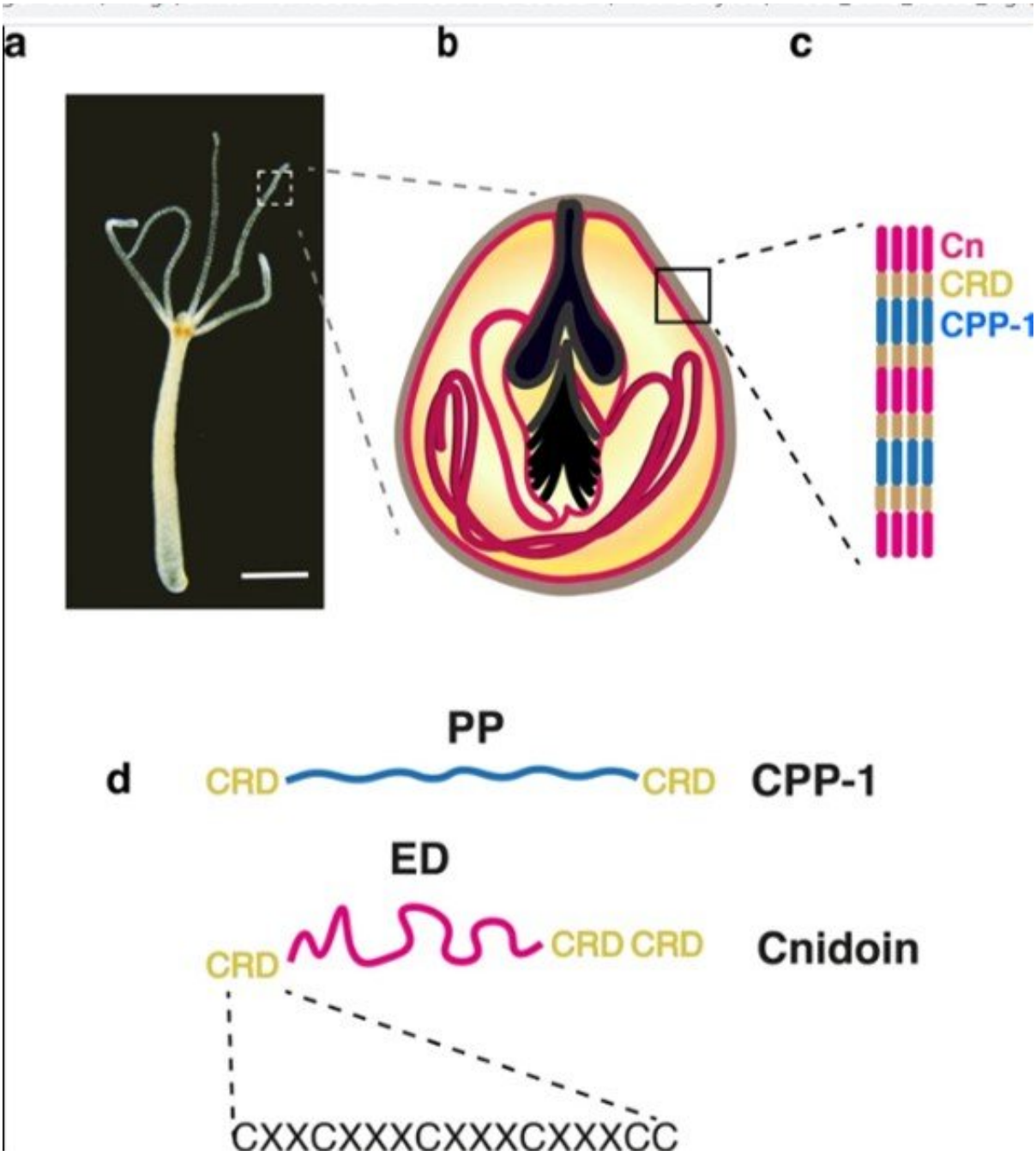


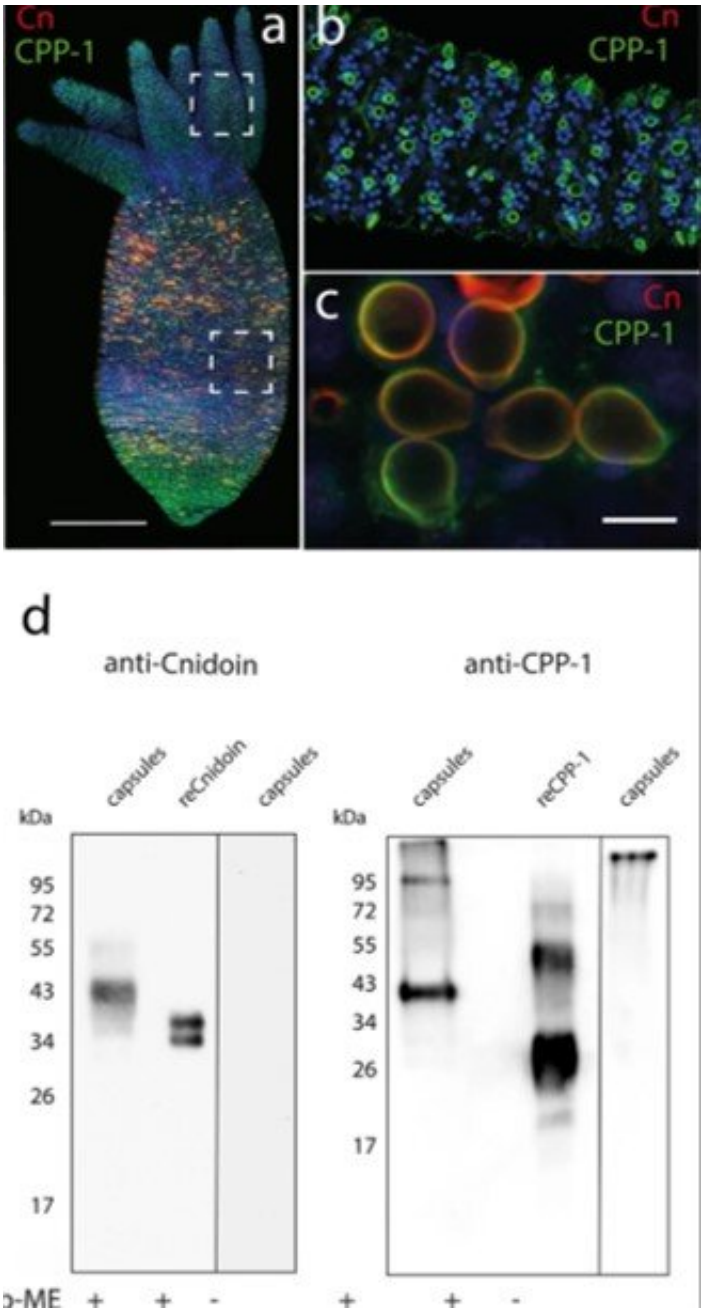
(a) Bright field image of a Hydra polyp (scale bar: 500 µm). (b) Schematic representation of a stenothele-type nematocyst with a large stylet apparatus and a coiled tubule inside of the hollow capsule body. (c) The nematocyst capsule wall consists of CPP-1 and Cnidoin (Cn), linked via cysteine-rich domains (CRDs). (d) CPP-1 has a "rigid" polyproline domain (PP) flanked by two CRD units, while Cnidoin consists of an "elastic", silk-like domain (ED) flanked by CRD units. Each CRD unit has six cysteine residues in a conserved pattern (X denotes a non-cysteine residue). Credit: Scientific Reports, doi: 10.1038/s41598-019-55655-0

Nematocysts are stinging organelles of cnidarians that have remarkable mechanical properties to undergo 50 percent volume changes during explosive exocytosis (process by which cells excrete waste and large molecules), while withstanding osmotic pressures beyond 100 bar. Researchers had recently identified two novel protein components that built up the nematocyst wall in Hydra to include (1) a cnidarian proline-rich protein-1 (CPP-1) with a rigid polyproline motif, and (2) an elastic Cnidoin possessing a silk-like domain. In a new study, now on *Scientific Reports*, Theresa Bentele and a team of researchers in the departments of Medicine, Molecular Evolution and Genomics and the Institute of Physical Chemistry in Germany, Australia and Japan, expressed recombinant Cnidoin and CPP-1 proteins in *Escherichia coli*.

