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Physical stimuli-responsive DNA hydrogels: design, fabrication strategies, and biomedical applications

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Abstract Physical stimuli-responsive DNA hydrogels hold immense potential for tissue engineering due to their inherent biocompatibility, tunable properties, and capacity to replicate the mechanical environment of natural tissue, making physical stimuli-responsive DNA hydrogels a promising candidate for tissue engineering. These hydrogels can be tailored to respond to specific physical triggers such as temperature, light, magnetic fields, ultrasound, mechanical force, and electrical stimuli, allowing precise control over their behavior. By mimicking the extracellular matrix (ECM), DNA hydrogels provide structural support, biomechanical cues, and cell signaling essential for tissue regeneration. This article explores various physical stimuli and their incorporation into DNA hydrogels, including DNA self-assembly and hybrid DNA hydrogel methods. The aim is to demonstrate how DNA hydrogels, in conjunction with other biomolecules and the ECM environment, generate dynamic scaffolds that respond to physical stimuli to facilitate tissue regeneration. We investigate the most recent developments in cancer therapies, including injectable DNA hydrogel for bone regeneration, personalized scaffolds, and dynamic culture models for drug discovery. The study concludes by delineating the remaining obstacles and potential future orientations in the optimization of DNA hydrogel design for the regeneration and reconstruction of tissue. It also addresses strategies for surmounting current challenges and incorporating more sophisticated technologies, thereby facilitating the clinical translation of these innovative hydrogels.

Keywords Physical stimuli, DNA hydrogel, Extracellular matrix, Biomechanical cues, Tissue regeneration

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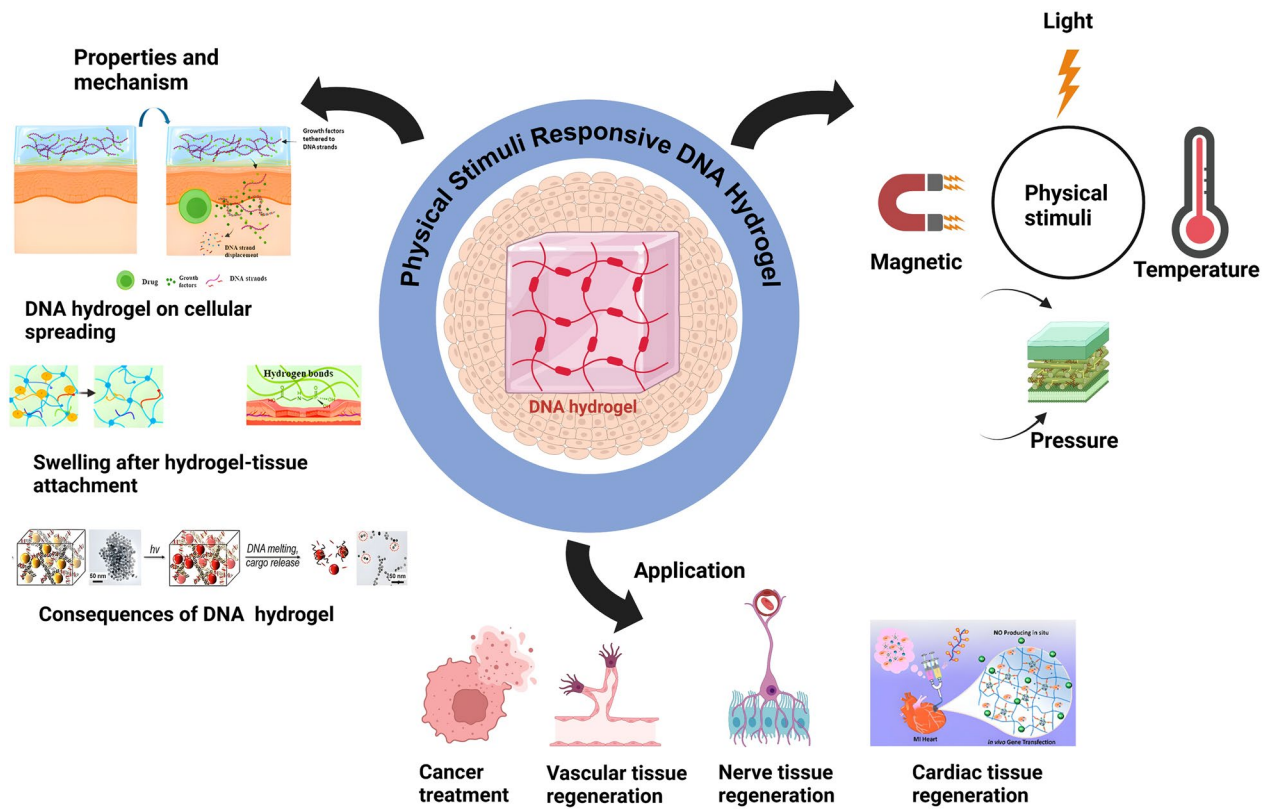
Graphical Abstract



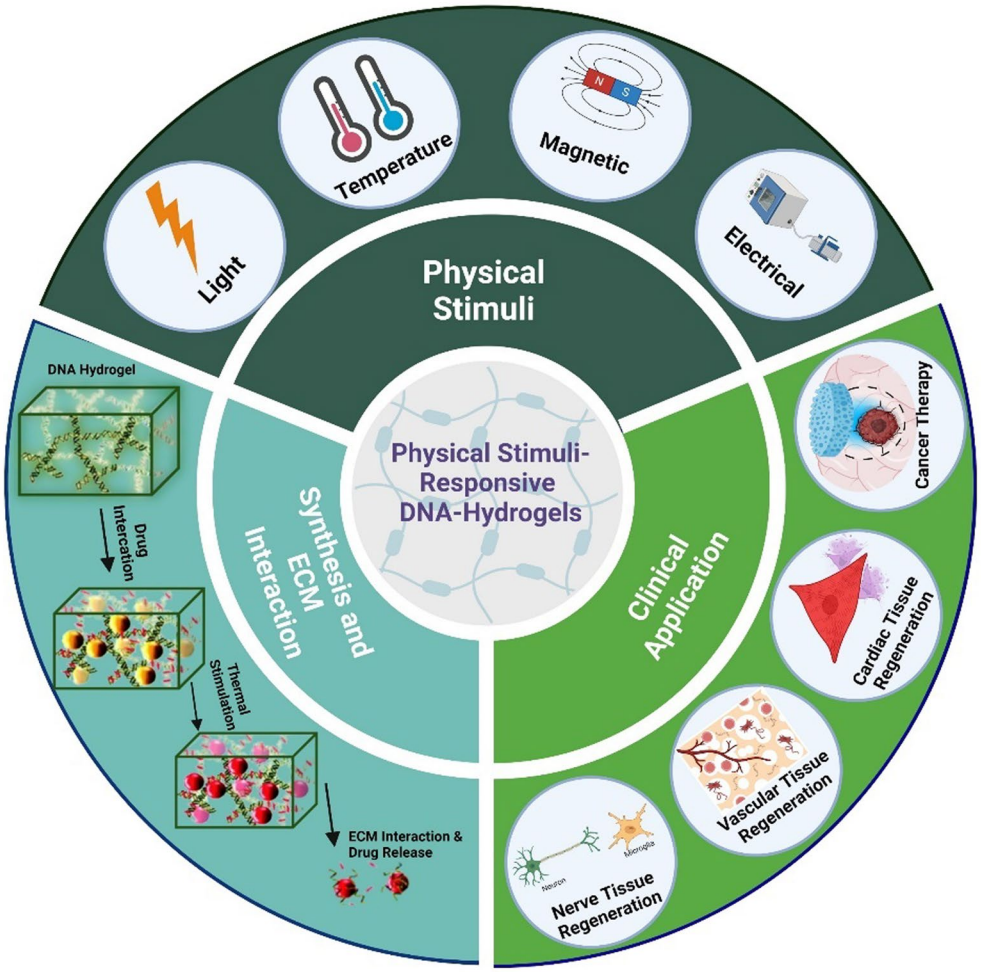
Introduction

Hydrogels in tissue engineering aim to develop strategies for repairing or replacing damaged tissues and organs by combining cells, biomaterials, and bioactive molecules [1–3]. With their large water absorption capability and mechanical tunability, hydrogel networks can have a large number of biological fluids, elasticity, and flexibility without dissolution compared to normal tissue and provide the same conditions as living tissue [3]. These hydrogels share similarities with the natural ECM, providing a supportive structure for cell attachment, proliferation, and differentiation. In recent years, there has been increasing interest in the development of physical stimuli-responsive DNA hydrogel as an ECM platform for multifunctional tissue regeneration. With recent advancements in biomaterials, DNA hydrogels have emerged as a novel and versatile platform for tissue regeneration. These DNA hydrogels offer unique advantages over traditional hydrogels due to their inherent biological properties. These hydrogels can undergo structural modifications and respond to environmental changes, making them ideal for tissue regeneration applications. DNA hydrogels are composed of synthetic or natural DNA molecules that can self-assemble into a three-dimensional network through base pairing interactions [4, 5]. DNA as a natural polymer has received increasing attention for constructing hydrogels compared to other natural polymer-based hydrogels. DNA can be considered a negatively charged hydrophilic polymer, and it has good biocompatibility, biodegradability, unique programming, and versatility [6, 7]. Molecules of DNA that act as a polymer backbone, polymer cross-linker, or functional tag to design hydrogels for customized properties. With consistent efforts, researchers have strived to engineer these hydrogels by modifying their physical and chemical properties, referring to them as stimuli-responsive hydrogels or smart hydrogels. In the presence of physical triggers such as light, temperature, an electric or magnetic field, or mechanical force, the responsive DNA hydrogels are stimulated to change cross-linking density at the microscopic level [7–9]. This ensures the reversible and switchable gel-to-sol or sol-to-gel transition at the macroscopic scale, which makes the hydrogel highly promising in multifunctional tissue regeneration applications [8–10]. For physical stimuli-responsive DNA hydrogel, this DNA hydrogel can be synthesized in two possible ways. One is all-DNA hydrogel, and another is hybrid DNA hydrogel. Here, all-DNA hydrogels mean

