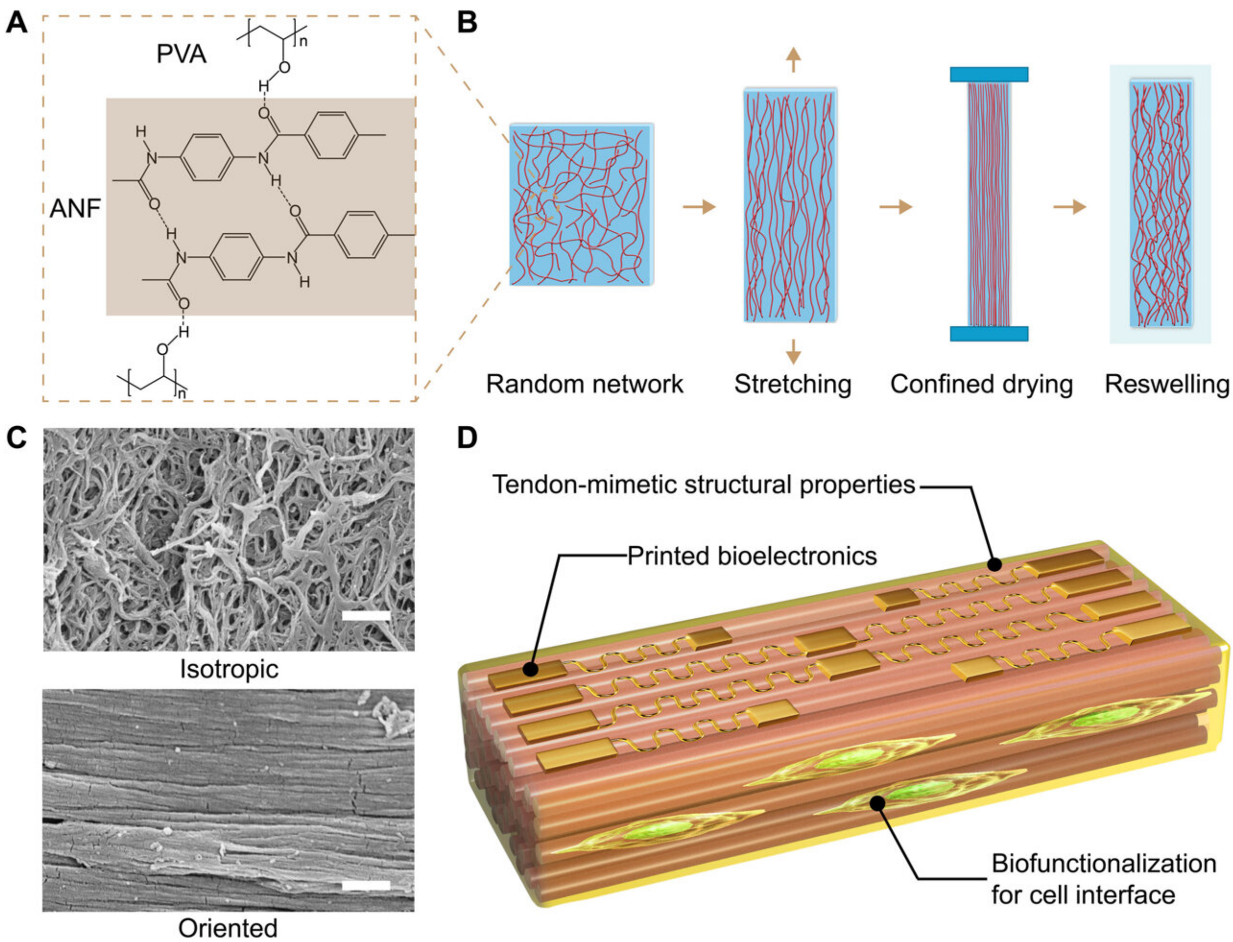
Tissue engineering: Developing bioinspired multi-functional tendon-mimetic hydrogels

February 27 2023, by Thamarasee Jeewandara



Design and processing of tendon-mimetic anisotropic composite hydrogels (ACHs). (A) Chemical structures of ANF and PVA and their intermolecular hydrogen bonding. (B) Schematics of the processing steps for ACH involving stretching and confined drying for the orientation of nanofiber assembly. (C) SEM images of isotropic ANF-PVA hydrogel (top) and ACH-80 (bottom). Scale bars, 1 μm. (D) Schematics of the multifunctional tendon-mimetic ACHs.

Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade6973

In a new report now published in *Science Advances*, Mingze Sun and a research team in physics, mechanical engineering, electrical and electronic engineering in Hong Kong China reported the development of multifunctional tendon-mimetic hydrogels by assembling aramid nanofiber composites.

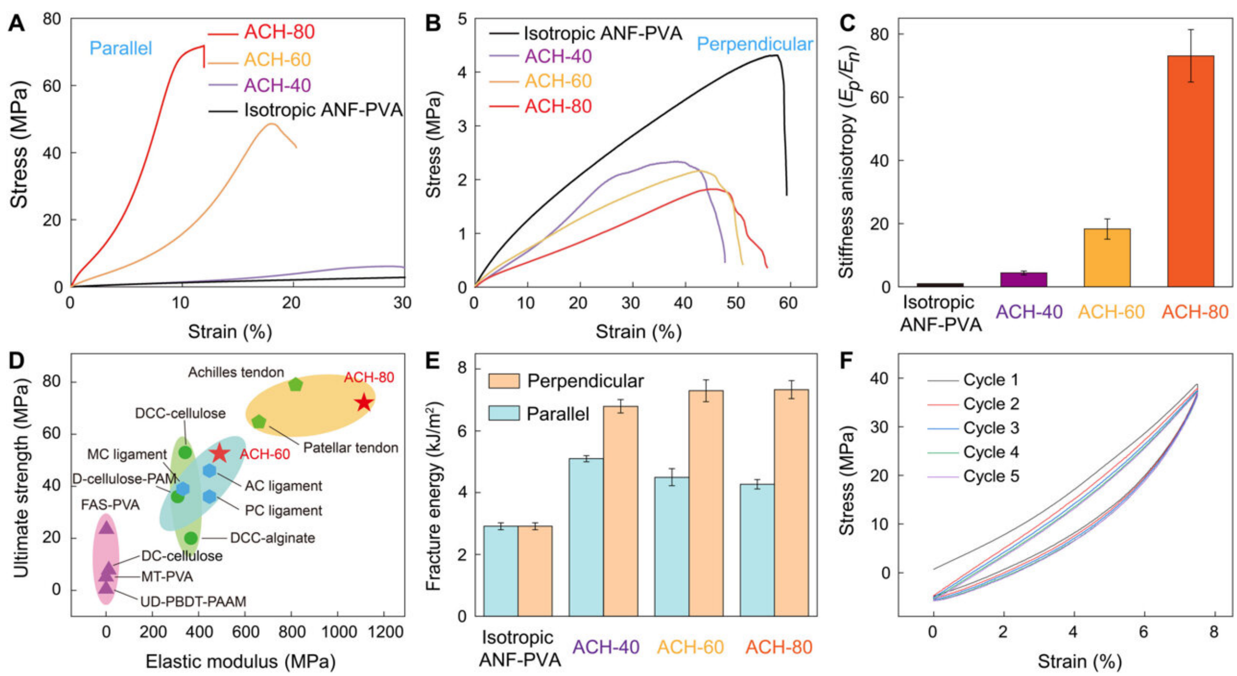
The anisotropic composite hydrogels (ACH) contained stiff nanofibers and soft polyvinyl alcohol moieties to mimic biological interactions that typically occur between collagen fibers and proteoglycans in tendons. The team was bioinspired by natural tendons to develop hydrogels with a high elastic modulus, strength and fracture toughness.

The researchers biofunctionalized these material surfaces with bioactive molecules to present biophysical cues to impart behavioral similarities to those of cell attachment. Additionally, the soft bioelectronic components integrated on the hydrogels facilitated a variety of physiological benefits. Based on the outstanding functionality of the tendon-mimetics, the team envisioned broader applications of the materials in advanced tissue engineering to form implantable prosthetics for human-machine interactions.

Materials engineering a biomimetic tendon

Materials scientists work to develop advanced biological materials for medical devices and tissue engineering platforms to emulate natural biological tissue architectures via materials engineering. However, the natural tissue architecture has a variety of characteristics that are difficult to synthetically replicate. The architecture of tendons relies on the load-bearing capacities of the musculoskeletal system to provide biophysical cues that translate into cellular behaviors via interfacial interactions. In the past decade, researchers had devoted extensive research efforts to engineer tendon-mimetic materials with high structural anisotropy.

Sun and colleagues developed an advanced materials platform for their work to construct hybrid anisotropic hydrogels with tendon-like behaviors and multifunctionality at the bio-interfaces. During the experiments, they established reconfigurable interactions between the stiff and flexible polymers to form a highly oriented framework that emulated a microstructural interplay between aligned collagen fibers and the soft proteoglycans. The biomimetic results of the anisotropic biophysical cues thereby regulated the cell behavior.