They compared the elastic modulus of spontaneously crosslinked bulk proteins with that of isolated nematocysts. The researchers systematically optimized the fabrication of uniform protein nanofibers using electrospinning and preparative conditions. Both fibers remained stable even after rigorous washing and immersion in bulk water, due to simultaneous crosslinking of cysteine-rich domains. The resulting nanofibers were clearly different from other protein nanofibers that

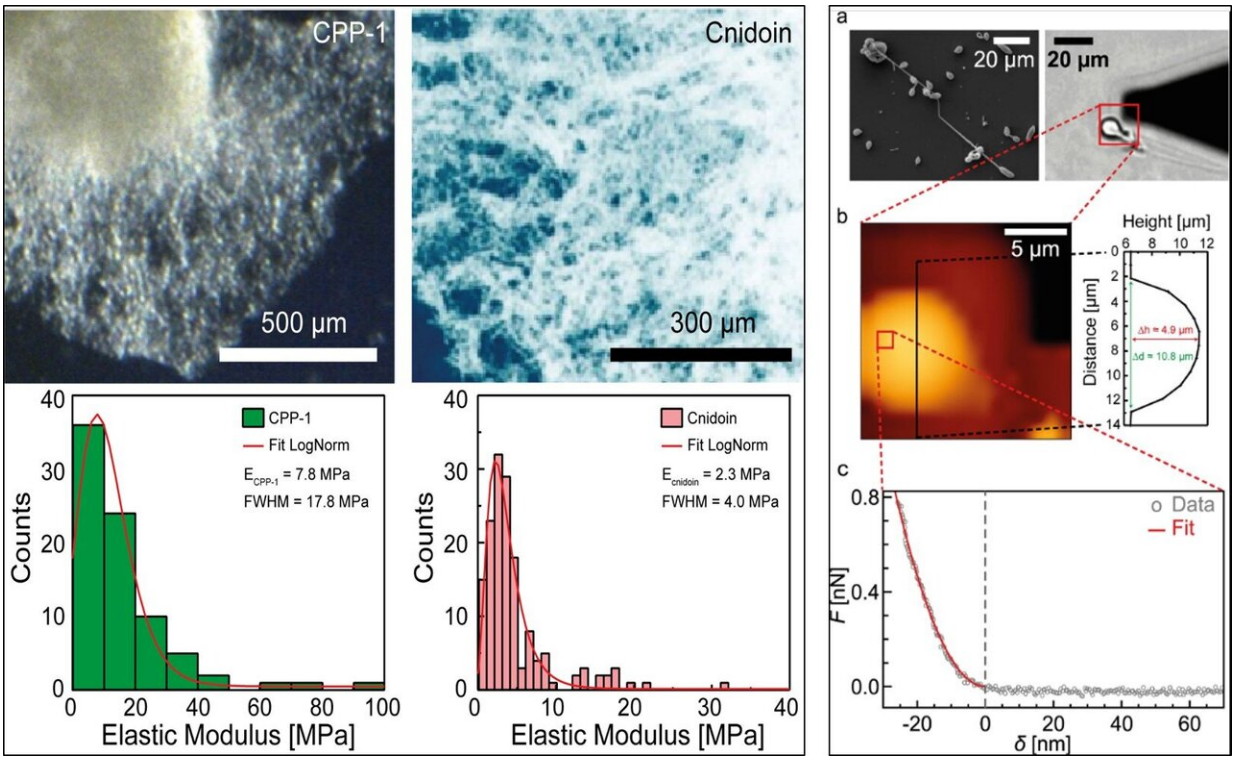
were unstable without chemical crosslinker procedures. After quantitative assessment of mechanical properties, they examined applications of Cnidoin and CPP-1 nanofibers to promote the growth of human mesenchymal stem cells.

Hydra nematocysts comprise four variants that develop in the body column of polyps in specialized cells known as nematocytes. The outstanding mechanical toughness of the capsule wall structure make nematocysts unique to form bioinspired materials in the lab. The capsule contains protein complexes crosslinked by intermolecular disulfide bonds between cysteine-rich domains (CRD), which can be used as a versatile crosslinker to create linear or branched polymers among diverse proteins. The scientists had already identified two new capsule proteins including CPP-1 and Cnidoin while studying Hydra nematocysts in their previous work. The potential to combine elastic Cnidoin and rigid CPP-1 proteins was a promising strategy to design new biomaterials capable of forming stable structures with spontaneous crosslinking to realize outstanding flexibility and toughness, similar to biological nematocyst capsules. Synthetic bioinspired protein nanofibers have gained increasing attention as an artificial matrix to culture stem cells for tissue engineering applications. Electrospinning offers a common method to fabricate such fibers using silk proteins, collagen and gelatin. The thin fiber products have multiple applications in wound healing and tissue engineering.



