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Supramolecular Hydrogels for 3D Biosensors and Bioassays

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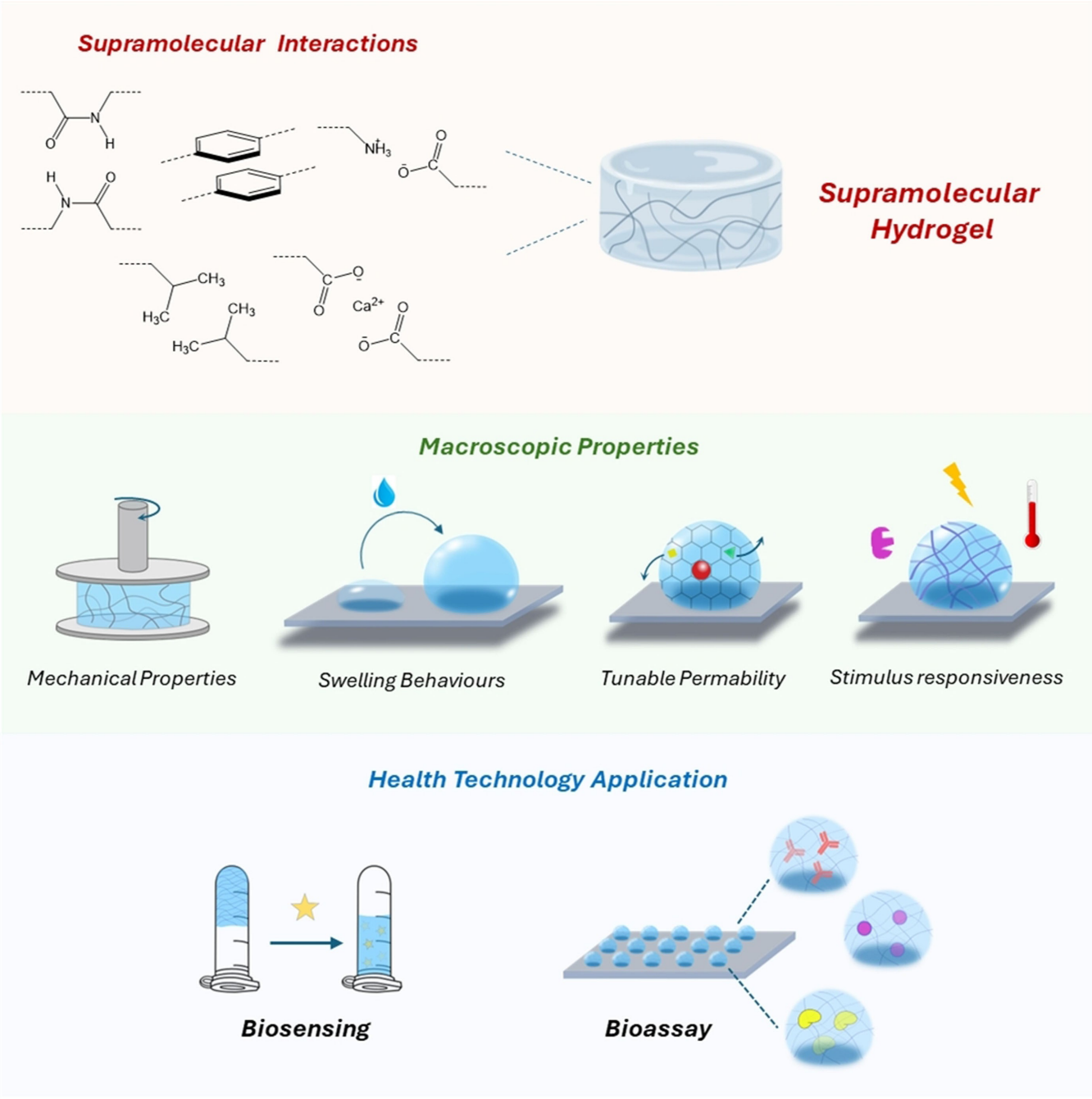

Supramolecular hydrogels play a pivotal role in many fields of biomedical research, including emerging applications in designing advanced tools for point-of-care testing, clinical diagnostics, and lab-on-chip analysis. This review outlines the growing relevance of supramolecular hydrogels in biosensing and bioassay devices, highlighting recent advancements that deliver increased sensitivity, real-time monitoring, and multiplexing capabilities through the distinctive properties of these nanomaterials. Furthermore, the exploration extends to additional applications, such as using hydrogels as three-dimensional matrices for cell-based assays.

1. Introduction

The term "supramolecular" was coined in 1987 by Nobel prize laureates, Jean-Marie Lehn, Donald J. Cram and Charles J. Pedersen to describe the directional and ordered non-covalent organization of molecules through defined recognition motifs.[1] Since its inception in the development of cryptates, supramolecular chemistry has considerably expanded beyond the "original" host-guest chemistry field, finding applications at the interfaces with many other disciplines, and offering novel perspectives and solutions to several questions in biology, physics, and engineering. In fact, while it started with the study of systems under thermodynamic control, the field of supramolecular chemistry is presently experiencing a shift toward kinetically controlled and far-from-equilibrium systems, where the richest functions can be harnessed, focusing on dynamic non-covalent interactions between molecules that give rise to molecular recognition and self-assembly processes.[2]

Within this new concept, supramolecular hydrogels are formed when molecular (or macromolecular) gelators spontaneously self-assemble in aqueous solvents, to form a well-ordered 3D structural network via synergistic intermolecular noncovalent interactions, entrapping a high amount of water. Common supramolecular interactions involved in this process include hydrogen bonds, hydrophobic and van der Waals interactions, π-π bonding, electrostatic interactions, and metal-ligand coordination (Figure 1).[3]

In the early stages of supramolecular hydrogels development, most hydrogelators comprised small organic molecules, such as urea derivatives and amino acid amphiphiles.[4] Ongoing exploration in this field has led to the emergence of small molecules with low molecular weight (LMWGs) that spontaneously self-assemble in water, forming nanofibers that bundle into 3D networks.[5]

Aligning with the fact that biological systems extensively rely on self-assembly processes to perform biological functions,[6] the use of biomolecular building blocks has become highly attractive in the pursuit of new soft materials with designed biomimetic functions. For example, self-assembling peptides (SAPs) are unparalleled units for constructing supramolecular hydrogels with several favorable and distinctive features. Peptides can indeed be easily synthesized and functionalized, offering virtually unlimited design possibilities to control monomer assembly and tune resulting hydrogel properties.[7]

Similarly, significant progress in DNA synthesis has triggered a transformative shift by integrating DNA as a functional component in the creation of supramolecular materials. The latest DNA synthetic techniques enable rapid preparation or amplification of DNA sequences in large quantities through automated techniques, polymerase chain reaction (PCR), or production in microorganisms.[8] Despite being composed of a limited set of nucleic acid monomers, the precise and predictable interactions between the monomer units and their respective complementary nucleic acids (AT and GC) facilitate the spontaneous and convenient programming of synthetic 2D and 3D structures in solution.[9]

On the contrary, noncovalent polymeric hydrogels derived from natural sources, such as alginate or agarose, lack the same level of versatility and structural programmability. To address the limitations of mono-component hydrogels and achieve synergistic properties, the use of hybrid hydrogels-formed by an interpenetrating network of two distinct components-can be a viable approach.[10]

The transient and modular nature of noncovalent interactions can confer extremely versatile properties to supramolecular hydrogels, including self-healing and shear-thinning behavior, responsiveness to external physical/chemical stimuli, and tunable mechanical properties.[11] This stands in stark contrast to the traditional use of cross-linked hydrogels that, while usually characterized by excellent mechanical strength and stability, lack an exploitable dynamic nature.[12]

As a result, there has been an exponential progress in advancing supramolecular hydrogel technologies for a multitude of biomedical applications within the fields of tissue engineering, drug delivery, microfabrication techniques, and biosensing technologies.[12c–g]

While many applications of supramolecular hydrogels have been extensively reviewed elsewhere,[13] this review specifically delves into their emerging role in biosensing and bioassay areas, highlighting recent advances in achieving high sensitivity, real-time monitoring, and multiplexing capability through the distinctive properties of these nanomaterials. A special emphasis is placed on clinical diagnostics, point-of-care testing and lab-on-a-chip applications, where a substantial demand exists for the development of efficient biosensors and bioanalytical detection methods for the early detection of diseases.[14] Additionally, we will explore some extensions into other fields, such as hydrogels as three-dimensional matrices for cell culture. A

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selection of the presented studies and applications is listed in Table 1.

2. Supramolecular Hydrogels: Favorable Properties to 3D Platforms Design

Traditionally, most analytical platforms have relied on planar, bidimensional (2D) assay schemes, wherein the molecular interaction between the bioprobe and its targeted (bio)analyte occurs at the interface of a solid support to which the capturing bioprobe is bound.

In this context, the immobilization strategy of the molecular ligand becomes a critical factor in determining the final performances and robustness of the entire analytical device. Depending on the nature of the bioprobes, a range of conjugation strategies can be used, including random adsorption, covalent cross-linking, or chemoselective oriented binding.[30] For instance, molecules of limited molecular size (e.g. peptides) may partially or totally lose their binding capacity if a spatial orientation favorable to target interaction is not preserved, such as in the case of random absorption. This consideration applies more generally and can also impact larger molecules, leading to suboptimal analytical performances resulting from insufficient loading capacity, poor accessibility of immobilized bioprobes, and partial denaturation of probes upon surface binding. These limitations leave room for considerable performance and robustness improvements, which have been the rationales to sustain the development of 3D sensing and bioassay technologies.

