Single Cell Mechanoprofiling of Aging and Cancer

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Key Findings

- **RGD-functionalized ultrashort peptide nanofibers reveal cell-specific adhesion mechanophenotypes.**
- **Young fibroblasts respond to RGD primarily through increased adhesion energy (EA), whereas healthy MSCs respond through enhanced mechanical reinforcement (FA/Dmax).**
- **Cancer-derived and high-passage (aged) cells exhibit convergent adhesion behaviors, characterized by attenuated RGD responsiveness.**
- **Most RGD-mediated adhesion maturation occurs within the first 10 s of contact.**

Main Conclusion

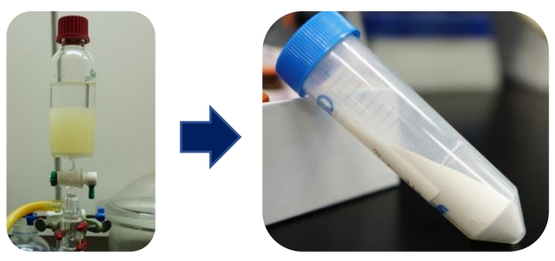
AFM mechanoprofiling using RGD-functionalized ultrashort peptide nanofibers discriminates healthy, aged, and malignant cell states through distinct adhesion maturation strategies.

Background on Synthetic Ultrashort Self-assembling Peptides (USP)

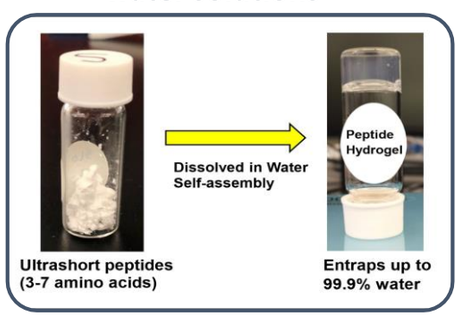
Why ultrashort self-assembling peptides?

- **Chemical Synthesis**: •Creating novel materials (peptidomimetics, cyclic peptides, etc.)
- **New Functionalities**: •Glycan moieties •Cellular recognition motifs •Cross linkers & metal coordination
- **Tunable Properties**: •Viscoelasticity •Adhesion •Surface modification •Degradability

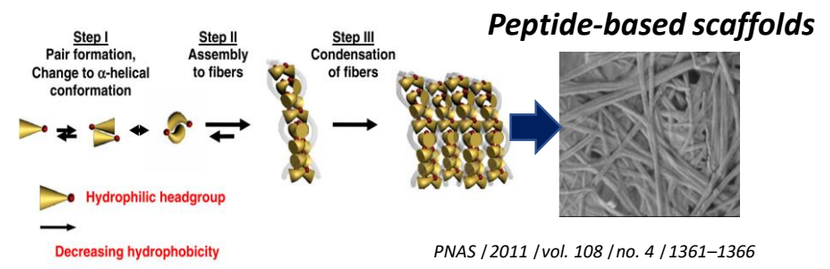
Chemical Synthesis of Ultrashort Self-assembling Peptides



Spontaneous self-assembly upon addition of water solutions



Self-assembling Into a Porous Nanofibrous Structure



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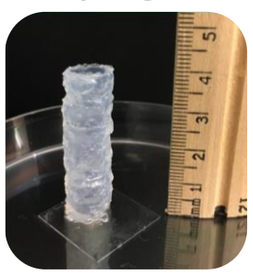
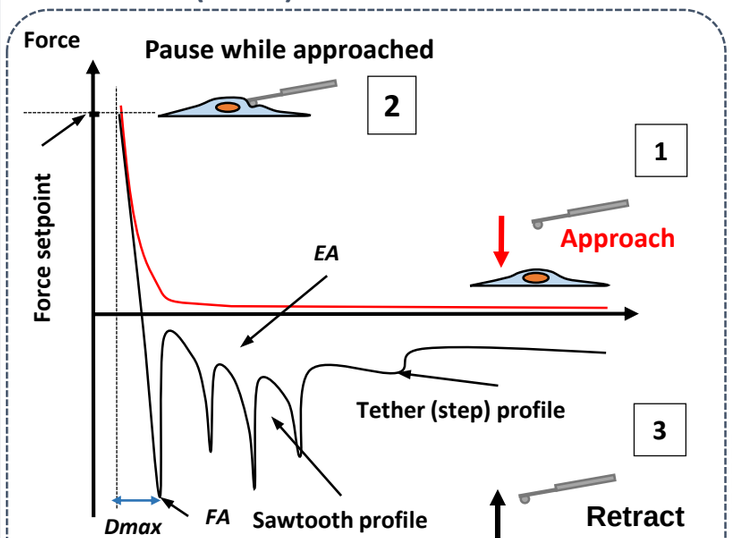
USP hydrogels support 3D cell and organoid culture growth

Printability of USP hydrogels



Single Cell Mechanoprofiling with USP and Atomic Force Microscopy

A plethora of information can be retrieved from force-distance curves performed on single cells of different types, including: Energy of Adhesion (EA); Maximum Force of Adhesion (FA); Distance at which the FA occurs (Dmax)



Moreover, the shape of the unbinding event sheds light on the type of interaction occurring at the interface