(a) Immunofluorescence image of a Hydra polyp stained with CPP-1 and Cnidoin antibodies; cell nuclei (blue), CPP-1 (green), and Cnidoin (red). (b) Mature capsules in tentacles showed only CPP-1 signals. (c) Zoom-in images of capsules in the gastric region indicated co-localization of CPP-1 and Cnidoin in nematocyst walls. (d) Western blot analysis of CPP-1 and Cnidoin in isolated nematocysts and after recombinant expression in E. coli (reCPP-1, reCnidoin). (+) and (−) indicate the presence or absence of β−mercaptoethanol (β−ME) in the sample buffer. Credit: Scientific Reports, doi: 10.1038/s41598-019-55655-0

In the present work, Bentele et al. introduced a new class of synthetic crosslinker-free nanofibers based on the Hydra nematocyst proteins CPP-1 and Cnidoin using electrospinning. Based on the spontaneous crosslinking capacity of CRDs they systematically optimized the preparative conditions to bioengineer crosslinker-free protein nanofibers that are stable under water with potential applications for human stem cell culture. The research team obtained representative immunofluorescence images of a Hydra conjugated with CPP-1 (green) and Cnidoin (red) antibodies to co-localize the proteins in the capsule wall. The images indicated the presence of Cnidoin as more densely packed within mature nematocyst walls compared to CPP-1. Thereafter, Bentele et al. used Western blot methods to identify the isolated native nematocyst capsules and recombinant proteins (proteins expressed in other organisms); which they produced in E. coli. The results indicated considerable post-translational modifications of CPP-1 in Hydra. They confirmed the results using the CPP-1 protein as expressed in E. coli and deduced both CPP-1 and Cnidoin to be structural proteins of the nematocyst wall integrated during formation or morphogenesis.