In this scenario, confining analytical bioprobes within hydrogels offers remarkable advantages over conventional 2D systems, such as increased sensitivity due to higher loading capacity and minimization of surface effects detrimental to assays performance. Hydrogels, by mimicking the water-rich native environment of biomolecular interactions,[31] serve as ideal systems for encapsulating bioprobes and counterpart analytes (e.g. drugs, proteins, and other biomolecules),[31b–d] preserving their full structural integrity and functionality (Figure 1). These solution-like, benign confinement conditions contribute to the specificity, sensitivity and robustness of the assay/sensing platform.[32] Through physically immobilization in a 3D environment, it becomes possible to overcome the limited coverage of 2D surfaces by accommodating more recognition elements, unlocking enhanced immobilization (loading) capacity and accessibility of biomolecules. The resulting improved signal intensity usually leads to a lower limit of detection and better signal-to-noise ratios.[33]

The first examples reported for hydrogel-based assays exploited the use of conventional covalent hydrogels.[24,34] However, some characteristics of the crosslinked matrix still hamper their straightforward use in developing 3D biosensing and bioassays. In particular, the diffusion properties in terms of mass transport can be severely limited in dense cross-linked matrices, requiring extensive incubation times are required to allow large biomolecules to diffuse through the gel. This limitation inherently restrict versatility in assay design. Additionally, gel viscosity and stiffness pose severe obstacles to 3D printed assays. Moreover, the formation of cross-linked hydrogels constituent units usually involves chemical reactions and/or the use of reactants such as metal catalysts, photoinitiators, and UV light irradiation, processes that can harm to the functionality of sensitive biomolecules. In contrast, microfabrication using supramolecular hydrogels relies on spontaneous assembly of 3D matrices under mild conditions, without the need for chemical reactions, making them an attractive alternative. Additional figures of merit of supramolecular hydrogels include their tunable properties, which can be conveniently engineered to meet specific analytical needs. For instance, achieving tunable permeability to different biomolecules allows for the separation of the target analyte from other molecules in a sample.[35] Unlike crosslinked hydrogels, the dynamic nature of supramolecular interactions enables hydrogels to rapidly respond to various external stimuli, including physical factors (e.g., temperature, light, voltage, magnetic field) and chemical stimuli (e.g., pH, ionic strength, redox agent, glucose, enzyme, competitive host/guest). Generally, these external stimuli significantly impact the supramolecular network forming the hydrogel, leading to changes in the macroscopic properties (e.g., swelling, dissociation) that can even be favorably exploited for detection purposes. The dynamic nature of

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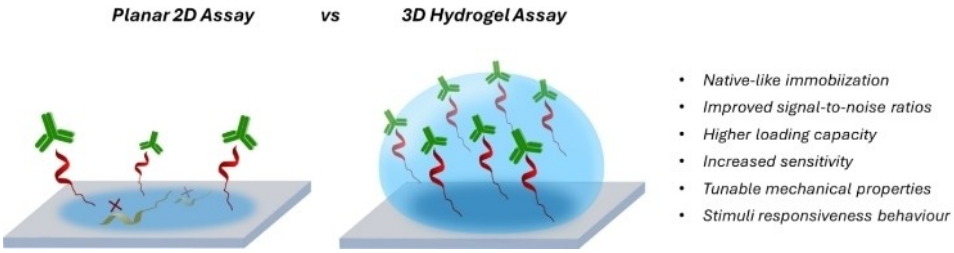

Figure 1. Comparative scheme of planar (2D) vs. 3D assays. Immobilized bioprobes on 2D surfaces can suffer from suboptimal display and denaturation, limiting their targeting capacities. In contrast, 3D systems based on hydrogels can preserve the full functionality of confined probes, providing unimpaired recognition properties and additional advantages.

Table 1. The selection of the discussed supramolecular hydrogel materials in the context of biosensing and bioassay is categorized according to specific applications.

Hydrogelators	Target/Application	Ref
Hydrogel for Biosensing		
Fmoc–FF	H_2O_2	[15]
BPmoc–F3	H_2O_2, glucose and uric acid	[16]
Fmoc–FEFK	HER2	[17]
Oligo-recognition sequence CGATTCTGTGTT and VKVKVEVK	Multiple nucleotide-polymorphisms	[18]
Antibodies-antigens couples in a PAM	PKA	[19]
OPD/agarose	Organophosphate pesticides	[20]
Hydrogel for Bioassay		
PVA	Hg(II), Cu(II), Pb(II), and Cd(II)	[21]
YFQ11	ZIKV	[22]
Q3/Agarose	Covid-19 and EVs	[23]
POH/POA/PO	β-lac inhibitors	[24]
κ-carrageenan, agarose, and gelatine	LFA_Covid-19	[25]
DNA hydrogels	Microfluidic Chip_Covid-19	[26]
Fibrin	IL-6_CARD-dELISA	[27]
PAM_fibrin	IL6 dependent glycaemic control	[28]
CPHs and Fmoc–FF	Cardiomyocyte growth	[29]

supramolecular interactions also imparts tunable mechanical functions to the 3D network, including self-healing properties, shape memory ability, and printability. The physical properties of hydrogels can be combined with modifications of the structural 3D template by components used to induce specific functionalities (e.g., optoelectronic properties)[36] or enhance the biocompatibility of implantable devices.

The following sections will showcase representative examples, illustrating how this virtually unlimited flexibility can be exploited.

2.1. 3D Hydrogel Biosensors

Biosensors are defined as analytical devices that integrate a biological active element within a physicochemical transducer component (e.g., optical, electrochemical, thermometric, piezoelectric) to detect reversibly and selectively the concentration of specific chemical species in any type of sample (e.g. blood, urine, saliva, environmental waters).[37] The sensing of a specific target, both in vitro and in vivo, holds paramount importance across various applicable domains, encompassing clinical diagnosis, biomedical tests, and the identification of trace analytes in personalized healthcare, food analysis, and environmental monitoring.

In a typical biosensor, the biosensing element (bioprobe) selected to specifically interact with the target analyte is immobilized on a solid support (e.g., glass, metal, microbeads, etc.),[38] and the binding event triggers a signal that, depending on the detection method, can undergo amplification, ultimately being processed for the final readout.[39] Examples of widely used biosensors include glucose monitoring devices for diabetic patients and pregnancy tests, which detect human chorionic gonadotropin in urine.

In hydrogel-based biosensors, the specific binding of the target to the 3D confined probe can either induce measurable changes in hydrogel properties (e.g., swelling volume, mass, mechanical properties) or the increasing applications of hydrogel-based biosensors are favored by advancements in the manipulation of hydrogels in micro- and nano-patterns to

deliver lab-on-a-chip, portable, and implantable devices.[41] In this regard, SAP hydrogels have garnered significant interest due to their exceptional biocompatibility, tunability, water-absorbing capacity, and stability, offering a multitude of versatile options for biosensor design.[42]

Some illustrative examples here follow. release of an encapsulated cargo generating a readout signal of different natures (e.g., colorimetric, electrochemical).[40] The self-assembling N–fluorenylmethoxycarbonyl–diphenylalanine (Fmoc–FF) unit was successfully exploited to realize a smart electrochemical biosensor to detect H_2O_2 in living cells.[15] The supramolecular hydrogel matrix served a dual function by encapsulating an active enzyme (horseradish peroxidase, HRP) and acting as a robust scaffold that promotes direct cell adhesion on the electrochemical surface (glass carbon electrode GCE) (Figure 2). Cyclic voltammetry experiments demonstrated that the HRP enzyme was stably immobilized in the gel-like 3D matrix, retaining its activity, and the biosensor provided a calculated limit of detection of 18 nM. The high selectivity of the HRP/Fmoc–FF hydrogel/GCE biosensor was proved through a competition experiment, highlighting the specific detection of H_2O_2 even in the presence of possible interfering species (e.g., dopamine, ascorbic acid, uric acid). Thanks to the biocompatible properties of the hydrogel scaffold, Hela cells were shown to adhere to the sensor surface, enabling the direct in situ detection of the H_2O_2 release from living cells. This electrochemical device represents an illustrative example of hydrogel-based biosensors for the real-time detection of intracellular signals with good analytical performance, reproducibility, and selectivity. This example highlights the possibility of detecting molecular signals immediately upon secretion from living cells by exploiting the proximity between the hydrogel-confined enzyme and hydrogel-adhered cells. Additionally, incorporating all components within a confined 3D matrix resulted in a concentrated effect of the analyte, with benefits for analytical sensitivity.

The fabrication flexibility afforded by the self-assembly strategy, enabling the incorporation of enzymes within hydrogel matrices without the need of a covalently linked immobilization strategy, was further explored to design electrochemical biosensors. For example, Lian et al. reported on the use of superoxide dismutase as a sensing element in an electrochemical biosensor for in-situ detection of cell-released superoxide anions (O_2).[43] The same concept was also leveraged to develop biosensors able to undergo naked-eye transitions in response to a variety of physiologically relevant biomolecules.[44]

To illustrate, redox-sensitive hydrogels were employed to detect the presence of different biomarkers (e.g., H_2O_2, glucose, lactose, uric acid, choline, acetylcholine, sarcosine). The general detection principle involves a cascade enzymatic reaction within the 3D networing response to the analyte, leading to a visible decomposition of the hydrogel microstructure due to the disassembly of the structural peptide nanofibers.

