A SELF-ASSEMBLING PEPTIDE HYDROGEL FOR ULTRARAPID 3D BIOASSAYS

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Background

Hydrogels are appealing 3D semi-wet systems to locally confine biomolecules on (micro)analytical surfaces while preserving their structural integrity and function

Mass transport can be severely limited in dense cross-linked matrices. Accordingly, extensive incubation time can be required to allow large molecules to diffuse through the gel aqueous cavities

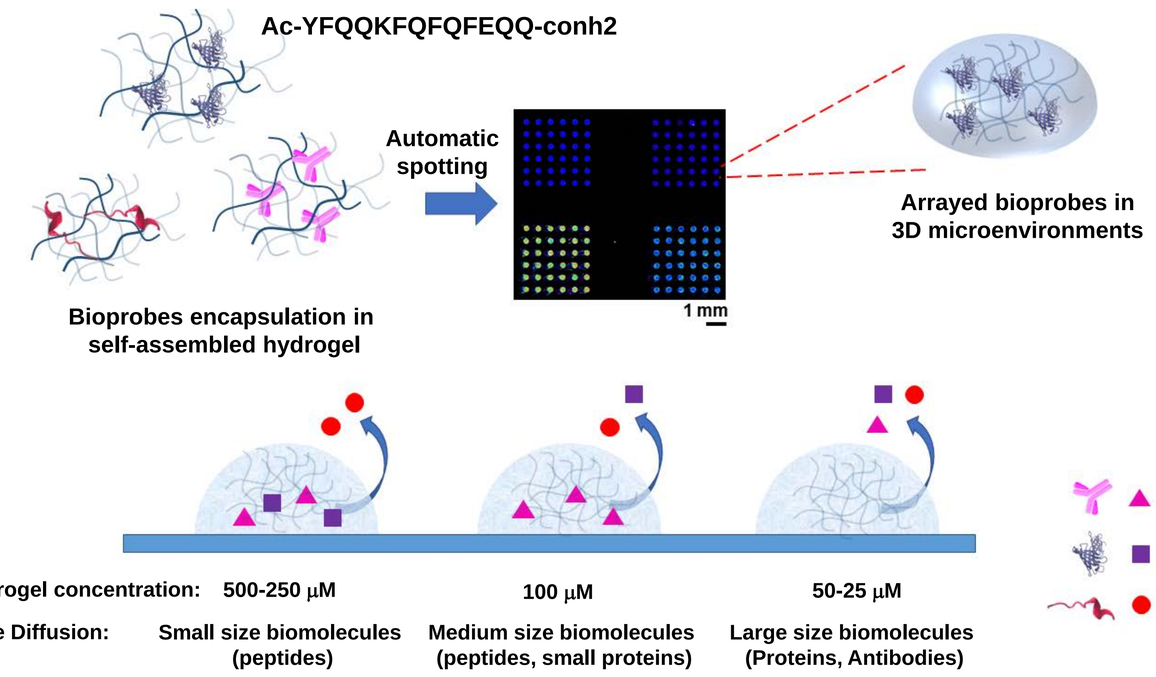
Gel viscosity can be a severe obstacle to (micro)chip fabrication; laborious and multi-step strategies may be required to circumvent this limitation

It is highly desirable to develop hydrogel materials able to match fast biomolecule diffusion rates with stable probe entrapment, while avoiding difficult handling and poor versatility of use