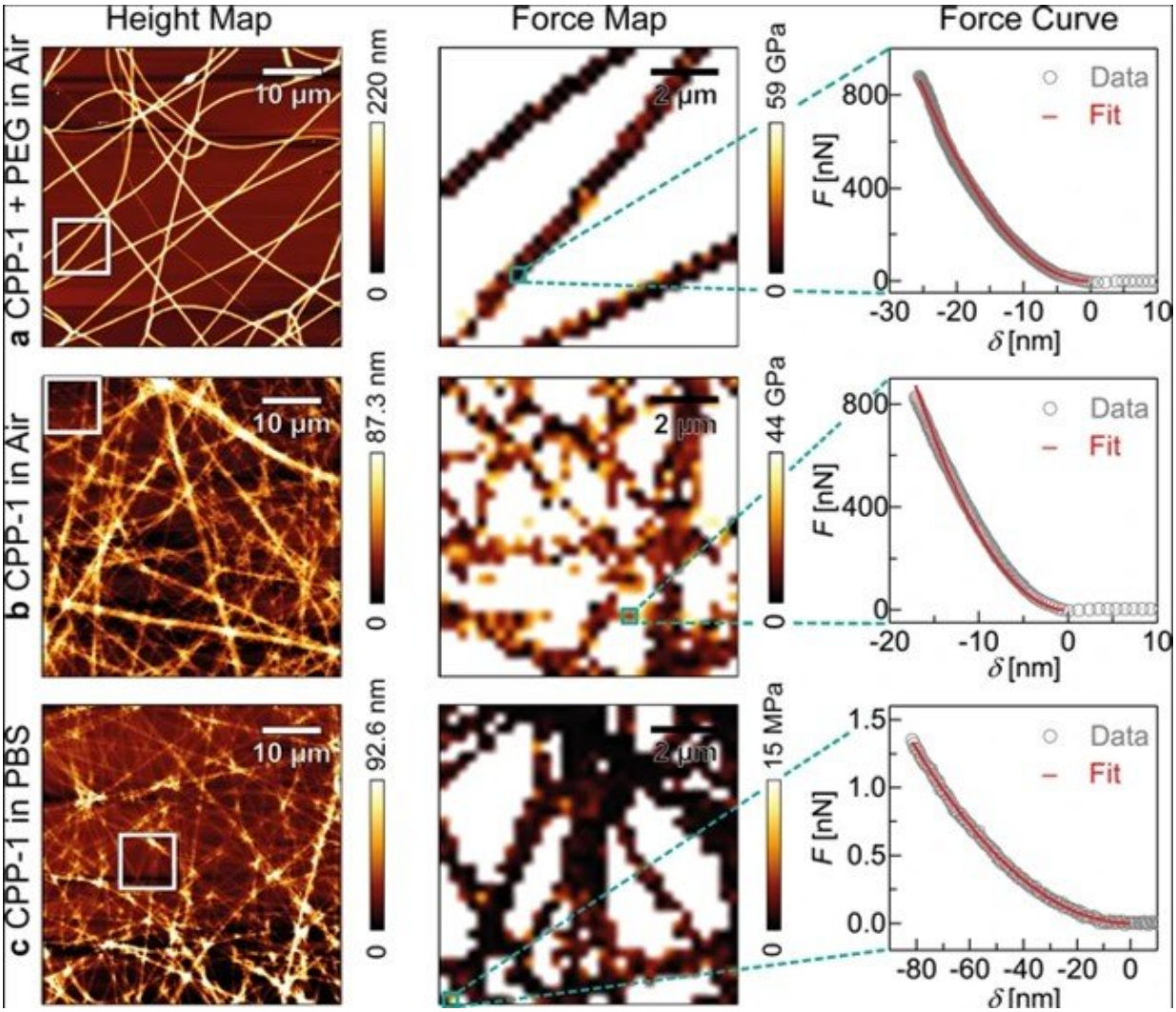


LEFT: Effective elastic moduli of recombinant CPP-1 and Cnidoin in PBS. Aggregates from purified and oxidized reCPP-1 and reCnidoin proteins were subjected to AFM indentations. The distributions of the effective elastic moduli were fitted using a log normal distribution. The peak positions and FWHM are shown as the legends. RIGHT: (a) Left: SEM image of isolated, partly discharged nematocysts. Right: Bright field microscopy image of an isolated discharged stenothele. The black triangle shadow corresponds to the AFM cantilever. (b) Height map of the discharged nematocyst collected from the red square in (a) (17 × 17 µm2). (c) A typical force-indentation curve measured on the nematocyst at the position indicated by the red square in (b) (1.1 × 1.1 µm2). The force-indentation data (gray circles) was fitted with the Bilodeau model for pyramidal tips (red curve). Credit: Scientific Reports, doi: 10.1038/s41598-019-55655-0

The researchers then tested the mechanical properties of Hydra nematocysts and bulk proteins using scanning electron microscopy

(SEM) and atomic force microscopy (AFM). The scientists extracted the distribution of the elastic moduli and further measured the elasticity of purified recombinant CPP-1 (reCPP-1) and Cnidoin (reCnidoin) expressed in E. coli. They then optimized nanofiber production by introducing polyethylene glycol (PEG) 900 kDa to the pure solution to obtain higher viscosity of the product. The team investigated the influence of relative humidity—which significantly affected the quality of nanofibers, while the ionic strength or conductivity of spinning solutions showed no influence on the nanofibers.