In this context, peptides containing H_2O_2-reactive boronoarylmethoxycarbonyl (BAmoc) groups have been extensively exploited to design stimuli responsive hydrogels. The removal of the BAmoc unit through an oxidation/elimination reaction triggered by specific enzymes and H_2O_2, induces collapse of the 3D matrix and a naked-eye gel-sol transition.[16] This strategy was exploited by Yoshii et al. to realize a multicomponent supramolecular hydrogel for the sensitive, naked-eye detection of H_2O_2, glucose, and uric acid in human samples. Utilizing the H_2O_2-responsive BPmoc–F3 peptide (Figure 3), the authors physically incorporated a dendritic amplifier molecule (**1** in Figure 3) and a specific sarcosine oxidase (SOx) within the 3D scaffold. As such, active components were constrained in close proximity, generating a cascade mechanism of signal amplification, ultimately enhancing the sensitivity of the system. The sensing detection in this case was associated with BPmoc–F3 decomposition, occurring in response to the presence of H_2O_2, causing the gel-sol transition. Additionally, the same principles were demonstrated using enzyme components like glucose oxidase (GOx) or urate oxidase (UOx), enabling the sensing of desired target analytes like glucose and uric acid. Importantly, this strategy was also adapted to realize logic gates (OR and AND) output into the hydrogel–enzyme hybrid materials, enabling simultaneous sensing of multiple specific biochemicals and executing controlled drug release in accordance with the logic operation.[45]

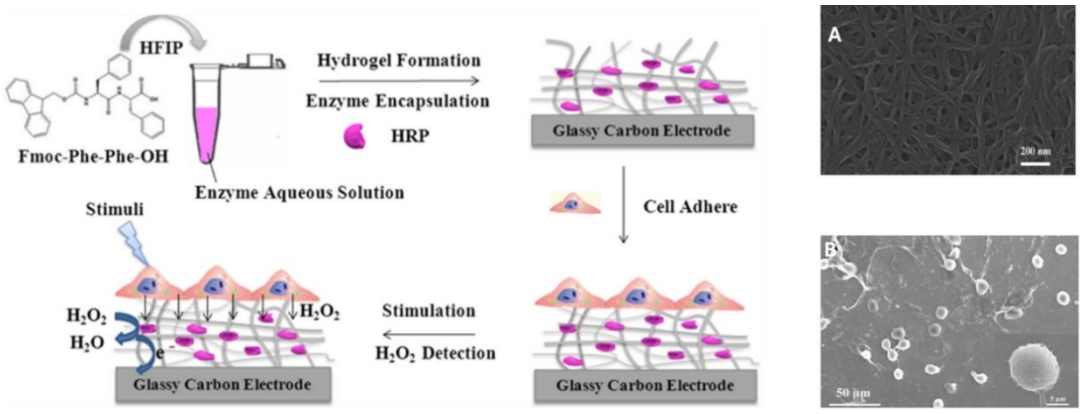

Figure 2. Fmoc–FF hydrogel based electrochemical biosensor to detect H2O2 in living cells. SAM images of the A) Fmoc–FF hydrogel and B) HeLa cells adhered to the hydrogel scaffolds. Reprinted and adapted with permission from [15]. Copyright (2016) American Chemical Society.

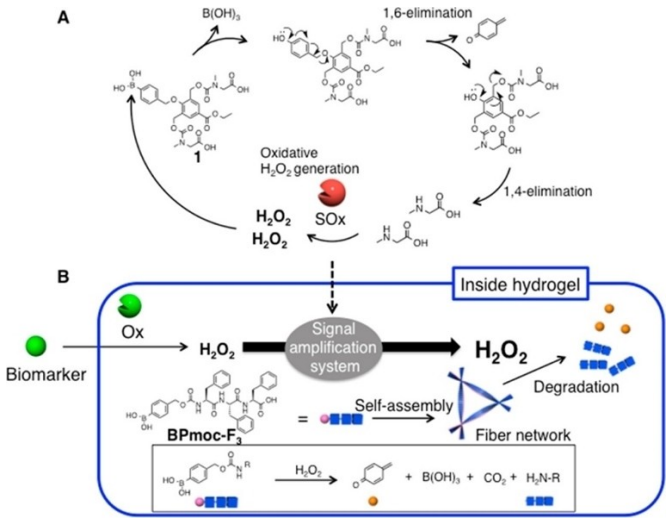

Figure 3. Cascade mechanisms involved in the BPmoc–F3 based biosensing of H2O2. Thanks to the physical entrapment of the active components in the supramolecular hydrogel, a significant enhancement of sensitivity was achieved. Reprinted and adapted with permission from [16]. Copyright 2015 American Chemical Society.

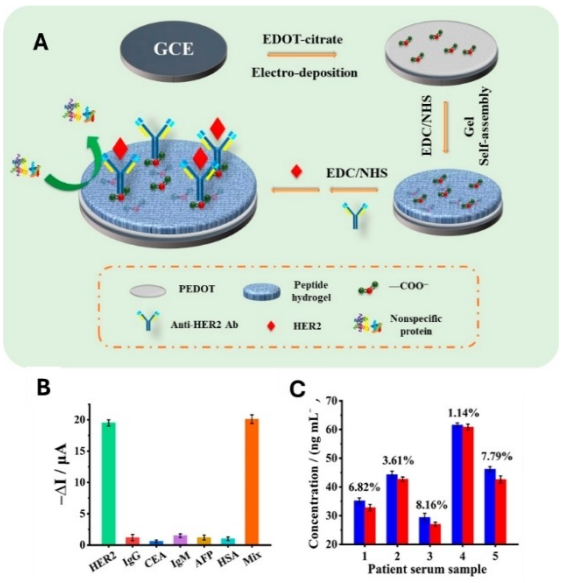

Figure 4. A) Schematic representation of the PEDOT/peptide hydrogel sensor. B) Responses of the HER2 biosensor to 0.1 mg mL^{-1} of IgG, CEA, IgM, AFP, HSA and a solution mixture (Mix) of those proteins. C) HER2 determination in breast cancer patient serum using a commercial ELISA Kit (blue) and the PEDOT/peptide biosensor (red). Reprinted and adapted with permission from. [17]. Copyright 2022 American Chemical Society.

The good hydrophilicity typical of water-rich hydrogels, besides preserving the structural and functional integrity of entrapped macromolecules, can also facilitate the effective alleviation of nonspecific interactions or biofouling events, that may otherwise occur with the solid support. Wang et al. have developed an immune electrochemical biosensor for the detection of human epidermal growth factor receptor 2 (HER2), whose overexpression has been associated with 20–30% of breast cancer development.[17] The biosensing is performed by a hydrogel scaffold composed of a conductive polymer poly(3,4-ethylenedioxythiophene) (PEDOT) and a peptide hydrogel (FEKF functionalized with a fluorene methoxycarbonyl group). (Figure 4) The hydrogel layer was then functionalized with a specific antibody. The decrease in readout signal in the biosensor was recorded by differential pulse voltammetry (DPV) and correlated to the amount of the HER2 biomarker present in the serum sample. The limit of detection (LOD) of the biosensor (45 pg/mL) was among the lowest reported for HER2 analytical detection. Moreover, the biosensor showed excellent specificity for the target HER2 even in the presence of a large amount of human serum proteins (e.g., IgG, IgM, CEA, AFP and HAS) and excellent accuracy in the detection of HER2 in serum samples from breast cancer patients.

These outstanding performances could be attributed to the high surface area and an excellent biocompatible environment for the immobilized antibody.

Self-assembled nanostructures can also serve as direct solid support for molecular biosensing. For example, King and co-workers exploited peptide fibers as a 3D solid support onto which oligonucleotides were immobilized for capturing complementary sequences. In biosensor design, the oligo-recognition sequence (CGATTCTGTGTT) was chemically conjugated to a self-assembling peptide motif (VKVKVEVK) and then incorporated into the hydrogel matrix through co-assembly with the unmodified peptide. The result was a supramolecular structure with the DNA aptamer exposed on the fiber surface. To assess the binding properties toward complementary sequences, a molecular beacon (MB) fluorescent probe, F-5′-CGATTCGCCAAA-CACAGAATCG-3′-D (F = fluorescein, D = dabcyl), was designed. Interaction with the functionalized hydrogel resulted in a remarkable increase in fluorescence, indicating hybridization of the MB with the DNA recognition motif on the hydrogel fibers and inhibition of the FRET quenching (detection limit of 22 pM). This pilot work demonstrated that SAP–hydrogel, in combination with a DNA aptamer, can be exploited as a biosensing tool to identify single or multiple nucleotide polymorphisms, with the potential to recognize susceptibility to certain monogenic or complex diseases in patients.[18]

The programmable volume changes in macroscopic properties (i.e., shrinking and swelling) upon external stimuli are another convenient output readout of stimuli-responsive hydrogels. Exploiting the selective antibody-antigen interaction, Quin et al. reported a versatile competition-based photonic crystal hydrogel (PCH) biosensor capable of naked-eye detection of various biomolecules (e.g., proteins, peptides, and small molecules) with high sensitivity, selectivity, and excellent reversibility.[46]

The incorporation of photonic crystals (PCs) into a hydrogel network, which alters the periodicity layout of PCs, allows the transformation of a biophysical change into an optical change (e.g., colorimetric variation). In the authors report, the biosensors were fabricated by immobilizing antibody – antigen couples in a polyacrylamide (PAM) matrix containing colloidal photonic crystals. Three different combinations covering antibody-based recognition of proteins (IgG–Protein A, PrtA),

peptides (anti-L-glutathione reduced, Ab^{GSH}–GSH), and small molecules (anti-biotin antibody, Ab^{biotin}–biotin) were realized, exhibiting excellent performance in reliably and quantitatively detecting GSH and PrtA from biological samples. Using a similar strategy, the authors also developed a kinase PKA sensor that showed excellent performance with a lower detection limit compared to the reported PCH–PKA biosensor.[19] In addition to the biomedical field, there is a growing demand for technological advancements in biosensing, particularly in the development of sensitive, reliable, and portable sensors for detecting pollutants. Organophosphate pesticides (OPs), crucial for high crop production, have garnered increased public attention due to their persistent health risks, even at trace concentrations. In this context, Jin et al.[20] have developed an easy-to-use and cost-effective test kit that integrates Ag^+-gated stimuli-responsive hydrogel, enabling portable and visually quantitative analysis of OPs (Figure 5). This system takes advantage of a hybrid hydrogel composed of O–phenylenediamine (OPD) and silicon quantum dots (SiQDs) embedded in agarose, immobilized on the cap of an Eppendorf tube. The detection mechanism relies on silver ions (Ag^+) triggering the oxidation of OPD into a yellow compound (2,3-diaminophenazine, DAP) emitting light at 557 nm. Simultaneously, SiQDs' fluorescence at 450 nm is quenched by DAP through an inner filter effect (IFE), resulting in a ratiometric fluorescence response. The hydrogel responds to acetylcholinesterase (AChE) inhibition caused by OPs, releasing Ag^+ and initiating the oxidation of OPD. To detect OPs, the tube is inverted, allowing Ag^+ to diffuse into the hydrogel, triggering OPD oxidation and IFE formation. A smartphone records the fluorescence color change, and a software analysis establishes a linear relationship with pesticide concentration. This SiQDs/OPD-based platform offers on-site OP identification with high sensitivity (<10 ng/mL) and accurate pesticide recognition. The use of a smartphone makes the system cost-effective and environmentally friendly. The portable kit has successfully monitored OPs in food samples, presenting a novel approach for pesticide detection.