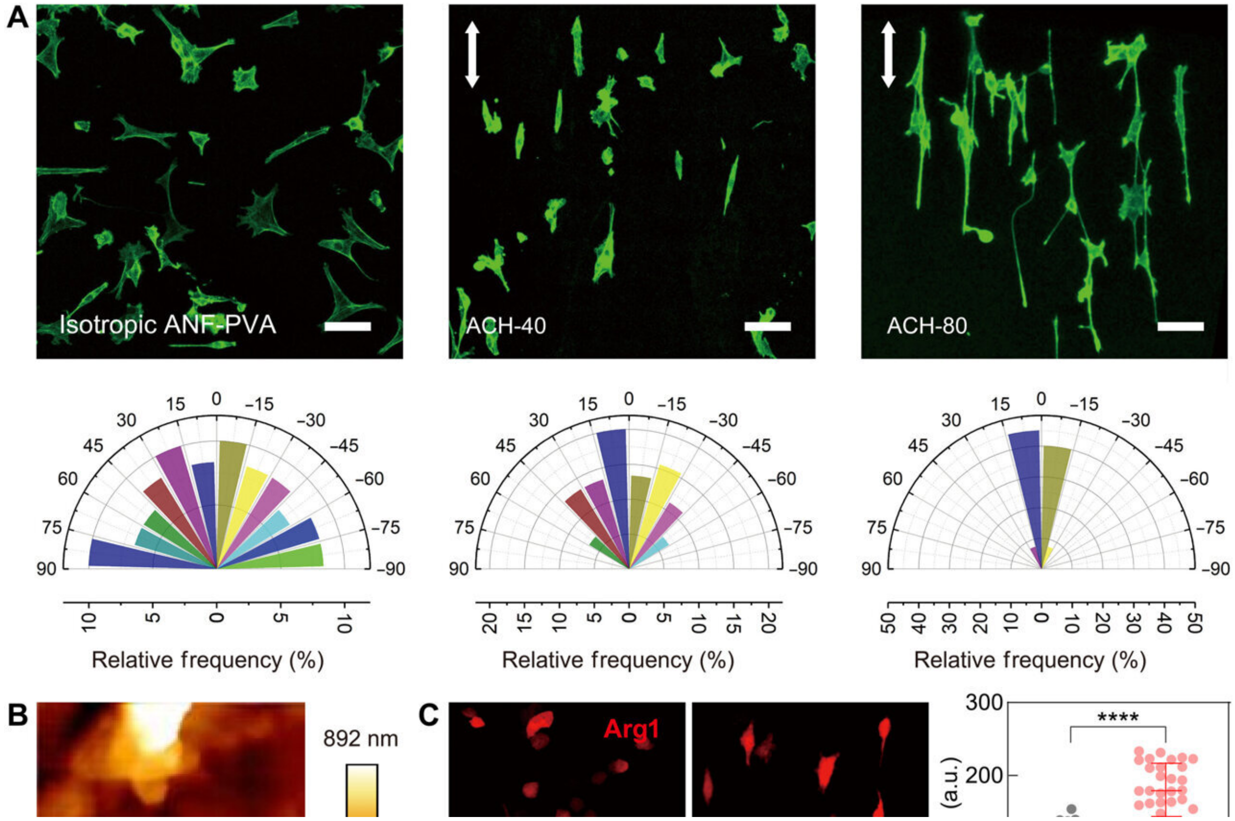


Mechanics of ACHs. (A and B) Tensile stress-strain curves of ACHs in the directions parallel (A) and perpendicular (B) to the fiber orientation, respectively, as compared with the responses of isotropic ANF-PVA hydrogels. The sample denoted as ACH-x corresponds to x% of imposed elongation during the prestretching-drying process. (C) Stiffness anisotropy of ACHs, as characterized by the ratio between initial tensile moduli parallel (Ep) and perpendicular/normal (En) to the fiber alignment. (D) Moduli and strengths of ACH-80 and ACH-60 as compared with those of natural tendons, ligaments, and other anisotropic hydrogels with tendon-mimetic characteristics. (E) Fracture energies of ACHs measured in the directions parallel and perpendicular to the fiber alignment, as compared with those of isotropic ANF-PVA hydrogels. (F) Cyclic tensile tests on ACH-80 in the direction parallel to the fiber alignment, with 7.5% of maximum imposed strain. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade6973

Creating an advanced polymer material in the lab

The research team developed anisotropic composite hydrogels by stretching and confining the material consisting of stiff and flexible polymer constituents. The resulting material demonstrated branched microstructures that mimicked collagen-like building blocks.