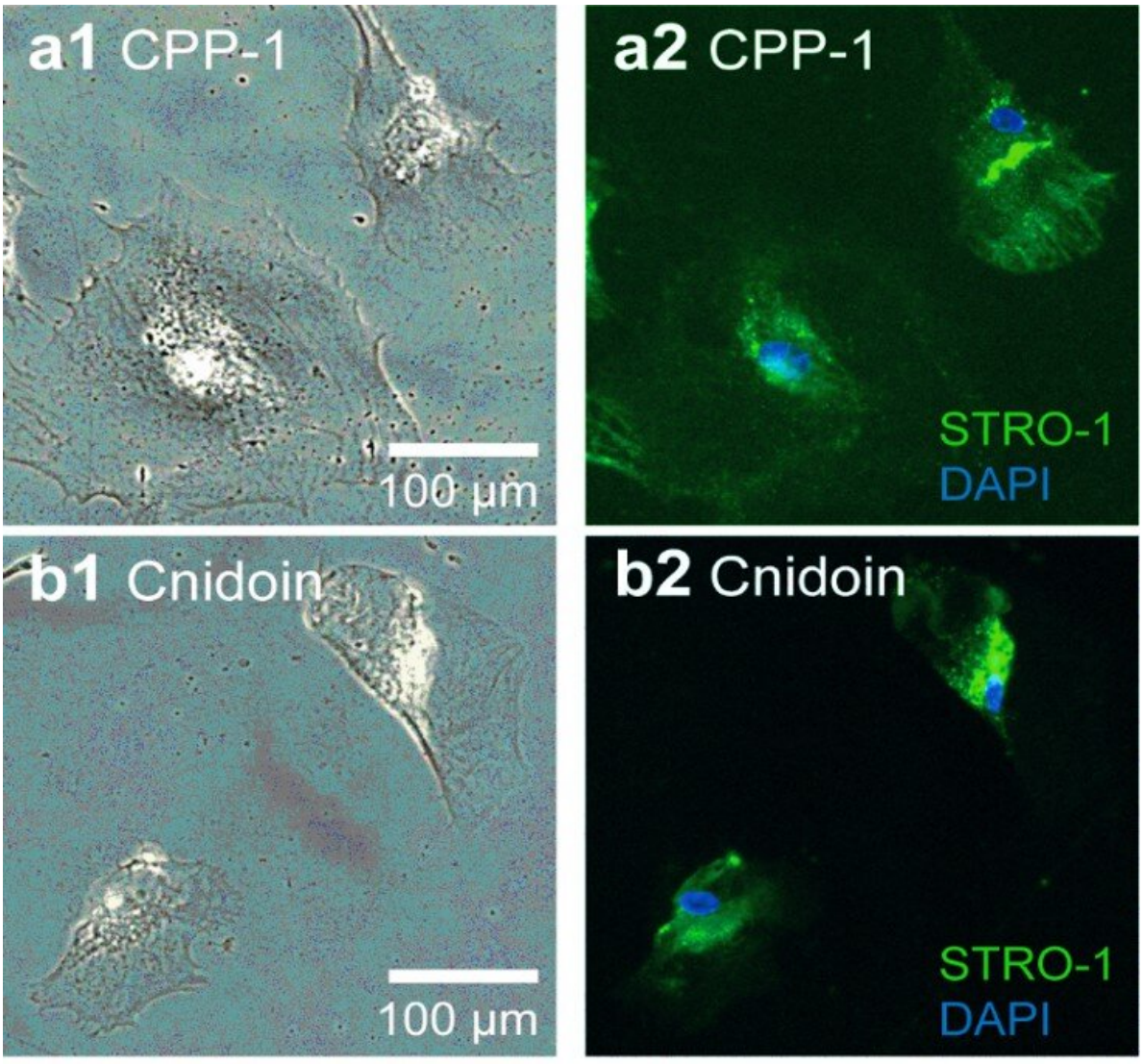
Based on the preliminary materials development and characterization results, Bentele et al. fabricated protein nanofibers by electrospinning the protein-PEG solution on glass coverslips. The freshly spun recCPP-1-PEG nanofibers showed a uniform width and height across a 50 x 50 μm^2 area and displayed a uniform elastic modulus. The team then measured the surface topography, obtained an elasticity map and a characteristic force-indentation curve for reCPP-1 and reCnidoin nanofibers (a) in air, (b) in air after washing with water, and in (c) physiological buffer solution. They could remove PEG by washing with water to obtain a significantly decreased fiber thickness for reCnidoin nanofibers, although the dimensions were less pronounced compared to reCPP-1 after water treatment.