Overall, the examples described are intended to be purely representative of the possibilities and versatility that the use of supramolecular hydrogels allow. The scenario thus appears highly transversal for application purposes, and with the progressive consolidation of synthetic and engineering technologies, increasingly greater future opportunities are perceived.

2.2. 3D Hydrogel Bioassays

The quantification of multiple biomarkers in physiological fluids has become a crucial tool in clinical applications, encompassing diagnostics, therapy monitoring, and follow up. Given the diverse functions of specific biomarkers (e.g., proteins, antibodies, extracellular vesicles) in an organism, measuring their levels can provide valuable information about its biological state, including cell and organ activities, the onset and progress of a disease, or drug action. With the increasing demand for simultaneous measurement of multiple biomarkers in a single sample, the development of multiplex and high-throughput techniques is desirable to enhance analysis performance and alleviate the burden of time and costs associated with investigating individual components.[47] In response to this demand, bioassay technologies have become a highly active area of research, integrating various assays to address complex biological questions related to gene expression profiles, signaling pathway events, and indicators of cell health and proliferation.

Supramolecular hydrogel bioassays have garnered significant attention owing to their fluid-phase kinetics, high precision, and flexible target selection properties. These platforms make use of hydrogel-coated analytical surfaces (e.g., glass, silicon, polymethylmethacrilate) or of discrete hydrogel droplets that entrap bioprobes in hydrophilic and three-dimensional environments. In comparison to 2D platforms, the hydrophilic and 3D nature of hydrogels is beneficial to the stability of biomolecules, preserving their functionality. This is crucial for key assay properties like specificity and sensitivity, along with favorable binding kinetics. Additionally, supramolecular hydrogels can be optically transparent, enabling standard staining and labeling protocols for colorimetric and fluorescence-based analytical assays.[48] This section explores recent reports showcasing technological advancements in bioassay platforms based on supramolecular hydrogels.

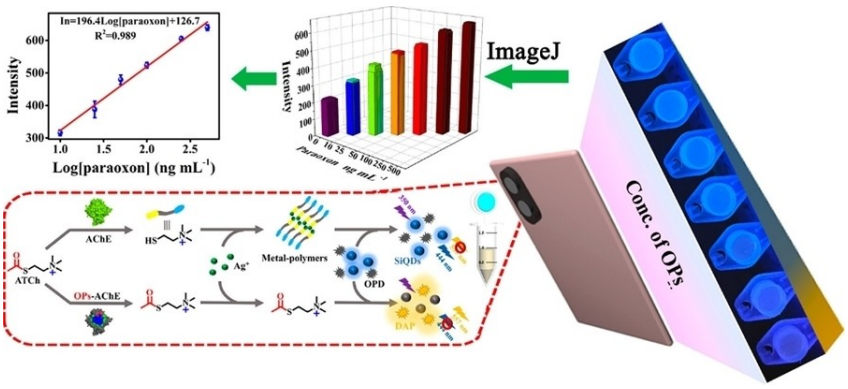

Figure 5. Schematic illustration of the SiQDs/hydrogel-based device for the detection of organophosphate pesticides. Reprinted and adapted with permission from [20]. Copyright 2019 American Chemical Society.

He and co-workers developed a smart colorimetric bioassay to detect toxic metal ions in real samples by physically encapsulating the enzyme β-galactosidase and its substrate CPRG (chlorophenol red-β-D-galactopyranoside) within a PVA hydrogel.[21] (Figure 6). In the absence of metal contaminants, the β-galactosidase catalyses the substrate, producing chlorophenol red, allowing a colorimetric readout. However, in the presence of toxic heavy metal ions, the enzyme activity is inhibited, impeding the colorimetric reaction. This bioassay allowed for the detection of different toxic heavy metal ions such as Hg(II), Cu(II), Pb(II), and Cd(II) with high sensitivity, selectivity, and fast kinetics. Notably, the hydrogel system provided a protective environment for the encapsulated enzyme, ensuring good performance even at high temperatures (up to 60 °C) and after 5 months of storage.

Thanks to their tunable mechanical features, supramolecular hydrogels can find applications on different multiplexed platforms, including microarrays.

In microarray fabrication, up to thousands of bioprobes can be deposited in discrete microspots, facilitating high-throughput screening of complex samples through a multiplexed and parallel process. Hydrogel microarrays, in this context, offer attractive systems by combining the inherent potential of microarrays with the ability to entrap biomolecules onto the microarray surface under solution mimetic conditions.[49]

In this field, our group recently exploited a series of self-assembling peptides to create a 3D matrix with controlled permeability properties for the selective confinement of biomolecular probes (e.g., peptides, proteins, and antibodies) in microarray immunoassays.

Using a printable and self-adhesive hydrogel (Ac–YFQQKFQFQFEQQ–conh2, YF–Q11), we obtained a user-friendly and robust nano-scaffold, enabling the execution of diagnostic immunoassays for the detection of Zika virus serological tests in human serum within an ultrashort (< 5 min) timeframe.[22] The tunable nature of the hydrogel was harnessed to modulate the accessibility of different probes, achieving a tunable permeability of the supramolecular matrix. The rheological and self-adhesive properties of the hydrogel material resulted in a higher surface binding capacity and improved exposure of the recognition site. Additionally, due to the high hydrophilicity of the hydrogel matrix, nonspecific binding and background signals were basically abolished.

In follow-up work, we evolved this system using a composite peptide–agarose hydrogel, where the two components were favorably combined to provide a material entailing unique robustness for 3D assays. While it is common for polysaccharides to act as gellants, the use of saccharides for supramolecular hydrogels has been less explored due to difficulties in functionalization and unfavorable mechanical properties. Experimental results demonstrated improved robustness and high sensitivity in both model antibody–epitope recognition assays and in real-case IgG immunoreactivity profiling of Covid-19 patients.[23] The 3D assay outperformed conventional 2D assays, exhibiting more favorable interactions between epitope probes and antibody analytes, along with increased loading capacity and stability over extended incubation times (Figure 7). Moreover, in a proof-of-concept study, the same composite hydrogel was successfully applied for the gentle confinement and analysis of extracellular vesicles (EVs).[50]

The application of 3D microarray technology also found significance in high-throughput screening approaches to accelerate the drug lead optimization process. Mateen et al.[24] reported the fabrication of a printable hydrogel microarray capable of immobilizing and stabilizing a diverse set of enzymes on a cellulose substrate for drug screening, specifically identifying β-lac (β-lactam antibiotics) inhibitors. The authors used a polymeric hydrogel to entrap enzymes (alkaline phosphatase, urease, and β-Lac), preserving their hydration mobility and flexibility while maintaining physiologically mimetic conditions for optimal enzyme-catalyzed reactions (Figure 8). To assess the capability of the printed hydrogel platform to distinguish between specific and nonspecific species, various promiscuous inhibitors (rottlerin, kinase inhibitors and tetraiodophenolphthalein) were tested against TEM-1 β-lac, a commonly encountered beta-lactamase in Gram-negative bacteria. The findings demonstrated the platform's ability to make quantitative predictions of the IC50 values for well-established β-lac inhibitors, effectively

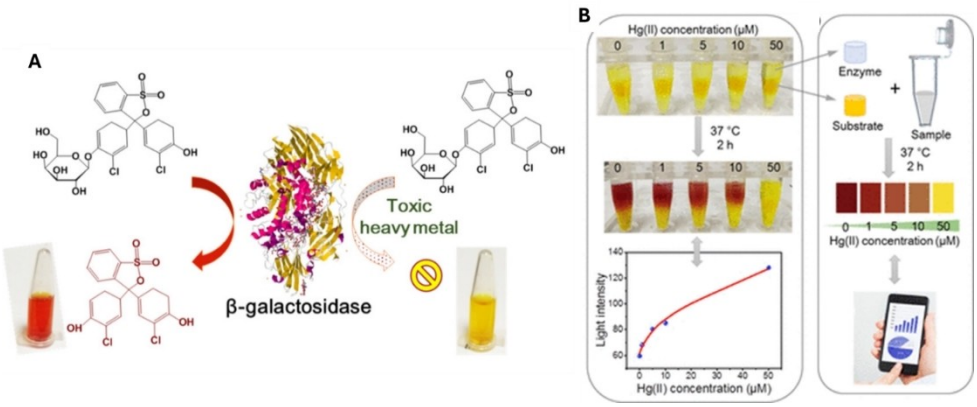

Figure 6. (A) The bioassay components for the detection of toxic metal ions; (B) Quantitative and macroscopic changes of the hydrogel assay varying Hg(II) concentrations. Reprinted and adapted with permission from [21]. Copyright 2018 American Chemical Society.