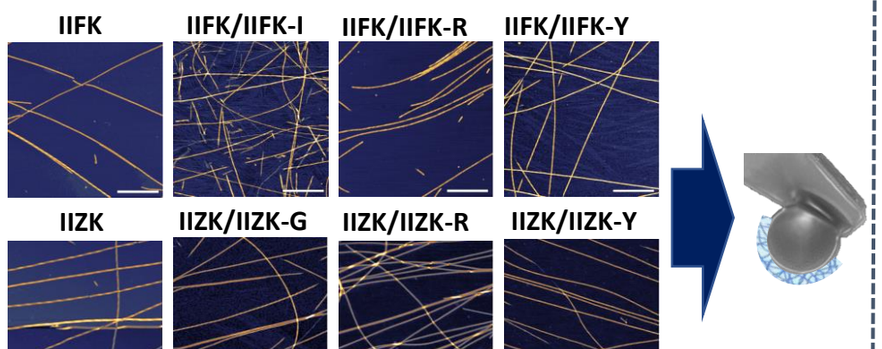
Verification of fiber formation capacity of USP hydrogels by AFM topography and coating of the AFM spherical probe

Parent peptides

$Ac\text{-}IIFK\text{-}NH_2$ | $Ac\text{-}IIZK\text{-}NH_2$

Biofunctional peptides

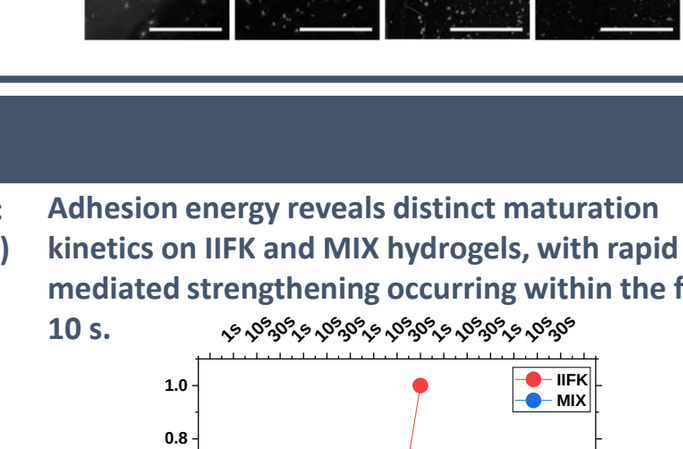
$Ac\text{-}IIFKGGGIKVAV\text{-}NH_2$ (IIFK-I)	$Ac\text{-}IIZKGGGGFOGER\text{-}NH_2$ (IIZK-G)
$Ac\text{-}IIFKGGGRGDS\text{-}NH_2$ (IIFK-R)	$Ac\text{-}IIZKGGGRGDS\text{-}NH_2$ (IIZK-R)
$Ac\text{-}IIFKGGGYIGSR\text{-}NH_2$ (IIFK-Y)	$Ac\text{-}IIZKGGGYIGSR\text{-}NH_2$ (IIZK-Y)



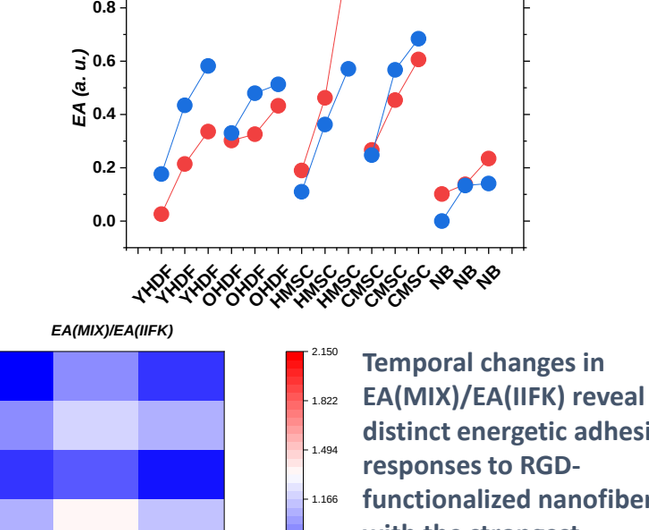
Scale bar 500 nm

Reducing complexity by selecting the most adhesive biofunctional USP hydrogel

Screening of USP hydrogels by F-D AFM and work of adhesion on human dermal fibroblasts



Example of two USP hydrogels screening by cell growth effect



Results

The most effective peptide combination is used to investigate the single cell response of different cell types: human dermal fibroblasts at low passage (YHDF), human dermal fibroblasts (OHDF), healthy mesenchymal stromal cells (HMSC), cancer mesenchymal stromal cells (CMSC), neuroblastoma cells (NB). The parent peptide IIFK is used for comparison.

Scale bar 35 µm

Normalized AFM adhesion parameters reveal cell-specific and time-dependent mechanophenotypes on native (IIFK) and RGD-functionalized (MIX) peptide nanofibers.

All the values are: Normalized median values (0, 1)

Distinct adhesion-response phenotypes reveal cell-specific strategies of energetic engagement (EA) and mechanical reinforcement (FA/Dmax).

Adhesion energy reveals distinct maturation kinetics on IIFK and MIX hydrogels, with rapid RGD-mediated strengthening occurring within the first 10 s.

Temporal changes in EA(MIX)/EA(IIFK) reveal distinct energetic adhesion responses to RGD-functionalized nanofibers, with the strongest enhancement observed in young fibroblasts.

Temporal changes in FA/Dmax(MIX)/FA/Dmax(IIFK) reveal differential mechanical reinforcement strategies, highlighting a pronounced RGD-dependent stiffening response in healthy MSCs.

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