the hydrogel is entirely made from DNA self-assembling or enzymatic ligation, whereas DNA hybrid hydrogels mean natural and synthetic polymers synthesize the hydrogel with nanoparticles and other small molecules [11, 12]. Talking about physical stimuli-responsive DNA hydrogel, researchers introduce various physical stimuli responses, such as temperature, light, magnetic field responsiveness, electric field responsiveness, and others, by incorporating methods into the DNA hydrogel and the formation of physical stimuli-responsive DNA hydrogels are available. These hydrogels have shown great potential in tissue engineering and regenerative medicine, as they can be used to create scaffolds or matrices that mimic the ECM of native tissues. The ECM is a complex network of proteins and other molecules that provides structural support and biochemical signals to living cells. By mimicking the ECM, physical stimuli-responsive DNA hydrogels can provide a suitable environment for cells to grow and differentiate and promote tissue regeneration [13, 14]. By leveraging the responsiveness of DNA hydrogels to these stimuli, researchers can design and control their structural properties, drug release kinetics, and cellular behavior within the hydrogel matrix [7, 15]. Most importantly, they can make hydrogel scaffolds for tissue engineering, especially in the tissue regeneration field [4]. These hydrogels offer several challenges associated with traditional tissue regeneration strategies, such as limited control over drug release and cell behavior, as well as the lack of multifunctionality in scaffolds. In this way, we can say that the use of physical stimuli-assisted or stimuli-responsive DNA hydrogels as ECM platforms offers several advantages for multifunctional tissue regeneration. Firstly, these hydrogels can mimic the natural extracellular matrix in tissues, providing a supportive and bioactive environment for cell growth and tissue development. Secondly, the physical stimuli-responsiveness of these hydrogels allows for precise control over drug release and cellular responses, enabling tailored regenerative therapies. Thirdly, physical stimuli-assisted DNA hydrogels can be engineered to have multifunctional properties, allowing for the integration of different therapeutic agents and growth factors within the hydrogel matrix. This integration of multiple functionalities within the DNA hydrogel platform enhances its potential for tissue regeneration as it enables the delivery of a combination of factors that can promote cell growth, angiogenesis, and tissue remodeling1 [16–18].

DNA hydrogels were first explored for their potential in tissue engineering in the early 2010s, leveraging the unique properties of DNA to create biocompatible and programmable scaffolds [19, 20]. Recent studies have further expanded the applications of DNA hydrogels in tissue engineering. For instance, researchers have developed DNA hydrogel-based scaffolds that mimic the extracellular matrix, enhancing cell adhesion and proliferation in soft tissue engineering applications [21]. Additionally, innovative approaches using DNA hydrogels for controlled drug delivery in bone tissue engineering have shown improved osteogenic differentiation and bone regeneration. Researchers demonstrated the ability of DNA hydrogels to promote mineral growth, support osteogenic cell viability, and facilitate bone repair in animal models without inducing inflammation [19]. Over the past decade, advances in molecular design principles and synthesis strategies have enabled the rational engineering of DNA hydrogels with tailored responsiveness, mechanical properties, and functionalities [22]. Recent studies have integrated DNA hydrogels with other biomaterials like nanoparticles and polymers to create multifunctional constructs that synergistically promote angiogenesis and osteogenesis for enhanced bone regeneration outcomes [23]. With their versatility, biocompatibility, and potential for spatiotemporal control over therapeutic release, DNA hydrogels hold immense promise as next-generation scaffolds for various tissue engineering applications and control over drug delivery and cell behavior [24]. Moreover, recent advancements have addressed one of the key limitations of DNA hydrogels by developing new crosslinking strategies that significantly improve their stability and durability under physiological conditions. This breakthrough potentially expands their use in load-bearing tissue engineering applications [25].

As an example of physical stimuli-responsive, temperature-responsive DNA hydrogels are particularly attractive for tissue regeneration applications. These responsive DNA hydrogels offer a revolutionary platform for tissue regeneration. Within their delicate networks, the essence of life, DNA, intertwines with synthetic moieties, birthing a material miming the dynamic interplay of mechanics and biology in cancer therapy, cardiac tissue regeneration, vascular tissue regeneration, natural bone, nerve, chronic wound healing, and drug delivery. Physical stimuli-responsive DNA hydrogels harness the unique properties of DNA to create dynamic, programmable scaffolds that can mimic the complex microenvironments of living tissues [26]. By leveraging the intrinsic responsiveness of DNA to these external cues, these hydrogels can be precisely tailored to guide cellular behavior, promote tissue regeneration, and unlock new frontiers in personalized medicine. This cutting-edge approach holds immense promise for revolutionizing regenerative therapies, paving the way for more effective treatments and improved patient outcomes across a wide range of clinical applications. With the help of growth factors, hydrogel amplifies and guides cellular differentiation and tissue remodeling. Physical stimuli-assisted DNA hydrogels can be engineered to have multifunctional properties, allowing for the integration of different therapeutic agents and growth



Scheme 1 Schematic representation of physical stimuli-responsive DNA hydrogel in bone tissue regeneration. Obtained a 3D hydrogel network of DNA by crosslinking and showing different phase changes of hydrogel triggered by various stimuli applicable in bone tissue regeneration by mimicking their ECM

factors within the hydrogel matrix. Integrating multiple functionalities within the DNA hydrogel platform enhances its potential for tissue regeneration by delivering a combination of factors that can promote cell growth, angiogenesis, and tissue remodeling [27].

This article discusses the various approaches to physical stimuli-responsive DNA hydrogels, including the role and types of physical stimuli, methods of incorporating them into DNA hydrogels, and the formation of these hydrogels (Scheme 1). It also emphasizes the importance of an ECM platform and the specific applications of physical stimuli-responsive DNA hydrogels in tissue regeneration, such as cancer therapy, cardiac tissue regeneration, and nerve and vascular tissue regeneration. We will incorporate current advancements in weaving responsive features, such as light-sensitive components and magnetic manipulation sites, into these scaffolds, enhancing their precision and quality. Emerging trends in DNA hydrogel research for tissue engineering applications include an increasing focus on multifunctional hydrogels that combine tissue scaffolding with drug delivery capabilities, growing interest in developing DNA hydrogels with tunable degradation rates to match tissue regeneration timelines, and exploration of hybrid systems combining DNA hydrogels with other biomaterials to enhance overall performance.

Physio-chemical properties of DNA hydrogel

DNA hydrogels are synthesized using DNA molecules, cross-linked together with chemical linkers an appropriate buffer. The cross-linker molecules bind to DNA at various sites, forming the hydrogel network. The structure,

observable via scanning electron or atomic force microscopy, heavily depends on the preparation method. Unlike other hydrogels, DNA hydrogels are not thermodynamically driven; their structure is determined by the rate of gelation rather than the minimal energy state of the system. This affects how the structure and composition of DNA hydrogels can be tuned. The cross-linking mode plays a crucial role in determining the mechanical properties of DNA hydrogels. Higher concentrations of cross-linkers result in harder hydrogels due to increased network interconnectivity. Typically, these hydrogels are fabricated in a mesh form to enhance water content and porosity. The degree of cross-linking significantly affects the elasticity, stiffness, and overall mechanical strength of the hydrogel structure.

DNA hydrogel networks exhibit stability for several weeks but can become unstable under physiological conditions, particularly at elevated temperatures. This instability stems from DNA's nature as a biopolymer, with its structure dependent on base pairing. However, this characteristic also enables the design of stimuli-responsive hydrogels. These responsive hydrogels can undergo gel-sol transitions in response to temperature changes, allowing controlled release of bioactive molecules. pH changes can trigger the release of drugs or growth factors for tissue regeneration [28]. Light-sensitive DNA sequences can be incorporated to enable spatiotemporal control over drug release and tissue regeneration [29]. Magnetic nanoparticles can be integrated for external manipulation of hydrogel properties and drug delivery [30]. Electrical fields can induce reversible changes in mechanical properties like elasticity and stiffness. Additionally, DNA hydrogels can be designed to respond to mechanical forces such as shear stress or compression, which is crucial for load-bearing tissue regeneration [13, 23].