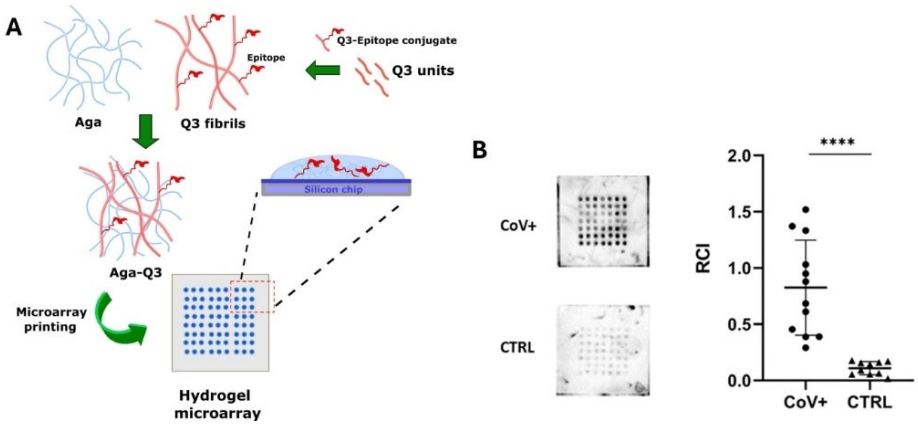

Figure 7. (A) Two-step process strategy to produce 3D hydrogel arrays. SAP–Q3 peptides are first mixed with the epitope-functionalized sequence, serving as scaffolds for introducing active functionalities. Subsequently, the obtained fibers are blended with agarose to yield composite hydrogels that are directly printed onto a microarray. B) IgG reactivity specific epitope in a panel Covid + patients' sera (CoV +) compared to that in healthy controls (CTRL). The left panel shows representative images of the array of 3D spots; the right panel shows CL signals detected for the two panels. The test was able to discriminate between the two populations at a statistically significant level ($p < 0.0001$) with 100% specificity and sensitivity. Reprinted and adapted with permission from [23]. Copyright 2018 American Chemical Society.

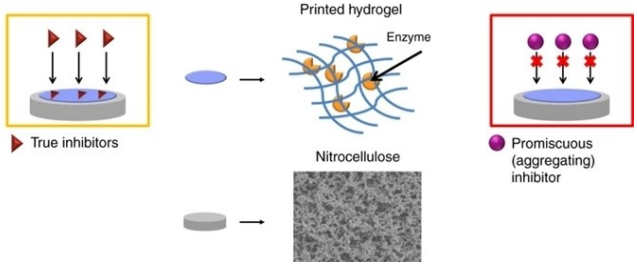

Figure 8. The scheme illustrates the size selectivity of the enzymes-based printed hydrogel for the identification of β-lac β-lactam inhibitors. Reprinted from [24], copyright Mateen et al., 2018. CC-BY 3.0 (https://creativecommons.org/licenses/by/3.0/).

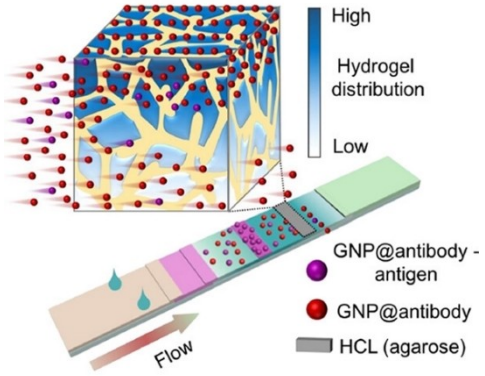

Figure 9. Mechanism of the hydrogel control line (HCL) in a LFA assay. Reprinted and adapted with permission from [25]. Copyright 2023 American Chemical Society.

preventing the occurrence of false-positive hits by promiscuous nonspecific inhibitors.

The modulation of the permeation properties in hydrogel microstructures enables precise control over the retention of bioprobes within the 3D matrix based on their size, bringing favourable applications. For example, in lateral flow assays (LFA), this strategy was exploited to prolong the interaction time of the target analytes in the samples with antibodies on the test line, resulting in an increased number of capture events. Tao and coworkers recently applied this strategy in the development of a novel LFA strip, utilizing a biomolecule-free hydrogel line (HL) as the CL directly coated onto the nanocellulose membrane[25] (Figure 9).

They demonstrated that incorporating a hydrogel as control line enhances the sensitivity of the analysis by capturing colloidal gold nanoparticles (GNPs), which serve as signal transduction elements. Various natural hydrogels, including κ-carrageenan, agarose, and gelatine, were evaluated at different concentrations to optimize the sensitivity of substrates in the detection of COVID-19 antigens. The resulting LOD was 2–3 times lower than those achieved using the goat anti-mouse antibody (IgG) directly grafted onto the analytical surface as the test control. The use of the HCL exploits the 3D structure of the hydrogel, slowing the sample flow in the test and extending the interaction with antibodies, thereby enhancing sensitivity. Adhering to the same principle, hydrogels can serve to impede uncontrolled fluid movement within a microfluidic device, promoting the accumulation of the target analytes. This concept has proven successful in achieving rapid detection of SARS-CoV-2 through DNA hydrogels in nylon microfluidic chips, as reported by Kim et al. In this approach, the target DNA pathogen in a sample specifically binds to the padlock probe immobilized on the nylon mesh, triggering a rolling circle amplification (RCA) process. The subsequent induced formation of a DNA-based hydrogel occurred, blocking the pores and impeding fluid movement (Figure 10). The presence of the virus was therefore analysed by measuring the fluid characteristics such as flow velocity, incubation time, and viscosity in the microfluidic channel. Following optimization of pore size, mesh location, and precision microfluidics, the LOD for SARS-CoV-2 was determined to be 0.7 aM.[26]

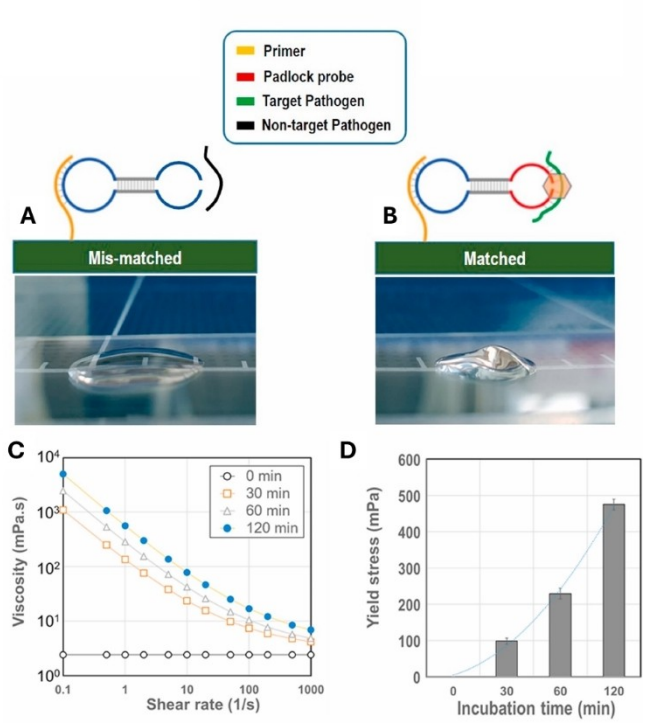

Figure 10. Macroscopic variations of the DNA supramolecular hydrogel upon A) a mismatched experiment using a non-target pathogen (dengue) and B) a matched experiment in the presence of a target pathogen (COVID-19). (C) Increase in viscosity and (D) in yield shear stress upon matched targeting at different incubation times (0, 30, 60, and 120 min). Reprinted from [26], copyright Mateen et al., 2018. CC-BY 3.0 (https://creativecommons.org/licenses/by/3.0/).

Enzyme-based digital bioassays have emerged as a novel class of multiplex bioanalytical technology with single-molecule detection sensitivity.[27,51] The primary principle of digital bioassays involves micro-compartmentalizing assay solutions to stochastically encapsulate individual target molecules within reactors in a "one-or-none" manner. In addition to their exceptional sensitivity, the highly parallelized nature of micron-sized reactor systems enables quantitative measurements across a broad range of target concentrations in a high-throughput fashion. To circumvent the expensive microfabrication process of microreactor supports, compartmentalization-free formats have been developed, exploiting fibroin hydrogel to promote fluorescence signal amplification. In pursuit of this objective, Maley et al. introduced a simplified digital ELISA assay format wherein the sealing substance was replaced with hydrogel, allowing for the direct encapsulation of beads in a 3D environment.[52]

As illustrated in Figure 11, the authors designed the platform using tyramide signal amplification (TSA), a method for catalyzed reporter deposition (CARD), for on-bead signal generation. Subsequently, the beads were immobilized in fibrin hydrogels, and single-molecule counting was performed in a simplified workflow format. The CARD-dELISA demonstrated the capability for ultrasensitive protein detection (IL-6: ~1 fM) in

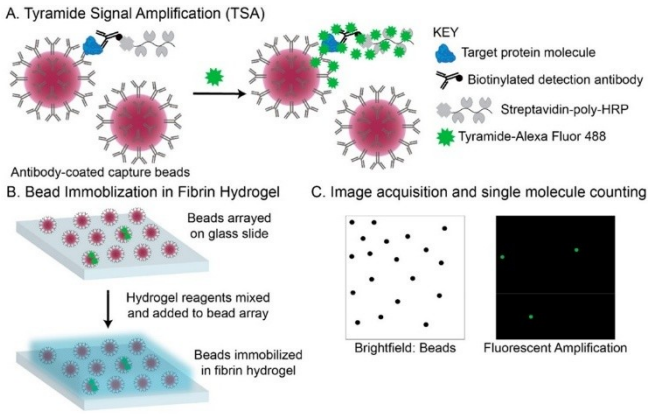

Figure 11. Illustration of the CARD-dELISA platform using fibroin hydrogel. Reprinted from [27], license number 5742390206024.

saliva samples, exhibiting a dynamic range comparable to the conventional Simoa assay.