Highlights

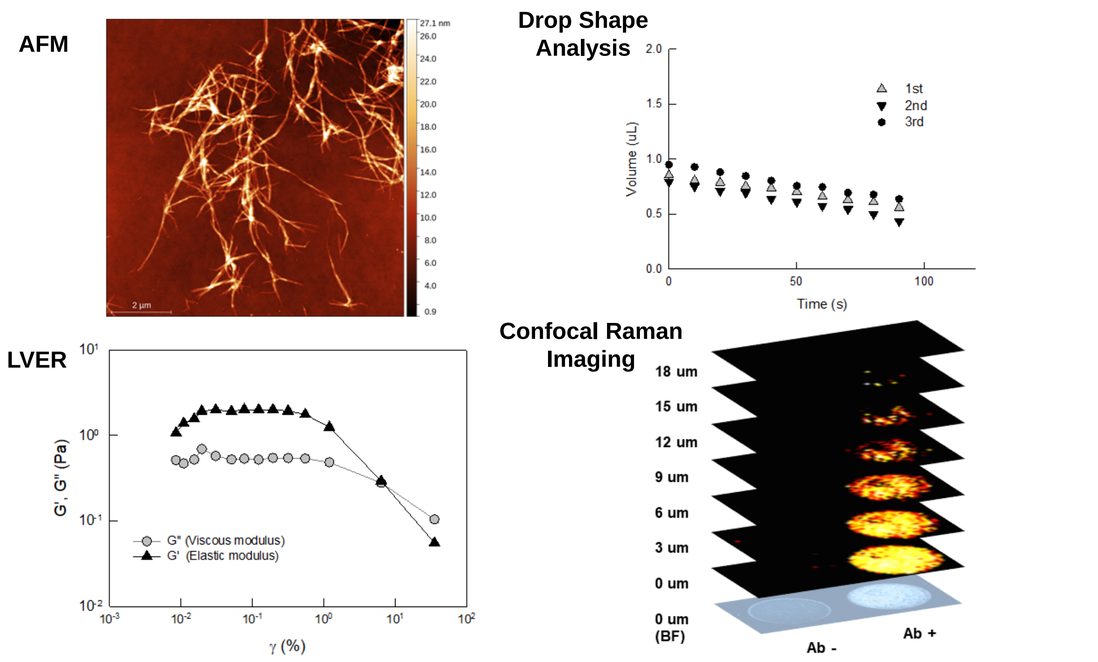
- **A self-assembling peptide hydrogel (Ac-YFQQKFQFQFEQQ-conh2) is used as a 3D matrix for bioassays**
- **Permeability to differently sized biomolecules is easily controlled by varying the concentration of the gel-forming peptide monomer, i.e. enabling selective bio-probes confinement/access**
- **Ultrafast diffusion of biomolecules can be obtained under tuneable conditions**
- **Fully compatible with microarrays fabrication protocols due to favourable viscoelastic properties and self-adhesiveness on different materials**
- **Overall, the hydrogel overcomes many limitations that plague hydrogel for bioassays while being user-friendly, robust and cost-effective**

The platform



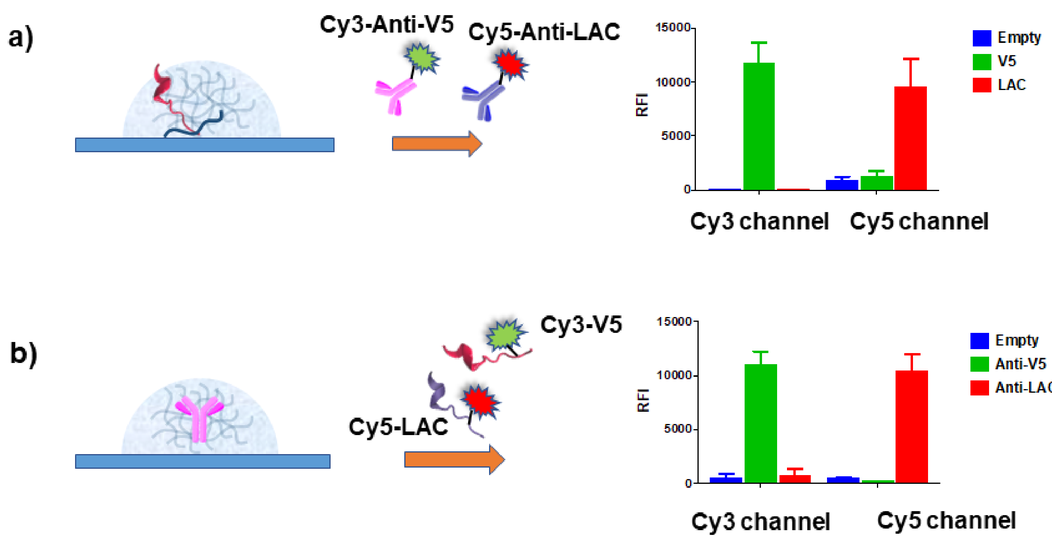
- Bioprobes are added to a self-assembled peptide hydrogel and then spotted on microarray chips using an automated robot. As a result, bioprobes are locally confined within aqueous 3D microenvironments
- Permeation to differently sized biomolecules can be easily tuned by varying the concentration of the gel forming peptide monomer
- In selected hydrogel concentrations, free diffusion occurs with ultrafast kinetics (<2min)