The unique properties of DNA hydrogels make them particularly suitable for tissue engineering and regenerative medicine applications. Their tunable stiffness and elasticity can mimic natural tissue, while injectable delivery minimizes invasiveness. The biocompatibility and cell-adhesiveness of these hydrogels promote cell attachment and growth, which is particularly beneficial for bone-cell interactions [4, 31–33]. They offer the controlled release of drugs or growth factors, enhancing healing and tissue regeneration [13]. Their shear stress responsiveness mimics the natural tissue response to physical activity, triggering the release of beneficial agents. Furthermore, their biodegradability allows for seamless integration with newly formed tissue over time.

These characteristics make DNA hydrogels a versatile and promising tool for tissue regeneration, with potential applications in treating complex wounds, tissue defects, and even tumor cell demolition [34]. By responding to specific physical stimuli, these materials provide precise control over their properties and functions, enabling their use in repairing a wide range of tissue injuries and diseases.

Properties of physical stimuli-responsive DNA hydrogels

Physical stimuli-responsive DNA hydrogel has many properties that make it suitable for multifunctional tissue regeneration. For example, they have been used for cancer therapy, cardiac tissue regeneration, nerve tissue regeneration, wound repair, and bone tissue regeneration. Here are some of the specific properties we have explored in this section that serve a great role in biomedical applications, especially in tissue regeneration.

Sol–gel phase transition

Physical stimuli-responsive DNA hydrogels undergo sol–gel phase transitions in response to specific physical stimuli, such as changes in light, temperature, or mechanical stress. For example, hydrogels are made by hybridizing DNA sequences with complementary sticky ends that undergo sol–gel transitions upon temperature changes like heating or pH changes [13]. In addition, some of the DNA hydrogels exhibit both shape-memory behavior and sol–gel transitions. The sol–gel phase transition is a reversible process in which a hydrogel can switch between a liquid-like sol state and a solid-like gel state. This phase switch is typically driven by changes in the hydrogel's cross-linking density, which external stimuli control [35]. For example, in a DNA hydrogel made by hybridizing complementary sequences, the sol–gel phase transitions are triggered by heating the hydrogel above a certain temperature, which causes the DNA strands to denature and the hydrogel to become more fluid. The sol–gel phase transition is an important property of physical stimuli-responsive DNA hydrogels because it allows the hydrogel to switch between different states, which can be useful for a wide range of applications. For example, DNA hydrogels that undergo sol–gel phase transitions in response to changes in temperature or pH can be used as smart hydrogel drug delivery systems, where the hydrogel releases a drug payload in response to specific stimuli. Similarly, DNA hydrogels that exhibit shape-memory behavior and sol–gel transitions can be used as scaffolds for tissue regeneration, where the hydrogels are molded into a specific shape and then triggered to solidify in situ [13, 36–38].

Self-crosslinking

DNA hydrogels are special because they can connect without complexity. Because of a process that involves

ligation. Besides this, these hydrogels made from DNA can become stronger by connecting in different ways using things like paraformaldehyde and other small molecules. This connection makes them turn into a kind of gel under normal body conditions, and they can also hold onto or combine with tiny parts of living things or medicine right where they are needed, helping make things simpler and faster. How well these hydrogels work, like how cells move or grow on them and how medicine works, depends on how strong and stiff they are. By changing how stiff they are, we can make them useful for different fields [39–41].

Mechanical properties

The mechanical properties of physical stimuli-responsive DNA hydrogels make them versatile materials for various biomedical applications, especially in tissue regeneration [42]. The mechanical properties of physical stimuli-responsive DNA hydrogels are tuned by controlling the hydrogel's cross-linking density, which is regulated by external stimuli such as changes in pH or temperature. An example provides that the DNA hydrogels made by the hybridization of complementary sequences can exhibit tunable mechanical properties by varying the length and concentration of the DNA strands used to form the hydrogel. Similar to those DNA hydrogels made by enzymatic ligation, they are also tuned by controlling the enzyme's activity, which changes in temperature or other physical stimuli can modulate [39]. Regarding their tunable mechanical properties, physical stimuli-responsive DNA hydrogels also show unique mechanical behaviors, like reversible deformation, shape-memory behavior. For example, some of the DNA hydrogels show shape-memory behavior, where the hydrogel can be deformed and then triggered to recover its original shape upon exposure to a specific stimulus. Similarly, some of the DNA hydrogels can undergo reversible deformation in response to changes in temperature or other physical stimuli, allowing the hydrogel to switch between different shapes or conformations. This way, we can say that the mechanical properties of physical stimuli-responsive DNA hydrogels are important for a wide range of applications, including tissue regeneration drug delivery. For example, DNA hydrogels with tunable mechanical properties can be used as scaffolds for tissue regeneration, where the hydrogel provides mechanical support for cells to grow and cell differentiation. Similarly, DNA hydrogels with shape-memory behavior can be used in drug delivery systems, where the hydrogel can be molded into a specific shape and then triggered to release the drug payload upon exposure to specific stimuli [43, 44].

Surface properties

The surface properties of physical stimuli-responsive DNA hydrogels can be tailored to achieve specific functions, including cell adhesion, drug delivery, etc. [8]. The ability to modify the surface properties of DNA hydrogels makes them versatile materials for a wide range of biomedical applications, like tissue regeneration [45]. For example, DNA hydrogels are functionalized with specific ligands or receptors to enable targeted binding to specific molecules or cells. Moreover, DNA hydrogels can be modified with chemical or physical cues to control cell behavior, such as proliferation, migration, and differentiation. One approach to modifying the surface properties of DNA hydrogels is incorporating specific functional groups or molecules into the hydrogel matrix. For example, DNA hydrogels can be modified with azobenzene groups to enable light-responsive behavior or with pH-sensitive groups to enable pH-responsive behavior. This type of modification can be used to control the swelling behavior of the hydrogel in response to specific physical stimuli, which can be useful for tissue regeneration and drug delivery applications [39, 43].

Porosity

The porosity property of physical stimuli-responsive DNA hydrogels is an important factor that affects their mechanical and biological properties [46]. The porosity of a hydrogel refers to the amount of space or voids within the hydrogel network that affect the diffusion of nutrients, oxygen, and other molecules through the hydrogel. The Porosity of physical stimuli-responsive DNA hydrogels is controlled by several factors, including the cross-linking density, the length and concentration of the DNA strands used to form the hydrogel, and the method of hydrogel formation [7]. For instance, DNA hydrogels made by hybridizing complementary sequences can exhibit tunable porosity by varying the length and concentration of the DNA strands used to form the hydrogel. This porosity of physical stimuli-responsive DNA hydrogel is also affected by external stimuli, like changes in temperature or pH. For example, DNA hydrogels made by hybridizing complementary sequences can undergo sol–gel transitions upon heating or changes in pH, which also affect the hydrogel's porosity. Overall, we can say that hydrogels with high porosity provide a favorable environment for cell growth and proliferation, while hydrogels with low porosity provide mechanical support for tissue regeneration applications. Similarly, hydrogels with tunable porosity can be used as drug delivery systems, where the porosity of the hydrogel can be controlled to regulate the release of drugs or other molecules [47].

Degradation

The degradation properties of physical stimuli-responsive DNA hydrogels are regulated by controlling the density of the hydrogel cross-link, which external stimuli like changes in pH, light, and temperature can be modulated. For example, DNA hydrogels made by hybridizing complementary sequences can show tunable degradation properties by varying the length and concentration of the DNA strands used to form the hydrogel [10, 39]. This degradation property of physical stimuli-responsive DNA hydrogels is important for various applications, including tissue engineering, drug delivery, and many more. By way of example, DNA hydrogels with tunable degradation properties are used as scaffolds for tissue regeneration, where the hydrogel provides temporary mechanical support for cells to grow and differentiate before being degraded and replaced by natural tissue. In this way, we can say that the degradation properties of physical stimuli-responsive DNA hydrogels make them versatile materials for a wide range of biomedical applications [48, 49].

Stiffness

The stiffness property of physical stimuli-responsive DNA hydrogels is a type of factor that affects their mechanical and biological properties. The stiffness of a hydrogel refers to its resistance to deformation under an applied force and is typically characterized by its elastic modulus or Young's modulus. This stiffness of physical stimuli-responsive DNA hydrogels is valuable for various applications in tissue engineering, drug delivery, and biosensing. Hydrogels with high stiffness can provide mechanical support for tissue engineering applications, while hydrogels with low stiffness can provide a favorable environment for cell growth and proliferation [39]. Similarly, hydrogels with tunable stiffness can be used as drug delivery systems, where the stiffness of the hydrogel can be controlled to regulate the release of drugs. So, it can be said that the stiffness property of physical stimuli-responsive DNA hydrogels is an important factor that affects their mechanical and biological properties. Several factors can also control it, including the method of hydrogel formation, the cross-linking density, and external stimuli [43, 49, 50].

Over and above, physically responsive DNA hydrogel shows a large variety of other properties such as stability, biocompatibility, swelling, cellular adhesion, and many more. These properties made the physical stimuli-responsive DNA hydrogel a promising material in multifunctional tissue regeneration.