In vitro bioassays offer an alternative to animal experiments for molecular and cellular physiological analysis. Conventional bioassays for cellular metabolic studies have typically focused on assessing the average variation in activity among cultured cells by analysing the concentration of specific metabolic molecules in a bulk medium. In contrast, the use of hydrogel-supported cell culture has demonstrated significant advantages, particularly in enhancing site-specific physiological analysis through the integration of in situ assays for cellular metabolic activity.[53]

Following this approach, Nagamine et al. developed a hydrogel-based bioassay for the simultaneous *in vitro* detection of oxygen consumption and IL-6 secretion in muscular metabolic activity.[28] To assess the activity of metabolic biomarkers, one sheet incorporated a photoresponsive sensor for oxygen, enabling the quantitative measurement of contraction-dependent change in the O_2 consumption rate of the myotubes, while the other was functionalized with a specific immunocapture probe (an anti-IL-6 antibody) for IL-6 detection (Figure 12). This bioassay proved successful in the *in vitro* study of IL-6-dependent glycaemic control through mitochondrial energy production in exercised skeletal muscle cells, proving insight into the mechanism underlying exercise therapy for type-2 diabetes.

The interest in hydrogel biomaterials as cell culture substrates for regenerative medicine and tissue engineering has experienced significant growth over the last decade.

Particularly noteworthy is the exploration of hydrogel design and development to mimic the natural extracellular matrix (ECM) environment specific to various cell types.[54] Through the careful tuning of material properties, such as stiffness or the inclusion of selective recognition motifs, it becomes possible to customize the cell microenvironment to provide information regarding cell proliferation and differentiation.

Pioneering research has uncovered that the chirality of hydrogel scaffolds can profoundly influence the adhesion and proliferation densities of cells. For example, utilizing the two

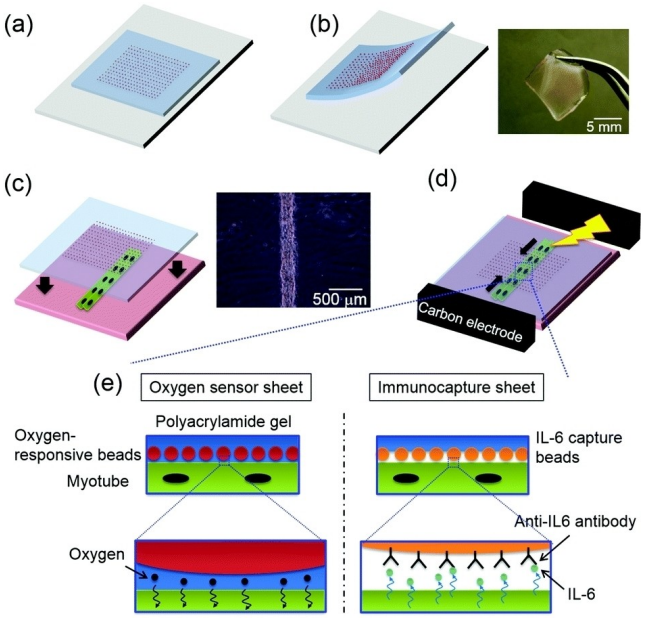

Figure 12. (a, b) Fabrication of the hydrogel-based bioassay for IL-6 detection and (c–e) applications on skeletal muscle cells. Reprinted from [28], license number 1454868-1.

enantiomers of a 1,4-benzenedicarboxamide phenylalanine derivative (D-Phe and L-Phe) as supramolecular gelators resulted in the creation of a new hydrogel material with a chiral nanofibrous structure, comprising left-handed nanofibers (l-Phe) and right-handed nanofibers (d-Phe) (Figure 13). Experimental results performed on fibroblast (NIH 3T3) and endothelial (HUVECs) cell lines, encapsulated within the 3D hydrogel scaffold, underscore that left-handed helical nanofibers increase cell adhesion and proliferation, whereas right-handed nanofibers have the opposite effect, demonstrating the crucial impact of chirality in ECM-based hydrogels.[55]

The combination of SAPs with commonly used polymers (e.g., polysaccharides, polyalcohols) represents a valuable strategy for creating new supramolecular hydrogels with mutually improved properties, thereby expanding the range of functional properties beyond mono-component systems.

A notable example is the development of conductive polymer hydrogels (CPHs), which leverage the benefits of conducting polymers while incorporating the mechanical properties of 3D nanostructure hydrogels.[29] However, the applications of CPHs as biomaterials face limitations in terms of biocompatibility, self-repair capability, and precise control over mechanical properties. To address these challenges, combining the self-assembling dipeptide Fmoc–FF with the conductive polymer polyaniline (PAni) emerged as a promising strategy.[10c] This composite hydrogel exhibited remarkable mechanical rigidity, boosting a high storage modulus (G′) value of approximately 2 MPa. Moreover, its stiffness could be finely tuned by adjusting the peptide concentration. Notably, the hydrogel displayed ohmic conductivity, pressure sensitivity, and self-healing properties. The integrated biocompatibility, conductivity, and soft nature of this composite hydrogel make it an ideal substrate for the growth of functioning electrogenic cardiac cells.

These distinctive attributes enabled its application in dynamic range pressure sensing and as a conductive interface for electrogenic cardiac cells. The composite hydrogel supported the organization of cardiomyocytes into a spontaneously contracting system, showcasing its potential for advanced applications in tissue engineering and biomedical devices.

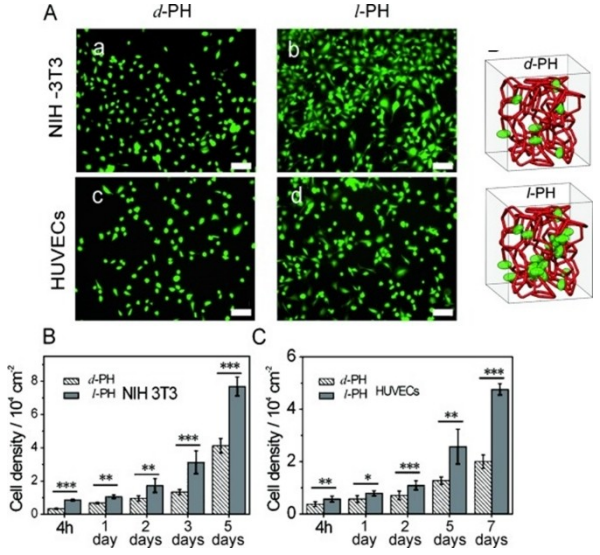

Figure 13. A) Fluorescence microscopy images of NIH 3T3 cells after culture for 3 days (a, b) and HUVECs after 5 days of culture (c, d) on d-PH and l-PH nanofibers. Right) Schematic representation of different cell-adhesion behaviors in the enantiomeric environment. B, C) Quantitative analysis of NIH 3T3 cells (B) and HUVECs (C) on left and right-handed nanofiber films after incubation for different times. Reprinted from [55], license number 5737090282405.

3. Summary and Outlook

In recent years, supramolecular hydrogels have progressively attracted significant attention in the biosensing and bioassay areas, leading to several advanced developments.

In our current work, we seek to provide a global view to the field by introducing some general principles that lay the foundation of hydrogels success, and showcasing relevant recent literature reports that build on the unique properties of supramolecular gels.

We have here presented different approaches wherein 3D platforms have been designed, adapted, and refined to deliver improved applicative performances. Even though no hydrogel formulation can provide a universal solution to address all the possible analytical challenges, supramolecular systems offer numerous advantages. Some of the most remarkable include tunable mechanical and permeation properties, loading capacity, prevention of nonspecific biomolecule adsorption and denaturation processes, ease, and versatile multiplatform integration. Overall, the possibilities for designing innovative solutions in the biosensing arena are almost unlimited. As such, the applications discussed in this review span from hydrogels

serving as immobilization matrices for biomolecules, to stimuli-responsive materials actively participating in the sensing process. We illustrated a wide range of analytical targets, showing that even though hydrogel-based systems are not always superior to existing techniques, they can contribute with additional functionalities, provide streamlined detection, or integrate multiple biosensor components into a unified system.

The confinement of different bioprobes or enzymes within supramolecular matrices was showcased to underline the potential of hydrogels in the development of healthcare platforms. Hydrogel based bioassays may indeed find broad applications as diagnostic tools, presenting new opportunities to improve traditional laboratory methodologies.

Despite the numerous advantages that supramolecular hydrogel-based systems offer, limitations persist in this field, indicating that significant progress is still needed before they can be broadly utilized as screening devices or in commercial healthcare products. Long-term stability, storage, and interlaboratory robustness are crucial challenges for the practical application of supramolecular hydrogels in clinical settings. These aspects remain, to date, largely neglected in the reported literature and deserve deeper investigation in the future. Additionally, when considering scalability and manufacturing challenges, the cost of building blocks and synthetic procedures plays a critical role. These limitations, along with issues related to shelf-life storage and compatibility with transducer formats, affect the commercial viability and widespread adoption of this technology. In this view, extensive investigations on sensitivity, specificity, response time, reproducibility, and durability must be performed to prompt the translation of supramolecular hydrogels from research to commercial products. Furthermore, implementation of quality control will be necessary for large-scale applications.