The team conducted extensive hydrogen bonding between the two polymer constituents to create a three-dimensional network with high toughness where the fibrillar network did not undergo structural disintegration even under high levels of strain, leading to their consistent alignment. They then observed the characteristic fibrillar network of isotropic hydrogels that resembled hierarchical structures as seen in natural tendons—such efforts weren't as feasible with pre-existing synthetic hydrogels.



Regulating cell morphology and phenotypes with biofunctionalized ACHs. (A) Fluorescence images of F-actin in NIH-3T3 fibroblasts cultured on various substrates (top) and the corresponding angular distribution of cell orientation (bottom) (n ≥ 30). Zero angle (0°) represents the direction parallel to the fiber alignment. Scale bars, 100 μm. (B) AFM images showing the surface topography of biofunctionalized ACH-80 (bottom) and isotropic ANF-PVA hydrogel (top). Scale bars, 1 μm. (C) Fluorescence images of RAW 264.7 macrophages cultured on isotropic ANF-PVA hydrogel (left) and ACH-80 (middle), immunostained for M2 biomarker Arg1, and statistics of the mean fluorescence intensity (MFI) of individual cells showing the differences induced by distinct substrates (right). The cell cultures were treated with IL-4 and IL-13 to induce M2 phenotype. Scale bars, 50 μm. a.u., arbitrary units(D) Immunostaining for iNOS (M1 biomarker) in RAW 264.7 showing the distinct effects induced by isotropic ANF-PVA (left) and ACH-80 (right), also characterized by MFI statistics (right). IFN-γ and LPS were used to induce M1 phenotype. Scale bars, 50 μm. n = 30, ****P Science Advances (2023). DOI: 10.1126/sciadv.ade6973

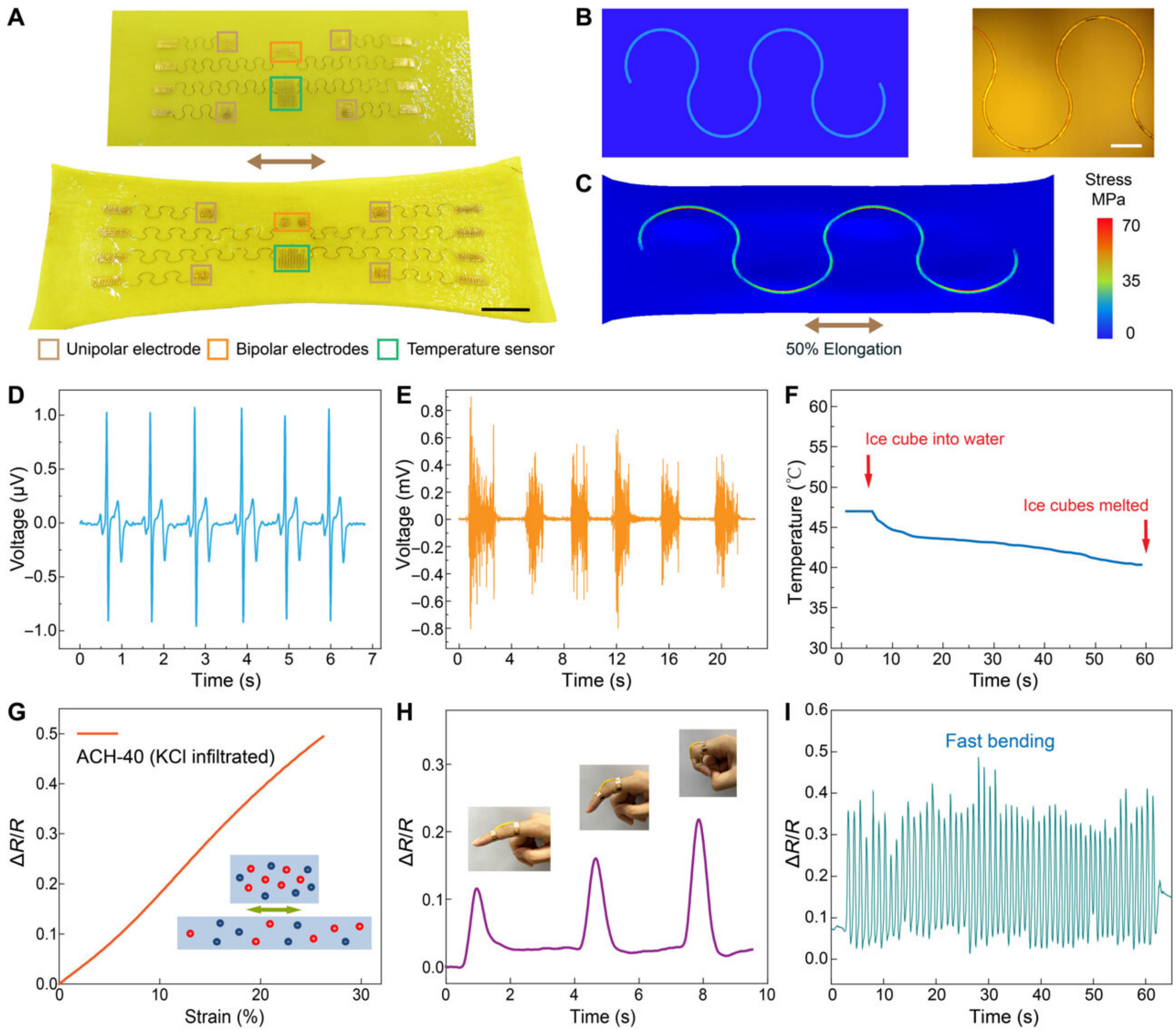
Biofunctionalization of the advanced polymers