Tunable DNA hydrogel under physical stimulation

Incorporation of physical stimuli-responsive function into DNA hydrogels is achieved by designing stimuli-responsive DNA cross-linkers that endow the hydrogel with smart features in response to environmental stimuli. This incorporation is a unique process that requires careful consideration of several key factors, including the selection of DNA sequences, the choice of cross-linking method, and the optimization of reaction conditions with various stimuli, especially the physical stimuli, according to this article. Researchers are spending time creating several techniques for controlling these factors and are now capable of creating Physical stimuli-responsive DNA hydrogels with a wide range of properties and applications [20, 51].

DNA hydrogel's design is composed of three basic components: (i) Parent polymer chains (natural and synthetic, mostly hydrophilic in nature, provide structural support to a gel. (ii) Linker moiety (DNA duplex to cross-link polymer chains to form gel linker moiety). (iii) DNA enzyme or DNA aptamer (functional tag for specific target area interacting with biomolecules and analytes) [52]. DNA hydrogels are mainly fabricated with the physical stimuli in two ways: physical crosslinking and chemical crosslinking. Chemical cross-linking belongs to the intermolecular covalent interaction of DNA, which is a permanent and irreversible linkage that maintains the mechanical strength, environmental stability, and shape memory of DNA hydrogel. Physical cross-linking depends only on non-covalent interactions, such as hydrogen bonding between complementary DNA, electrostatic interaction, coordination interaction, and electrostatic interaction to cross-link, which are dynamic and flexible [28]. By introducing physically responsive units to DNA hydrogels, the DNA hydrogel gets triggered and changes in the gel matrix's physiochemical properties, such as changes in cross-link density, volume, or mechanical properties. The key responsive units employed in the construction of smart and functional DNA hydrogels include i-motifs, aptamers, G-quadruplex, and triplex nucleic acid. In this section, we explore the types of physical stimuli and how they are incorporated with DNA hydrogels [53–56].

There are several types of Physical stimuli, but we mainly focus on those stimuli that play a major role and functionalize in tissue regeneration with DNA hydrogels equally [8, 57]. Concerning the incorporation method of the above-mentioned physical stimuli, they followed 2 different methods regarding the formation of physical stimuli-responsive DNA hydrogels (i) All DNA hydrogel; DNA that contains hydrogel is entirely made up of DNA i.e., DNA self-assembly and crosslinking of DNA strands and motifs. The construction depends on nucleic acid amplification techniques, including rolling circle

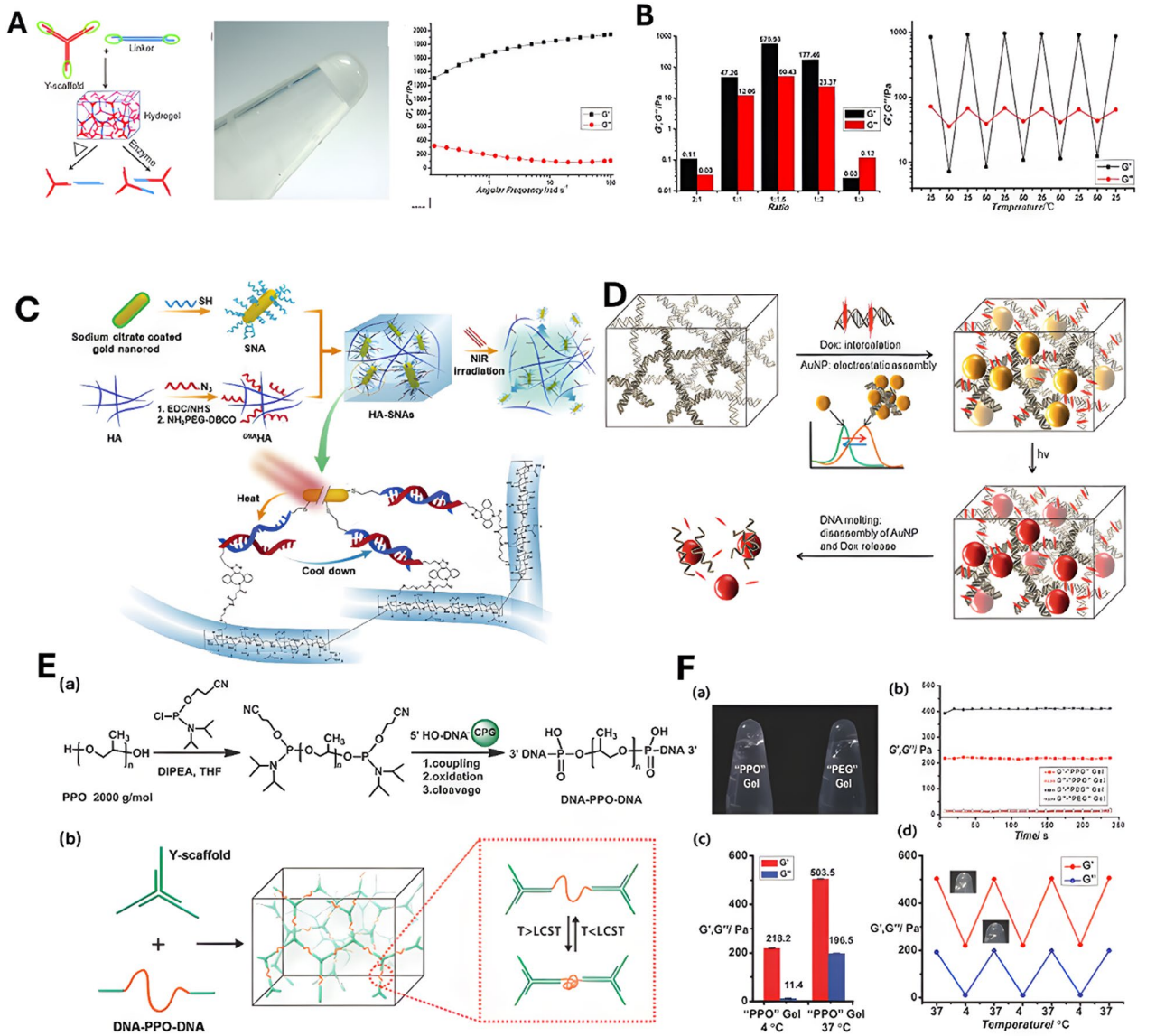


Fig. 1 Temperature-responsive DNA hydrogel formation. **A** DNA hydrogel exhibits phase transitions in response to temperature changes, DNA hydrogels synthesized from 500-μM of Y-scaffold and 750 μM of the linker with 8-base long "sticky ends," Frequency sweep test carried out between 0.05 and 100 rad $s.^{-1}$ at a fixed strain of 1% at 25 °C. **B** DNA hydrogels with different molar ratios (Y-scaffold/linker = 2:1, 1:1, 1:1.5, 1:2, and 1:3) with a total DNA content of 2% (w/v) were tested by time-scan rheological tests performed at a fixed frequency (1 Hz) and strain (1%) at 25 °C for 3 min. Reversible thermal response of DNA hydrogels formed from 400 μM of Y-scaffold and 600 μM of the linker with 8-base long "sticky ends." The rheological test was performed on the gel at a fixed frequency (1 Hz) and strain (1%) at 25 °C and 50 °C, respectively, for 5 cycles. **C** Near-infrared light triggers the release of SNAs encapsulated within nucleic acid (HA-SNAs) hydrogel by heating embedded gold nanorods, breaking the gel's internal hydrogen bonds [91]. **D** A temperature-responsive all-DNA hydrogel produced with Dox and AuNPs dissociates, releasing Dox and AuNPs. **E**(a-b) Engineering a DNA-based network with PPO reinforcements [44]. **F**(a-b) Image of DNA hydrogels with PPO and PEG domains, and Time sweep test at a certain frequency (1 Hz) and strain (1%) at 25 °C. (c-d) Mechanical properties of PPO gel incubated (4 °C or 37 °C) for 24 h tested by time sweep at a certain frequency (1 Hz) and strain (1%) and Reversible changes of mechanical properties of PPO gel when temperature changes(between 37 °C and 4 °C) [44]

amplification(RCA), polymerase chain reaction (PCR), hybridization chain reaction (HCR), and enzymatic ligation, hydrogen bond with physical entanglement [12, 51, 57]. (ii) Hybrid DNA hydrogel; synthesized by coupling and cross-linking of natural and synthetic polymers or biomolecules, nanomaterials attached to DNA. Construction is based on electrostatic interaction, coordination interaction, double bond polymerization, and so on [10, 39].

Temperature-responsive DNA hydrogels

Temperature-responsive DNA hydrogels show considerable potential in cancer therapy and tissue regeneration as a result of their distinctive capacity to perform sol–gel transitions in reaction to temperature fluctuations [58]. These hydrogels are specifically engineered to create stable networks when exposed to the temperature of the human body. It enables the precise and regulated release of therapeutic substances, such as chemotherapeutic medicines, siRNAs, and growth hormones, exactly at the intended location. Studies have shown that hydrogels may be filled with a mixture of pharmaceuticals and immunotherapeutic agents, which are then released in a controlled manner to improve the effectiveness of anticancer treatment and facilitate tissue healing. The origins of DNA hydrogels can be traced back to the revolutionary work of Nagahara in 1996, who introduced the concept of thermo-responsive DNA hydrogels to the scientific community [3, 55]. A study showed the formation of hydrogels from a succinimide copolymer, harnessing the thermal [3] dissociation of complementary DNA base pairs [3]. Specifically, a polyacrylamide chain incorporating DNA side chains was copolymerized with acrylamide-modified single-stranded DNA (ssDNA) and acrylamide monomers. Subsequently, a cross-linked DNA strand was added to the solution, and hydrogel formation was achieved through the hybridization between the ssDNA and the DNA chain (Fig. 1A, B) [59, 60]. This work demonstrated the remarkable ability of DNA hydrogels to undergo reversible sol–gel transitions in response to temperature changes. At room temperature, the hybridization between the complementary DNA strands resulted in a stable hydrogel network. However, as the temperature increased, the dehybridization of the DNA strands led to the dissolution of the hydrogel, showcasing the thermo-responsive nature of these biomaterials. This pioneering study paved the way for the exploration of DNA hydrogels in various applications, particularly in the field of tissue engineering, where their unique properties and stimuli-responsiveness hold immense potential for guiding cellular behavior and promoting tissue regeneration [48, 52, 61].