However, while hydrogels do not always outperform existing techniques, they offer additional functionalities, facilitate detection, and integrate several active components into one system. In this context, there is a substantial demand for the development of the next generation of biosensors and bioanalytical devices for use in clinical diagnostics, point-of-care testing, and personalized medicine. This emerging trend drives current efforts to address today's challenges in the application of supramolecular hydrogels, moving the field forward towards their wide adoption in routine healthcare.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: Supramolecular hydrogel · Self-assembly · 3D biosensing · 3D bioassays

[1] J. M. Lehn, *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89–112.
[2] a) E. Mattia, S. Otto, *Nat. Nanotechnol.* **2015**, *10*, 111–119; b) T. Banno, D. Sawada, T. Toyota, *Materials* **2022**, *15*, 2391.
[3] a) X. J. Loh, O. A. Scherman, Eds, *Polymeric and Self Assembled Hydrogels*, Royal Society of Chemistry, Cambridge (UK) **2012**; b) Y. H. Zhenfeng He, C. Wang, L. Zhang, Q. Xuan, S. Wei, Y. Wang, D. Pan, B. Dong, R. Wei, N. Naik, Z. Guo, *Chem. Commun.* **2021**, *57*, 1413–1429.
[4] a) M. Yamanaka, H. Fujii, *J. Org. Chem.* **2009**, *74*, 5390–5394; b) K. Hanabusa, M. Matsumoto, M. Kimura, A. Kakehi, H. Shirai, *J. Colloid Interface Sci.* **2000**, *224*, 231–244; c) L. A. Estroff, A. D. Hamilton, *Angew. Chem.* **2000**, *39*, 3447–3450; d) C. Boettcher, B. Schade, J. Fuhrhop, *Langmuir* **2001**, *17*, 873–877; e) M. Gradzielski, M. Bergmeier, M. Müller, H. Hoffmann, *J. Phys. Chem. B* **1997**, *101*, 1719–1722.
[5] P. R. A. Chivers, D. K. Smith, *Nat. Rev. Mater.* **2019**, *4*, 463–478.
[6] B. Yang, D. J. Adams, M. Marlow, M. Zelzer, *Langmuir* **2018**, *34*, 15109–15125.
[7] a) H. Kong, G. Yang, P. He, D. Zhu, X. Luan, Y. Xu, R. Mu, G. Wei, *Nano Futures* **2023**, *7*, 12001; b) F. Sheehan, D. Sementa, A. Jain, M. Kumar, M. Tayarani-Najjaran, D. Kroiss, R. V. Ulijn, *Chem. Rev.* **2021**, *121*, 13869–13914; c) J. Mitrovic, G. Richey, S. Kim, M. O. Guler, *Langmuir* **2023**, *39*, 11935–11945.
[8] a) F. Praetorius, B. Kick, K. L. Behler, M. N. Honemann, D. Weuster-Botz, H. Dietz, *Nature* **2017**, *552*, 84–87; b) S. Kosuri, G. M. Church, *Nat. Methods* **2014**, *11*, 499–507.
[9] a) J. Gačanin, C. V. Synatschke, T. Weil, *Adv. Funct. Mater.* **2020**, *30*, 1906253; b) V. Morya, S. Walia, B. B. Mandal, C. Ghoroi, D. Bhatia, *ACS Biomater. Sci. Eng.* **2020**, *6*, 6021–6035; c) J. Tang, X. Jia, Q. Li, Z. Cui, A. Liang, B. Ke, D. Yang, C. Yao, *Proc. Natl. Acad. Sci.* **2023**, *120*, e2303822120; d) I. Zare, R. Taheri-Ledari, F. Esmailzadeh, M. Mehdi Salehi, A. Mohammadi, A. Maleki, E. Mostafavi, *Nanoscale* **2023**, *15*, 10882–10903; e) Y. Li, R. Chen, B. Zhou, Y. Dong, D. Liu, *Adv. Mater.* **2024**, *36*, 2306570.
[10] a) E. Radvar, H. S. Azevedo, *Macromol. Bios.* **2019**, *19*, 1800221b) K. Firipis, M. Boyd-Moss, B. Long, C. Dekiwadia, W. Hoskin, E. Pirogova, D. R. Nisbet, R. M. I. Kapsa, A. F. Quigley, R. J. Williams, *ACS Biomater. Sci. Eng.* **2021**, *7*, 3340–3350; c) P. Chakraborty, T. Guterman, N. Adadi, M. Yadid, T. Brosh, L. Adler-Abramovich, T. Dvir, E. Gazit, *ACS Nano* **2019**, *13*, 163–175; d) R. Huang, W. Qi, L. Feng, R. Su, Zhimin He, *Soft Matter* **2011**, *7*, 6222; e) Z. Min, B. Xu, W. Li, A. Zhang, *Polym. Chem.* **2021**, *12*, 1898–1917.
[11] a) H. Xiong, Y. Li, H. Ye, G. Huang, D. Zhou, Y. Huang, *J. Mater. Chem. B* **2020**, *8*, 10309–10313; b) B. Zhang, J. He, M. Shi, Y. Liang, B. Guo, *Chem. Eng. J.* **2020**, *400*, 125994; c) T. Hu, X. Cui, M. Zhu, M. Wu, Y. Tian, B. Yao, W. Song, Z. Niu, S. Huang, X. Fu, *Bioact. Mater.* **2020**, *5*, 808–818.
[12] a) N. N. Ferreira, L. M. B. Ferreira, V. M. O. Cardoso, F. I. Boni, A. L. R. Souza, M. P. D. Gremião, *Eur. Polym. J.* **2018**, *99*, 117–133; b) L. Saunders, P. X. Ma, *Macromol. Biosci.* **2019**, *19*, 1800313; c) Y. Li, Q. Saiding, Z. Wang, W. Cui, *Prog. Mater. Sci.* **2024**, *141*, 101216; d) S. Sajjadi, A. Gholizadeh-Hashjin, F. Shafizadeh, S. Marefat, S. Hamidi, A. Farjami, *J. Appl. Polym. Sci.* **2023**, *140*, e54641; e) Z. Luo, Y. Wang, J. Li, J. Wang, Y. Yu, Y. Zhao, *Adv. Funct. Mater.* **2023**, *33*, 2306554; f) N. Falcone, M. Ermis, D. G. Tamay, M. Mecwan, M. Monirizad, T. G. Mathes, V. Jucaud, A. Choroomi, N. R. de Barros, Y. Zhu, N. Engin Vrana, H. Kraatz, H. Kim, A. Khademhosseini, *Adv. Healthcare Mater.* **2023**, *12*, 2301096; g) W. Feng, Z. Wang, *Adv. Sci.* **2023**, *10*, 2303326.
[13] a) X. Du, J. Zhou, J. Shi, B. Xu, *Chem. Rev.* **2015**, *115*, 13165–13307; b) K. Nakamura, R. Kubota, T. Aoyama, K. Urayama, I. Hamachi, *Nat. Commun.* **2023**, *14*, 1696; c) M. J. Webber, E. A. Appel, E. W. Meijer, R. Langer, *Nat. Mater.* **2016**, *15*, 13–26.
[14] a) K. Baryeh, S. Takalkar, M. Lund, G. Liu, In *Medical Biosensors for Point of Care (POC) Applications*, R. J. Narayan (Ed.), Woodhead Publishing, Elsevier, **2017**, pp. 3–25, ISBN: 978-0-08-100072-4; b) M. Hügle, O. Behrmann, M. Raum, F. T. Hufert, G. A. Urban, G. Dame, *Analyst* **2020**,