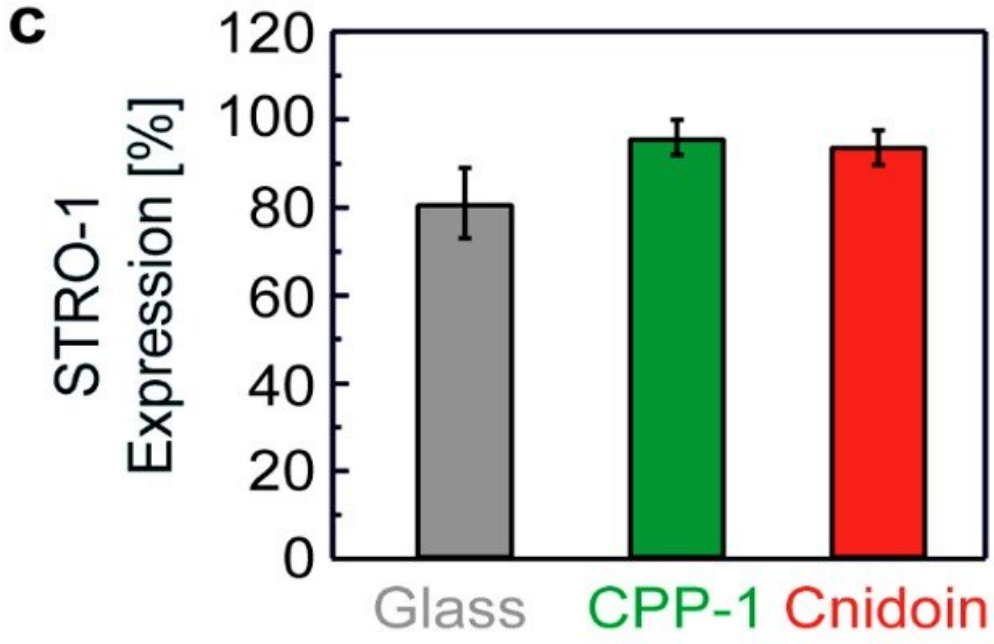


AFM measurements of electrospun reCPP-1 fibers. First, a reCPP-1:PEG (1:1) mixture was electrospun and characterized in air (a). Second, the reCPP-1:PEG fibers were washed by water, and the remaining reCPP-1 fibers were characterized in air (b), as well as in PBS (c). Each dataset consists of height maps (left column), force maps (middle column), and characteristic force-indentation curves (right column) fitted with the Bilodeau model (red curve). Credit: Scientific Reports, doi: 10.1038/s41598-019-55655-0

However, the fibers did not dissolve entirely after washing with water and retained their elastic moduli. The results suggest that the two

recombinant proteins can establish stable nanofibers by spontaneously forming disulfide bonds between CRD (cysteine-rich-domain) termini. The recombinant Hydra nematocyst proteins produced in this work also formed uniform and stable nanofibers through naturally occurring CRDs within air and in physiological buffer. The team examined the applications of these nanofibers with stable human mesenchymal stem cell culture for 20 days of incubation, during which approximately 95 percent of cells showed cell growth and viability on the new bioinspired materials.


a1 CPP-1
100 µm
a2 CPP-1
STRO-1
DAPI
b1 Cnidoin
100 µm
b2 Cnidoin
STRO-1
DAPI


c
STRO-1 Expression [%]
120
100
80
60
40
20
0
Glass
CPP-1
Cnidoin

Maintenance of hMSC on nanofiber substrates. Protein nanofibers substrates coated with (a) reCPP-1 and (b) reCnidoin nanofibers for 20 days. Phase-contrast microscopy images (a1 and b1) and the corresponding fluorescence images (a2 and b2) show the expression of STRO-1 (green) in the cytosol of hMSC. Cell nuclei were stained with DAPI (blue). (c) Fractions of hMSC immunoreactive to anti STRO-1, cultured for 20 d on glass (control), reCPP-1 and reCnidoin nanofibers (N > 30 for each samples). Credit: Scientific Reports, doi: 10.1038/s41598-019-55655-0

In this way, Theresa Bentele and colleagues proposed a new synthetic crosslinker-free nanofiber biomaterial, bioinspired by the nematocyst capsule proteins of Hydra. They expressed recombinant proteins of two recently identified CPP-1 and Cnidoin nematocyst capsule proteins within E. coli and prepared nanofibers via electrospinning. As a result of the cysteine-rich domains (CRD), the electrospun fibers could spontaneously crosslink via disulfide bonds. The reCPP-1 and reCnidoin recombinant proteins formed uniform nanofibers that were stable in water directly after electrospinning. The new material constructs demonstrated potential as biocompatible materials inspired by the tough and elastic Hydra nematocyst structure.

More information: Theresa Bentele et al. New Class of Crosslinker-Free Nanofiber Biomaterials from Hydra Nematocyst Proteins, *Scientific Reports* (2019). DOI: 10.1038/s41598-019-55655-0

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