Liu et al. showed their study based on all-DNA hydrogel assembly, the form of thermo-responsive DNA hydrogel. Here are two construction units that form hydrogel: one is the Y scaffold form of three single-stranded DNA with sticky ends, and another is the linker DNA, complementary to each other. Post interaction between these two building units, hydrogel formation can be obtained in situ within a fraction of a second, and the hydrogel is stiff in DNA duplex composition. Polymer enhancement is present in the molecular permeability. When the temperature rises from 25 to 50 °C, the gel transforms into a solution due to complementary sticky ends and base pairing (Fig. 1C) [38]. In another study, Song et al. used nanoparticles like AuNPs and DOX as thermo-responsive elements in the DNA hydrogel network based on DNA assembly. Researchers used X-shaped DNA with three sticky ends to undergo enzymatic ligation for cross-linking, where one arm was disabled to control the hydrogel size. The positively charged gold nanoparticles, AuNPs, were incorporated into the DNA hydrogel by electrostatic interactions. Here, DOX gets intercalated into the hydrogel network. When the visible light of about 660 nm is irradiated on the network, gold nanoparticles and AuNPs get excited at plasmon resonance and generate a heat shock, which causes the AuNPs and DOX to dissociate in hydrogel assembly and release the AuNPs and DOX (Fig. 1D) [62].

In addition, Wu et al. developed a thermo-reactive polypropylene oxide (PPO) polymer. In this study, PPO was incorporated into the DNA network, and this PPO has a low critical solution temperature (LCST). The interaction between the Y scaffold and the DNA-PPO-DNA network results in the formation of temperature-responsive DNA hydrogel. PPO becomes hydrophilic when its temperature goes below LCST and disperses uniformly into the DNA hydrogel network. However, when LCST rises, PPO becomes hydrophobic and collapses automatically. Because of this self-collapsing into the DNA hydrogel network, mechanical strength increased in the DNA hydrogel (Fig. 1E, F) [63].

Recently, Kang et al. developed an interesting type of temperature-responsive DNA hydrogel. This study used nanoparticles called gold-silver-based Nanorods (NRs) as templates for colloid-based polymerization. They found that certain chemical groups on the surface of these nanorods could help create long chains of polymer molecules when mixed with other specific molecules. Some of these molecules were modified with single-stranded DNA material called oligonucleotides, and these DNA-modified polymer chains were linked to the nanorods, forming a nano gel core–shell structure. The Nano rod core could absorb the energy from near-infrared (NIR) light and convert it to heat very efficiently [64]. The nanorods heated up when the nano gel was exposed to the NIR laser beam, causing the surrounding gel to warm. When the temperature reached a certain point, the double-stranded DNA molecules in the gel separated, and the gel collapsed. It also showed that the gel could be loaded with an anticancer drug, which could be released in a controlled manner when the gel collapsed due to the heat [59, 65].

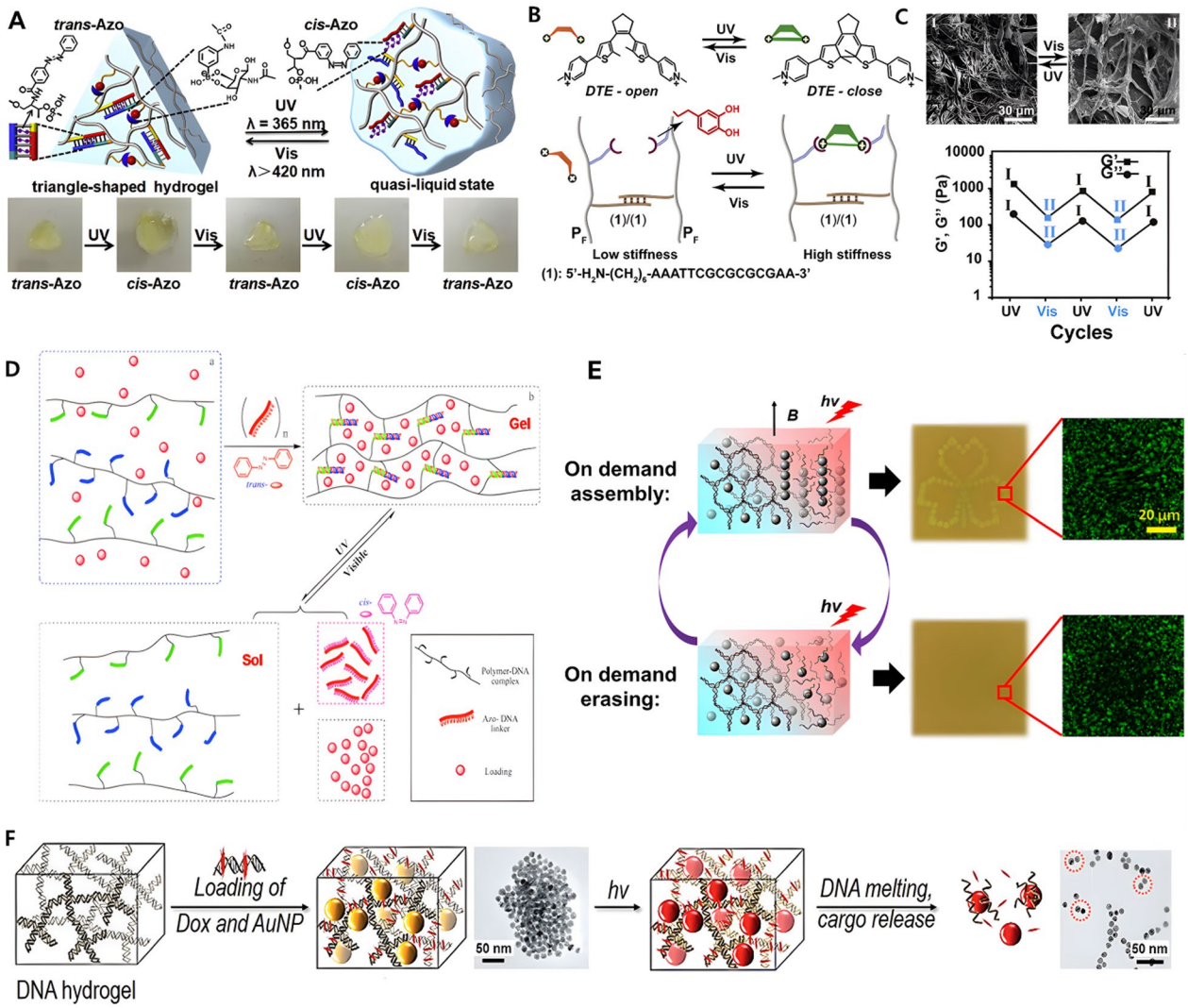


Fig. 2 Light-responsive DNA hydrogel formation. **A** Polyacrylamide DNA hydrogels of glucosamine-boronate eaters and G-quadruplexes are synergistically cross-linked in the presence of K^+/crown ethers during the cyclic hydrogel-solution transition [49]. **B**, **C** UV analysis of the hydrogel. **D** The synthesis and degradation of Light-responsive DNA hydrogels based on azobenzene cross-link. In visible light, cis-azobenzene-functionalized DNA cross-linked chains successfully cross-linked polyacrylamide-DNA complex chains and hydrogels were produced. However, under UV light irradiation, the cis-azobenzene conformation changed to trans-azobenzene, the crosslinking was released, and the hydrogel was dissolved [25]. **E** Schematic representation of the mechanism of in situ assembly of DNA supramolecular photo-responsive hydrogels [71]. **F** DNA-based hydrogels as a delivery vehicle for AuNPs and anticancer drugs combining triggered drug release and photothermal therapy. The DNA hydrogel is degraded after excitation-induced heat generation, which disperses the AuNPs. Co-loading of the anticancer drug Dox facilitated light-controlled cargo release from the hydrogel, which is attractive for, i.e., computer tomography (CT), imaging contrast, or radiosensitization [43]

Light-responsive DNA hydrogels

Light-responsive DNA hydrogels represent a cutting-edge approach in cancer therapy and tissue regeneration, leveraging their ability to undergo controlled structural changes and release therapeutic agents upon light exposure [66]. In cancer treatment, these hydrogels can be loaded with chemotherapeutic drugs, photosensitizers, or a combination of both, enabling localized and on-demand release upon light irradiation [66]. A molecule called azobenzene is commonly used to develop light-responsive DNA hydrogels. When present in the DNA hydrogel, this molecule can change its shape when exposed to UV light, transforming it from a trans to a cis form. This change helps separate the DNA strands in the hydrogel. Later, when the azobenzene returns to its trans form under visible light, the DNA strands rejoin and recover their original structure [29, 67].