145, 2554–2561; c) J. Kim, A. S. Campbell, B. Esteban-Fernández de Ávila, J. Wang, *Nat. Biotechnol.* **2019**, *37*, 389–406.
[15] M. Lian, X. Chen, Y. Lu, W. Yang, *ACS Appl. Mater. Interfaces* **2016**, *8*, 25036–25042.
[16] T. Yoshii, S. Onogi, H. Shigemitsu, I. Hamachi, *J. Am. Chem. Soc.* **2015**, *137*, 3360–3365.
[17] W. Wang, R. Han, M. Chen, X. Luo, *Anal. Chem.* **2021**, *93*, 7355–7361.
[18] P. J. S. King, A. Saiani, E. V. Bichenkova, A. F. Miller, *Chem. Commun.* **2016**, *52*, 6697–6700.
[19] K. I. MacConaghy, C. I. Geary, J. L. Kaar, M. P. Stoykovich, *J. Am. Chem. Soc.* **2014**, *136*, 6896–6899.
[20] R. Jin, D. Kong, X. Yan, X. Zhao, H. Li, F. Liu, P. Sun, Y. Lin, G. Lu, *ACS Appl. Mater. Interfaces* **2019**, *11*, 27605–27614.
[21] Y. Zhang, T. Ren, H. Tian, B. Jin, J. He, *ACS Appl. Mater. Interfaces* **2018**, *10*, 26705–26712.
[22] P. Gagni, A. Romanato, G. Bergamaschi, P. Bettotti, R. Vanna, C. Piotto, C. F. Morasso, M. Chiari, M. Cretich, A. Gori, *Nanoscale Adv.* **2019**, *1*, 490–497.
[23] G. Bergamaschi, A. Musicò, R. Frigerio, A. Strada, A. Pizzi, B. Talone, J. Ghezzi, A. Gautieri, M. Chiari, P. Metrangolo, R. Vanna, F. Baldelli Bombelli, M. Cretich, A. Gori, *ACS Appl. Mater. Interfaces* **2022**, *14*, 4811–4822.
[24] R. Mateen, M. Monsur Ali, T. Hoare, *Nat. Commun.* **2018**, *9*, 602.
[25] T. Ma, L. Peng, Q. Ran, Y. Zeng, F. Liang, *ACS Appl. Bio Mater.* **2023**, *6*, 5685–5694.
[26] H. Kim, N. Abbas, S. Shin, *Biosens. Bioelectron.* **2021**, *177*, 113005.
[27] R. Dong, N. Yi, D. Jiang, *Talanta* **2024**, *270*, 125529.
[28] K. Nagamine, K. Okamoto, S. Otani, H. Kaji, M. Kanzaki, M. Nishizawa, *Biomater. Sci.* **2014**, *2*, 252–256.
[29] L. Pan, G. Yu, D. Zhai, H. R. Lee, W. Zhao, N. Liu, H. Wang, B. C.-K. Tee, Y. Shi, Y. Cui, Z. Bao, *Proc. Natl. Acad. Sci. U S A* **2012**, *109*, 9287–9292.
[30] a) A. Gori, M. Cretich, R. Vanna, L. Sola, P. Gagni, G. Bruni, M. Liprino, F. Gramatica, S. Burastero, M. Chiari, *Anal. Chim. Acta* **2017**, *983*, 189–197; b) L. Sola, P. Gagni, I. D'Annessa, R. Capelli, C. Bertino, A. Romanato, F. Damin, G. Bergamaschi, E. Marchisio, A. Cuzzocrea, M. Bombaci, R. Grifantini, M. Chiari, G. Colombo, A. Gori, M. Cretich, *ACS Infect. Dis.* **2018**, *4*, 998–1006.
[31] a) B. Grigoryan, S. J. Paulsen, D. C. Corbett, D. W. Sazer, C. L. Fortin, A. J. Zaita, P. T. Greenfield, N. J. Calafat, J. P. Gounley, A. H. Ta, F. Johansson, A. Randles, J. E. Rosenkrantz, J. D. Louis-Rosenberg, P. A. Galie, K. R. Stevens, J. S. Miller, *Science* **2019**, *364*, 458–464; b) P. Dey, T. Schneider, L. Chiappisi, M. Gradzielski, G. Schulze-Tanzil, R. Haag, *Macromol. Bios.* **2016**, *16*, 580–590; c) N. A. Peppas, J. Z. Hilt, A. Khademhosseini, R. Langer, *Adv. Mater.* **2006**, *18*, 1345–1360; d) J. Li, D. J. Mooney, *Nat. Rev. Mater.* **2016**, *1*, 16071.
[32] a) A. Larsson, T. Ekblad, O. Andersson, B. Liedberg, *Biomacromolecules* **2007**, *8*, 287–295; b) S. H. Gehrke, L. H. Uhden, J. F. McBride, *J. Controlled Release* **1998**, *55*, 21–33.
[33] J. Chao, Z. Li, J. Li, H. Peng, S. o. Su, Q. Li, C. Zhu, X. Zuo, S. Song, L. Wang, L. Wang, *Biosens. Bioelectron.* **2016**, *81*, 92–96.
[34] a) C. Vigier-Carrière, F. Boulmedais, P. Schaaf, L. Jierry, *Angew. Chem. (Int. Ed. Engl.)* **2018**, *57*, 1448–1456; b) M. Moschallski, A. Evers, T. Brandstetter, J. Rühe, *Anal. Chim. Acta* **2013**, *781*, 72–79; c) S. Sharma, M. Floren, Y. Ding, K. R. Stenmark, W. Tan, S. J. Bryant, *Biomaterials* **2017**, *143*, 17–28; d) X.-H. Dong, A. C. Obermeyer, B. D. Olsen, *Angew. Chem. (Int. Ed. Engl.)* **2017**, *56*, 1273–1277; e) K. M. Mabry, S. Z. Payne, K. S. Anseth, *Biomaterials* **2016**, *74*, 31–41; f) S. Kiyonaka, K. Sada, I. Yoshimura, S. Shinkai, N. Kato, I. Hamachi, *Nat. Mater.* **2004**, *3*, 58–64.
[35] O. Andersson, A. Larsson, T. Ekblad, B. Liedberg, *Biomacromolecules* **2009**, *10*, 142–148.
[36] R. Dong, Y. Pang, Y. Su, X. Zhu, *Biomater. Sci.* **2015**, *3*, 937–954.
[37] R. Renneberg, D. Pfeiffer, F. Lisdat, G. Wilson, U. Wollenberger, F. Ligler, A. P. F. Turner, *Adv. Biochem. Eng./Biotechnol.* **2008**, *109*, 1–18.
[38] a) H. Filik, A. Aslıhan Avan, *Talanta* **2020**, *211*, 120758; b) S. M. George, S. Tandon, B. Kandasubramanian, *ACS Omega* **2020**, *5*, 2060–2068; c) S. Xu, A. C. Sedgwick, S. A. Elfeky, W. Chen, A. S. Jones, G. T. Williams, A. T. A. Jenkins, S. D. Bull, J. S. Fossey, T. D. James, *Front. Chem. Sci. Eng.* **2020**, *14*, 112–116; d) G. S. Kassahun, S. Griveau, S. Juillard, J. Champavert, A. Ringuedé, B. Bresson, Y. Tran, F. Bedioui, C. Slim, *Langmuir* **2020**, *36*, 827–836; e) I. Y. Jung, J. S. Kim, B. R. Choi, K. Lee, H. Lee, *Adv. Healthcare Mater.* **2017**, *6*, 1601475.
[39] a) Dhanjai, A. Sinha, P. K. Kalambate, S. M. Mugo, P. Kamau, J. Chen, R. Jain, *TrAC Trends Anal. Chem.* **2019**, *118*, 488–501; b) Q. Dou, Z. Zhang, Y. Wang, S. Wang, D. Hu, Z. Zhao, H. Liu, Q. Dai, *ACS Appl. Mater. Interfaces* **2020**, *12*, 34190–34197.
[40] a) H. M. El-Husseiny, E. A. Mady, L. Hamabe, A. Abugomaa, K. Shimada, T. Yoshida, T. Tanaka, A. Yokoi, M. Elbadawy, R. Tanaka, *Mater. Today Bio* **2022**, *13*, 100186; b) M. Chen, Y. Wang, J. Zhang, Y. Peng, S. Li, D. Han, S. Ren, K. Qin, S. Li, Z. Gao, *J. Nanobiotechnol.* **2022**, *20*, 40; c) D. Zheng, Z. Gao, T. Xu, C. Liang, Y. Shi, L. Wang, Z. Yang, *Nanoscale* **2018**, *10*, 21459–21465.
[41] K. Völlmecke, R. Afroz, S. Bierbach, L. J. Brenker, S. Frücht, A. Glass, R. Giebelhaus, A. Hoppe, K. Kanemaru, M. Lazarek, L. Rabbe, L. Song, A. Velasco Suarez, S. Wu, M. Serpe, D. Kuckling, *Gels* **2022**, *8*, 768.
[42] a) R. Binaymotlagh, L. Chronopoulou, F. H. Haghighi, I. Fratoddi, C. Palocci, *Materials* **2022**, *15*, 5871; b) V. Devika, P. J. Sreelekshmi, R. Niranjana, S. A. Lakshmi, A. Chandran, G. B. Gouthami, S. Sadanandan, *Mol. Pharm.* **2022**, *19*, 1999–2021; c) Y. Wang, W. Zhang, C. Gong, B. Liu, Y. Li, L. Wang, Z. Su, G. Wei, *Soft Matter* **2020**, *16*, 10029–10045; d) H. Zhou, Y. Zhu, B. Yang, Y. Huo, Y. Yin, X. Jiang, W. Ji, *J. Mater. Chem. B* **2024**, *12*, 1748–1774; e) R. Malta, A. C. Marques, P. Cardoso da Costa, M. H. Amaral, *Gels* **2023**, *9*, 802; f) N. Yadav, M. K. Chauhan, V. S. Chauhan, *Biomater. Sci.* **2020**, *8*, 84–100.
[43] M. Lian, L. Xu, X. Zhu, X. Chen, W. Yang, T. Wang, *Anal. Chem.* **2017**, *89*, 12843–12849.
[44] a) J. H. Kim, S. Y. Lim, D. H. Nam, J. Ryu, S. H. Ku, C. B. Park, *Biosens. Bioelectron.* **2011**, *26*, 1860–1865; b) S. Souza, S. Kogikoski Jr, E. Silva, W. Alves, *J. Braz. Chem. Soc.* **2016**, *28*, 1619–1629.
[45] M. Ikeda, T. Tanida, T. Yoshii, K. Kurotani, S. Onogi, K. Urayama, I. Hamachi, *Nat. Chem.* **2014**, *6*, 511–518.
[46] J. Qin, X. Li, L. Cao, S. Du, W. Wang, S. Q. Yao, *J. Am. Chem. Soc.* **2020**, *142*, 417–423.
[47] a) C. Dincer, R. Bruch, A. Kling, P. S. D. G. A. Urban, *Trends Biotechnol.* **2017**, *35*, 728–742; b) W. Teixeira, Y. Pallás-Tamarit, A. Juste-Dolz, A. Sena-Torralba, R. Gozalbo-Rovira, J. Rodríguez-Díaz, D. Navarro, J. Carrascosa, D. Gimenez-Romero, Á. Maquieira, S. Morais, *Biosens. Bioelectron.* **2022**, *213*, 114454; c) K. Gavina, L. C. Franco, H. Khan, J. Lavik, R. F. Relich, *J. Clin. Virol.* **2023**, *169*, 105613.
[48] A. Dutta, U. Maitra, *ACS Sens.* **2022**, *7*, 513–522.
[49] A. Sampieri, R. Monroy-Contreras, A. Asanov, L. Vaca, *Front. Bioeng. Biotechnol.* **2022**, *10*, 88197.
[50] G. Bergamaschi, R. Frigerio, A. Musicò, G. Lodigiani, P. Gagni, R. Vago, M. Cretich, A. Gori, *Sens. Diagn.* **2024**, *3*, 395–399.
[51] a) H. Noji, Y. Minagawa, H. Ueno, *Lab Chip* **2022**, *22*, 3092–3109; b) X. Zhou, B. Zheng, *Lab Chip* **2023**, *23*, 1151–1168.
[52] A. M. Maley, P. M. Garden, D. R. Walt, *ACS Sens.* **2020**, *5*, 3037–3042.
[53] a) E. Radvar, H. S. Azevedo, *ACS Biomater. Sci. Eng.* **2019**, *5*, 4646–4656; b) V. I. B. Castro, A. R. Araújo, F. Duarte, A. Sousa-Franco, R. L. Reis, I. Pashkuleva, R. A. Pires, *ACS Appl. Mater. Interfaces* **2023**, *15*, 29998–30007.
[54] X. Dou, C. Feng, *Adv. Mater.* **2017**, *29*, 1604062www.
[55] G. Liu, D. Z. Feng, *Angew. Chem. Int. Ed.* **2014**, *53*, 7789–7793.

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