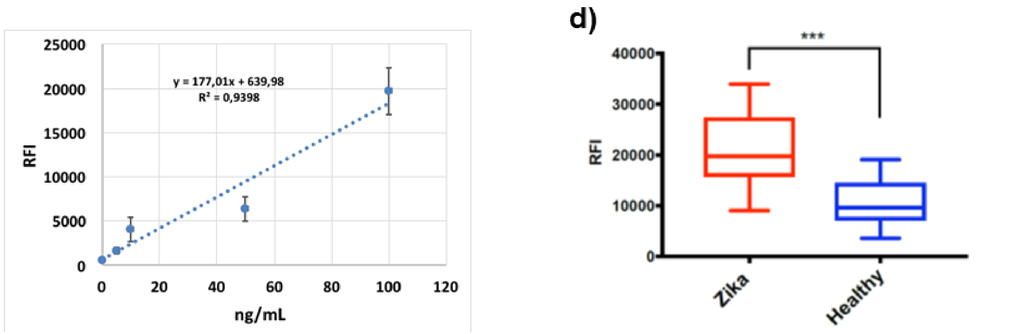
Material Characterization



The hydrogel microsctructure is formed by entangled nanofibers, as visualized by atomic force microscopy (AFM). Linear viscoelastic region (LVER) analysis shows the hydrogel behaves as a typical viscoelastic material and is characterized by loose fibers connection. Droplets volume observation upon repeated drying-rehydration cycles revealed that the hydrogel behaves like a supramolecular sponge. Confocal Raman imaging using a malachite-labelled antibody indicated it is stably embedded and uniformely distributed within the hydrogel spots (250μM gel conc.)

Functional assays





a, b) Symmetrical antibody-peptide recognition tests showed that probe-specific recognition occurred only between respective antibody-peptide pairs. Negligible fluorescence signals were detected in spots containing non-related antibodies/peptides or in empty spot controls

- Ultra-short incubation times (5 minutes) were used in the assays

- Biomolecules diffusion in our system is not hindered by the soft peptide matrix, which, oppositely, behaves as a solution-like environment

c) The calculated LOD of 2 ng/mL and the R2 (0.94) of the dose-response curve demonstrated linearity of the assay outcome in a clinically relevant antibody concentration range. d) a real immunodiagnostic assay to detect Zika Virus (ZIKV) infection in human serum samples effectively enabled the discrimination among two populations at a statistically significant level (p<0.001)

Remarks

- **The hydrogel formation is single component, spontaneous and rapid**
- **The favourable hydrogel rheological properties enable its direct integration with automated array production protocols**
- **The stunning diffusion properties of biomolecules through the soft hydrogel matrix are closely mimetic of solution conditions, so that ultrafast (<10 min) full immunoassays can be run using this platform**
- **High versatility of application for multiplexed bioassays**

Ref: P. Gagni, A. Romanato, G. Bergamaschi, P. Bettotti, R. Vanna, C. Piotto, C. Morasso, M. Chiari, M. Cretich and A. Gori* – A Self-assembled Peptide Hydrogel for Ultrafast 3D Bioassays – Nanoscale Adv., 2019, 1, 490-497*

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