The scientists next studied the structural characterization of the new polymer and its influence on cell behavior via interfacial interactions. They adopted chemical functionalization to present a cell adhesion motif such as the arginylglycylaspartic acid motifs to bind with integrins on the cell membrane. The researchers noted the successful biofunctionalization of the advanced materials by observing the adhesion of fibroblast cells on the material surfaces, while samples without surface functionalization did not demonstrate similar cell attachment.

The Rho-associated protein kinase (ROCK) molecules played a significant role by regulating the contractile machinery of cells during cell morphological responses to surface topography and substrate mechanics. The material constructs additionally regulated the macrophage cell differentiation between the pro-inflammatory M1 variants and the pro-healing M2 variants to establish bio-favorable implantable devices.

Advanced materials applications as bioelectronics

The research team ultimately demonstrated multimodal physiological sensing by integrating the advanced materials into soft bioelectronics. Among these iterations, they adopted a serpentine design to create wafer-based electronics with high stretchability to withstand the prewashing-drying process of the materials. They used finite element analysis to assess stress distribution across the device and enhanced the stretchability of the electronic component by modifying its geometrical design for improved mechanical integrity.



ACHs with integrated multifunctional bioelectronics. (A) Photographs of serpentine electronics transfer-printed onto an isotropic ANF-PVA hydrogel (top) and their stretched state with the processed ACH (bottom). The insets show various functional components. Scale bar, 2 cm. (B) FEA model (left) and microscope image (right) of a representative serpentine device bonded with isotropic ANF-PVA hydrogel. Scale bars, 1 mm. (C) FEA simulation on the stress distribution in the serpentine device under 50% elongation imposed to the hybrid structure. (D and E) ECG (D) and EMG (E) measured with bioelectrodes on a hybrid ACH. (F) Temperature variation in a water bath characterized with a temperature sensor on a hybrid ACH. (G) Schematics and the resistance response to tensile strain for an ionically conductive ACH sample. (H and I) Responses of an ACH-based strain sensor mounted on a finger under various amplitudes of deformation (H) and cyclic motion (I). Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade6973

Outlook

In this way, Mingze Sun and colleagues engineered tendon-mimetic hydrogels with outstanding mechanics and functionality that predominantly originated from the assembly of nanofibers. They used biophysical cues presented by the materials constituents to regulate the cell dynamics, useful for advanced tissue engineering applications. The tendon-mimetic behavior of the advanced materials were useful as implantable tissue prosthetics.

The researchers examined the physical integration between the advanced materials and natural tissues in vivo and envision the possibilities of using multifunctional bioelectronics integrated on advanced materials. These include delivering critical capabilities such as physiological monitoring and integrating wireless modules for two-way communications between the external hardware and the electronically active prostheses.

More information: Mingze Sun et al, Multifunctional tendon-mimetic hydrogels, *Science Advances* (2023). DOI: 10.1126/sciadv.ade6973

Jeong-Yun Sun et al, Highly stretchable and tough hydrogels, *Nature* (2012). DOI: 10.1038/nature11409

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