Peng et al. showed a light-responsive DNA hydrogel that undergoes volume changes by reversible DNA hybridization. This light-responsive DNA hydrogel was formed by incorporating azobenzene single-stranded DNA and the complementary strand within a polyacrylamide hydrogel network. By irradiating visible light, these two incorporated single-stranded DNA molecules get hybridized and form a DNA duplex structure, which causes the shrinkage in polyacrylamide hydrogel. However, when UV light irradiates the hydrogel, the duplex DNA dissociates, and an increase in the volume of polyacrylamide hydrogel can be observed (Fig. 2A–C) [52, 68]. In a study, the incorporation and formation of DNA hydrogel, where glucosamine-boric acid esters were cross-linked with a polyacrylamide chain, and the intercalation units of trans-azobenzene stabilized the DNA double-stranded bridge. Thus, hydrogels are formed with high stiffness. Photo-isomerization of trans azobenzene to cis azobenzene is caused by the irradiation of 365 nm UV light, which causes the separation of the double helix DNA bridge, but glucosamine-boric acid ester binding remains intact. Here, the low density of cross-linked hydrogel shows low stiffness, and this photo-triggered transition was reversible (Fig. 2D) [69]. In addition, Kandatsu et al. showed light-responsive DNA hydrogels that demonstrate that under different wavelengths of UV light, the phase transition of hydrogels like gel to sol is possible. The hydrogel was synthesized by hybridizing the sticky end of an X-shaped DNA motif. An artificial base called cnvK (3-Cyanovinylcarbazole Phosphoramidite) was incorporated into this hydrogel. This artificial base interacts with thymine under UV light at about 366 nm wavelength, but this hydrogel undergoes disintegration at 340 nm wavelength and causes the reversible hybridization of the sticky ends, resulting in the phase transition in the hydrogel from gel to sol [70].

Dong et al. developed a DNA hydrogel containing MNPs (Fig. 2E) that formed structural colors in response to heating and magnetic fields [71]. The spatially controlled photonic crystal coloring was accomplished using the photolithographic technique under NIR light. Without an external magnetic field, colors were "erased" by illuminating or heating the hydrogel. Under illumination circumstances, MNPs create heat, and the hydrogel was locally melted if they migrated and aligned with the external magnetic field to form the periodic PC structure. Due to the rapid heat dissipation, the DNA strands will rehybridize once the illumination conditions are removed. This method uses illumination, heating, and magnetic fields to achieve high spatial resolution (up to 10 μm). It allows for a direct inscription of colors and patterns, quick inscription (< 1 s), and erasing (< 10 s) without the need for a mask. This method produces more stable coloring than organic dyes. This technology offers a quicker reaction time than existing PC rewritable technologies, opening a new route for the application of stimuli-sensitive DNA hydrogels [71]. In another study, Song et al. used nanoparticles like AuNPs and DOX as thermo-responsive elements in the DNA hydrogel network based on DNA assembly. Researchers used X-shaped DNA with three sticky ends to undergo enzymatic ligation for cross-linking, where one arm was disabled to control the hydrogel size. The positively charged gold nanoparticles, AuNPs, were incorporated into the DNA hydrogel by electrostatic interactions. Here, DOX gets intercalated into the hydrogel network. When the visible light of about 660 nm is irradiated on the network, gold nanoparticles and AuNPs get excited at plasmon resonance and generate a heat shock, which causes the AuNPs and DOX to dissociate in hydrogel assembly and release the AuNPs and DOX (Fig. 2F) [62].

Magnetic field-responsive DNA hydrogels

In recent years, there has been growing interest in the development of novel therapeutic approaches for cancer treatment and tissue regeneration. One promising approach involves the use of magnetic field-responsive DNA hydrogels. These hydrogels are designed to selectively target cancer cells and promote tissue regeneration through the application of external magnetic fields. This innovative technology utilizes magnetic nanoparticles embedded within the DNA hydrogel, which can be remotely controlled and manipulated using external magnets [72]. A study introduces a fresh method for creating DNA-MNP hydrogel, which possesses special characteristics and can be controlled from a distance using magnetic fields. Additionally, regarding magnetic field-responsive DNA hydrogels, a study shows that magnetic nanoparticles are DNA-modified and that modified MNPs are incorporated into DNA hydrogel to produce magnetic field-responsive DNA hydrogel. There are several steps involved in developing DNA-modified magnetic nanoparticles. First, hydrophilic MNPs, small particles with a diameter of about 20 nm, add amino groups to these MNPs by mixing them with a substance called (3-aminopropyl)-triethoxysilane (APTES). After that, researchers take *N*-maleimido-caproyl-succinimide ester (EMCS) and combine one side with the amino groups on the MNPs [30, 73, 74].

Figure 3A–C shows a soft robot made of magnetic DNA and hydrogel [3]. The ultra-long ssDNA was synthesized using RCA and contained a capture sequence capable of hybridizing with the ssDNA on the modifying magnetic nanoparticles for the second amplification. This approach resulted in a stable magnetic DNA hydrogel. By altering and restoring its structure, this

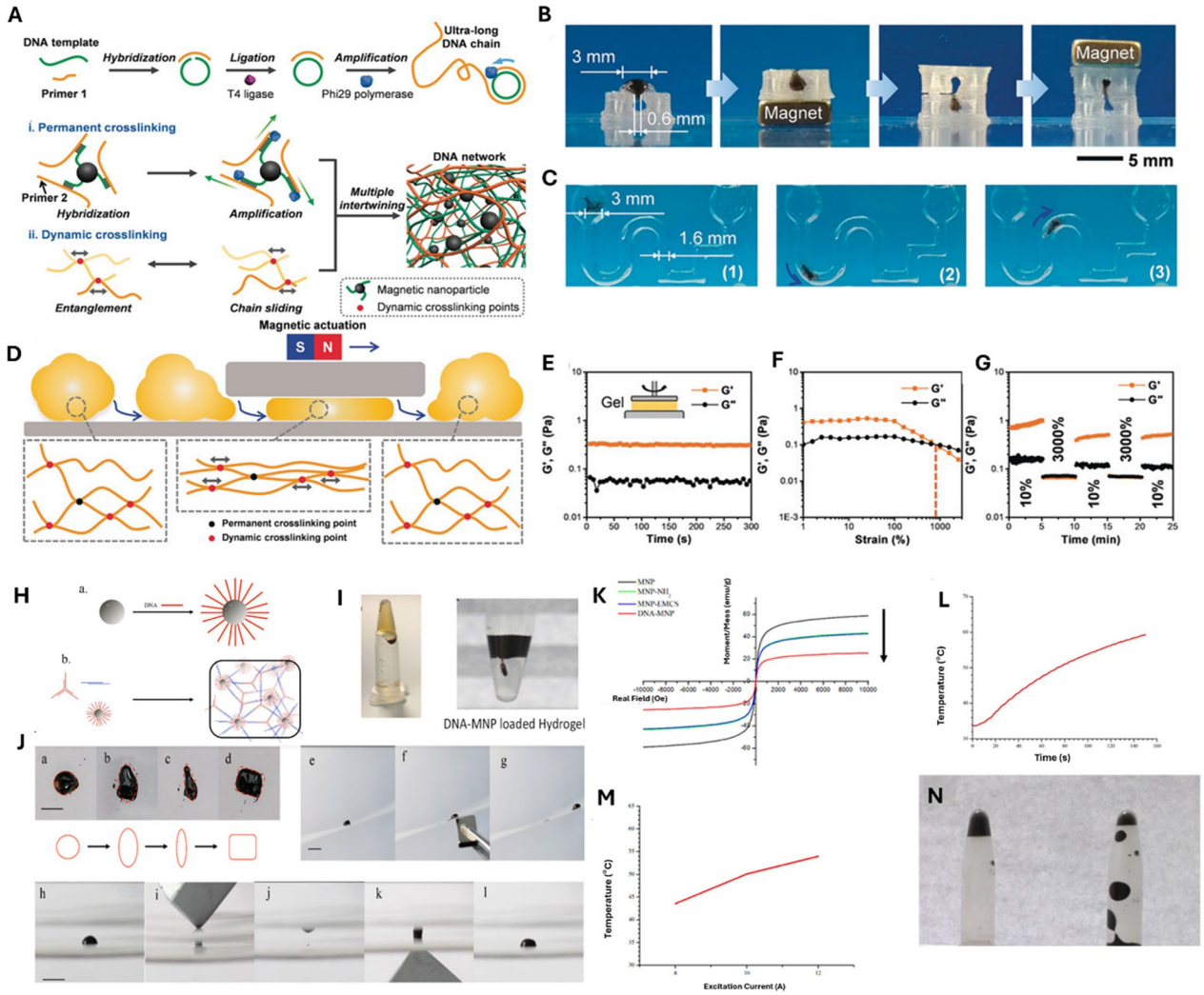


Fig. 3 Magnetic field responsive DNA hydrogel formation. **A** Schematic of magnetic driven DNA hydrogel synthesis. Enzymatic amplification of RCA to produce ultralong DNA strand products. Permanent crosslinking and dynamic crosslinking. Green strands represent the DNA chain product of secondary amplification [3]. **B**, **C** Navigational locomotion of DNA robot involving; entering rooves and (itinerating in a maze). **D** Schematic of the behavior of a robot when it strikes and passes through an obstacle [3]. **E** Time-dependent storage modulus (G') and loss modulus (G") of the hydrogel under constant strain, demonstrating its stability over time. **F** Strain-dependent rheological behavior of the hydrogel, showing its mechanical robustness under varying strain conditions. **G** Recovery behavior of the hydrogel after applying high strain, indicating its self-healing properties. Magnetic field responsive DNA hydrogel formation. **H** Diagrammatic representation of the DNA-MNP hydrogel production process. Y-scaffolds, DNA linker, and DNA-MNPs formed a three-component hydrogel after a. DNA was altered and added to the MNP surface. **I** DNA-MNPs after adding, DNA-MNP hydrogel was added on top of pure DNA hydrogel with a magnet. **J** The gel is exposed to a magnetic field, and it shows the phase transition, changing shapes, and moves around. Additionally, this magnetic field declines the effect of gravity on the gel [26]. **K-L** Rheological properties of the DNA-MNP hydrogel: **K** Shear-thinning behavior under increasing shear rate; **L** Temperature-dependent phase transition, showing gelation behavior with temperature increase. **M-N** Magnetic responsiveness of the hydrogel: **M** Temperature changes induced by magnetic excitation current; **N** Demonstration of shape transformation and movement under a magnetic field

DNA robot could navigate limited paths or a groove-like maze under magnetic actuation. It is worth mentioning that these paths were smaller than the robot, and the robot could return to its previous shape following deformation. DNA robots' accurate navigation and biocompatibility make them an intelligent carrying tool for live cells, with a potential future in diagnosis and therapy, implanted medical devices, and less invasive operations. Furthermore, researchers discovered that encapsulated multienzyme magnetic DNA hydrogels had dramatically increased enzymatic activity, cascade reaction efficiency, and stability for temperature, long-term storage, and organic solvents. In further investigations, researchers discovered that the coated multienzyme could detect

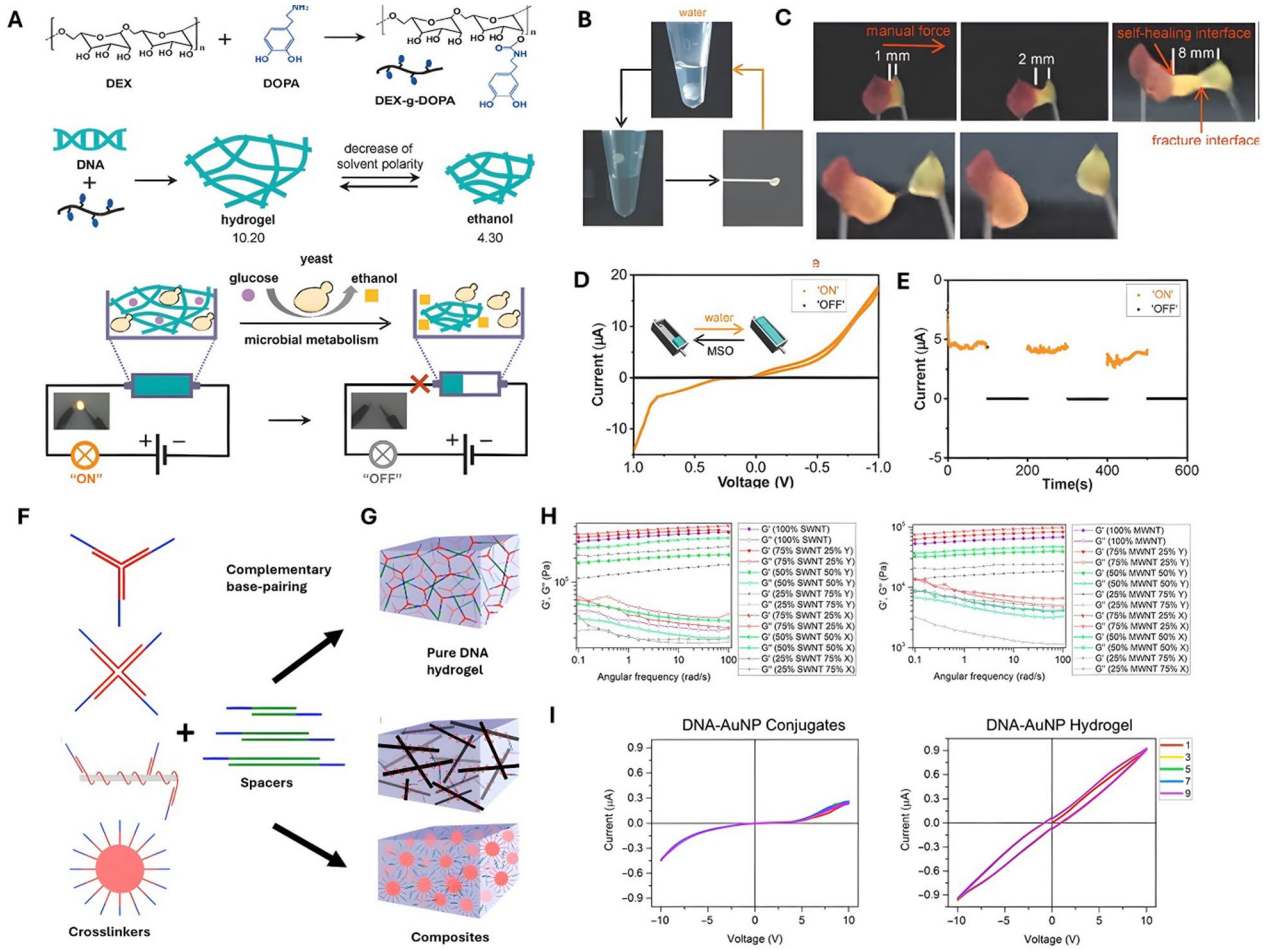


Fig. 4 Electrically responsive DNA hydrogel formation. **A** Molecular design and synthesis route of super-soft and dynamic DNA/DEX-g-DOPA hydrogel. **B** Digital photos of the hydrogel with volumetric responsiveness upon water. **C**, **D** When the hydrogel was taken out from the water, the volume of hydrogel was only one-tenth of that in water within 1 s. When soaked in water again, the hydrogel returned to its birth volume [78]. **E** Electrical (Current) response of the hydrogel under alternating "ON" and "OFF" states, showcasing its electrical responsiveness over time. **F**–**H** Schematic representation of DNA hydrogel; Y- and X-shaped DNA tiles, DNA–carbon nanotube (CNT) conjugate, and DNA–gold nanoparticle (AuNP) conjugate are crosslinkers. And Ss, Sm, and Sl spacers (33, 44, and 55 nt). Pure DNA hydrogel is made from Y- or X-shaped DNA tiles and spacers. DNA–CNT and DNA–AuNP hydrogel composites. **I** DNA–AuNP conjugates (left) vs. DNA–AuNP hydrogel (right) [84]

low-concentration glucose with high selectivity (Fig. 3E–G) [75]. Meanwhile, the other side of EMCS connects with thiolated DNA, securely attaching the DNA strands to the MNPs through a covalent bond. Furthermore, DNA hydrogel is developed by combining a DNA Y-scaffold and a DNA linker. The Y-scaffold is a branched DNA structure that can be hybridized with multiple DNA strands, while the linker is a single-stranded DNA molecule that can hybridize with the Y-scaffold and the DNA-modified MNPs. The DNA-modified MNPs are incorporated into the DNA hydrogel solution, and hybridization occurs between the DNA linker associated with the Y-scaffold and the DNA-modified MNPs. This results in the formation of a hybrid DNA-MNP hydrogel (Fig. 3H–J), where the MNPs are a precious part of the network, improving the stability of the particles in the gel (Fig. 3K, L). When the hydrogel is exposed to a magnetic field (Fig. 3M, N), it can change its shape from afar and perform different movements, such as jumping between two surfaces and climbing up a hill. By using different triggers like enzymes, temperature, and a magnetic field, this DNA-MNP hydrogel can transform from a liquid to a gel-like state in a specific way [30].

Electric field-responsive DNA hydrogels

Electrically responsive DNA hydrogels can be prepared using several methods, including enzyme-catalyzed ligation, DNA self-assembly, and electrostatic interactions. According to Li et al., DNA strands are modified with redox-active moieties, such as ferrocene or viologen,

which can undergo reversible oxidation and reduction in response to an applied electrical potential. Those modified DNA strands can be cross-linked using complementary DNA strands to form a hydrogel network that is responsive to electrical stimuli [76]. In another study, researchers used another approach involving DNA strands that are modified with conductive polymers, like polyaniline or polypyrrole, which can enhance the electrical conductivity of the hydrogel. These modified DNA strands can be cross-linked using complementary DNA strands to form a hydrogel network that is responsive to electrical stimuli [77]. Simply, making electrically responsive DNA hydrogels needs special knowledge and techniques. The process can be different based on how researchers plan to use the hydrogel and what properties they want it to have. Han et al. described a super-soft and dynamic nanofiber-assembled DNA/dopamine-grafted-dextran hydrogel made of natural long DNA chains and dopamine-grafted-dextran (DEX-g-DOPA), which demonstrated super-fast volume-responsiveness and great sensitivity to solvents with varied polarity (Fig. 4A). This hydrogel has synergistic permanent and dynamic double networks. DOPA-DOPA covalent contacts permanently crosslinked the hydrogel framework, whereas DNA and DEX-g-DOPA created dynamic associations through hydrophobic interactions, hydrogen bonds, and π-π stacking.

Because the hydrogel was so soft, changing the solvents caused the volume to alter in a matter of seconds. For instance, hydrogel responded quickly to water volume. In 1 s, the hydrogel's volume was one-tenth of that in water (Fig. 4B) [78]. In Fig. 4C, the manual force was applied to the right self-healed hydrogel. The initial green hydrogel length was 1 mm. The green hydrogel reached 8 mm without fracture at the self-healing interface when human force increased steadily. Water restored the hydrogel's volume. Hydrophobic interactions drove this largely. Hydrophobic interactions would reject water from hydrogel when removed from water. In the electric circuit that employed water and MSO as switches, DNA/DEX-g-DOPA hydrogel was used as dynamic wires because of its volumetric reactivity to change solvents (Fig. 4D). Gold nanoparticles were doped to improve hydrogel conductivity.

When MSO was introduced to the channel, the hydrogel aggregated and shrank, shutting off the electric circuit. After replacing MSO with water, the hydrogel expanded, and the electric circuit was switched on. Low solvent quantities caused the switch to the electric circuit because water and MSO have a substantial solvent polarity difference. The cyclical current–time curve (Fig. 4E) showed electric circuit stability. Triggering the electric circuit on/off kept the current value steady during the three water/MSO cycles. Switch pairs of solvent pairs with different polarities might turn the electric circuit on/off [78].

Incorporating nanomaterial conjugates changes hydrogel properties for mechanical and electrical engineering. Hydrogel-based composite mechanical properties can be improved by changing DNA tile concentrations and nanomaterial branch topologies. However, the electrochemical properties of hydrogel composites with embedded conductive nanomaterials have seldom been studied, and DNA as a 3D nanocircuit construction material must be understood. Consider carbon nanotubes. The mechanical strength and stiffness of CNTs enhance nanofiber networks [79]. Chemically stable CNTs with high aspect ratios alter electrical percolation in nanocomposites, making them suitable nanoelectronics materials [80]. Water insoluble CNTs lack surfactants or sidewall functionalization. Single-stranded DNA (ssDNA) wraps around nanotubes via strong noncovalent hydrophobic interactions between CNT walls and DNA nucleobases to form water-soluble supramolecular complexes in biomolecular dispersion [81]. These hybrids increase solubility and manageability by combining CNTs' electrical and mechanical properties with DNA's molecular recognition. CNTs may work for fast-switching, non-volatile memory. In CNT-based memory, voltage stimulates nanotube electromechanical interaction. Memristive structures made of CNTs alter electrical resistance based on current/voltage activity. DNA-functionalized gold nanoparticles and biomolecules are another traditional combination. These hybrids benefit biosensing and complicated nanostructure production. DNA–AuNP conjugates are first made via gold–thiolate attaching thiolated DNA to AuNPs [82, 83]. Polyadenine bases that interact strongly with gold surfaces have been employed to adsorb non-thiolated DNA strands onto AuNPs. Gao et al. adopted this technique for DNA-functionalized AuNP manufacturing because it covers AuNP surfaces quickly and effectively with unmodified ssDNA and has a high loading capacity [84]. Designed DNA sequences and then bottom-up fabricated macroscale hydrogels using the crosslinker plus spacer design, where oligonucleotides were sequence-directed hybridized with sticky ends on crosslinker un to form molecular networks (Fig. 4F–I) [84].

Ultrasound-responsive DNA hydrogels

Ultrasonic-responsive DNA hydrogels are a unique type of hydrogel that may undergo a reversible phase transition between a gel and a liquid state in response to ultrasonic vibrations [85]. These capabilities render them highly valuable for several applications, including medication delivery, tissue engineering, and biological

sensing. Ultrasound-responsive DNA hydrogels may be prepared using two methods: chemical cross-linking and physical cross-linking [86]. Chemical crosslinking is employed to facilitate medication release, whereas physical crosslinking is utilized for tissue regeneration. In the physical cross-linked approach, DNA strands are interconnected by the utilization of physical forces, such as ionic or hydrophobic bonds [86, 87]. Ultrasound waves disturb the forces acting on the hydrogel, leading to its collapse. A study was done where researchers performed tests to investigate the capacity of PMAA/PVPON hydrogel capsules to release G-quadruplex DNA when exposed to ultrasonic irradiation [88]. To begin, G-quadruplex DNA was synthesized using the conventional solid-phase DNA synthesis technique and subsequently purified by high-performance liquid chromatography (HPLC). Next, the PMAA/PVPON multilayer hydrogel capsule was fabricated utilizing the layer-by-layer (LBL) construction process. Subsequently, scientists introduced the G-quadruplex DNA into the capsule by immersing the capsule in a solution containing the G-quadruplex DNA. The DNA molecules exhibit electrostatic affinity towards the positively charged PAH and PMAA layers present on the surface of the capsule. The hydrogel capsule was subjected to ultrasonic stimulation to induce the release of G-quadruplex DNA [89]. The ultrasound irradiation was performed at a frequency of about 22 kHz and an intensity of 14 W/cm^2 for a duration of 60 s, with 20-s intervals between bursts. Following the exposure, the liquid portion of each capsule solution was analyzed using UV–Vis spectroscopy to quantify the concentration of G-quadruplex DNA released per capsule in the sample [88].

Pressure-responsive DNA hydrogels

DNA hydrogels sensitive to shear stress and pressure have become cutting-edge materials with enormous promise in tissue regeneration and cancer treatment [90, 91]. These hydrogels are intended to change structural and functional characteristics in response to mechanical stimuli, like pressure and shear stress, which can be used therapeutically. Cross-linked polymer networks that glide over one another under external pressure are the usual components of pressure-responsive hydrogels. With pressure stimulation, the hydrogels' mechanical reactivity enables the release of encapsulated medicinal chemicals. For example, Zhao and colleagues created a pressure-responsive adhesive hydrogel that shows promise for tissue regeneration by efficiently downregulating type I collagen production, encouraging scar fibroblasts, and reducing scar size. Reversible changes in viscosity and structure under variable shear rates are what define shear stress-responsive DNA hydrogels [92, 93]. Because of their shear-thinning behavior and viscosity drops under shear stress, hydrogels are very injectable and physiologically flexible. For instance, a study showed that the injectability and adaptability of a DNA hydrogel loaded with Apt02-modified tetrahedral framework nucleic acid (tFNA) were improved by its noticeable shear-thinning behavior. Pressure and shear-stress-responsive DNA hydrogels can be used in cancer therapy to distribute drugs locally and under control. In reaction to mechanical cues seen in the tumor microenvironment, these hydrogels can encapsulate and release chemotherapy drugs [94]. The medications' therapeutic effectiveness is increased, and this tailored release mechanism reduces systemic toxicity. Furthermore, these hydrogels offer a flexible foundation for customized cancer therapy since their mechanical characteristics may be adjusted to fit the needs of various cancer types. Pressure and shear stress-responsive DNA hydrogels have several benefits for tissue rehabilitation. Because they can react to mechanical stimuli, they are perfect for dynamic tissue settings where mechanical stresses greatly influence tissue formation and repair [95]. Through the use of mechanical stimuli, these hydrogels may be designed to release growth factors, cytokines, or other bioactive compounds, therefore stimulating cell proliferation, differentiation, and tissue healing. The injectability of DNA hydrogels, for example, is increased by their shear-thinning characteristics, enabling minimally invasive administration to target areas where they can promote tissue regeneration by offering a favorable environment for cell proliferation and matrix production [95, 96].

Lee et al. developed a pressure-responsive DNA hydrogel that uses a polymerase enzyme to extend DNA chains and interlace them noncovalently into a hydrogel. The resultant substance, referred to as a meta-hydrogel, exhibits fluid characteristics when removed from water and solid characteristics when immersed in water. Furthermore, when water is added, and the hydrogel undergoes full deformation, it may be restored to its initial shape. The meta-hydrogel possesses a hierarchical internal structure. As an illustration of its possible applications, it may be utilized to fabricate an electric circuit that employs water as a switch (Fig. 5A) [36]. The DNA hydrogel exhibited unique metaproperties, exhibiting liquid or solid properties based on its physical surroundings. When the gel was removed from the water, it became a 'liquid' that flowed freely in a tube (Fig. 5B) and conformed to differently shaped containers. Restoring it in water resulted in a solid gel. Moreover, it emphasizes that the hydrogel was a gel despite its liquid-like behavior. The substance quickly recovered to its former shape in water despite undergoing many changes throughout its liquid-like condition. To study this unique characteristic, R4M16 meta-hydrogel was made in D, N, and

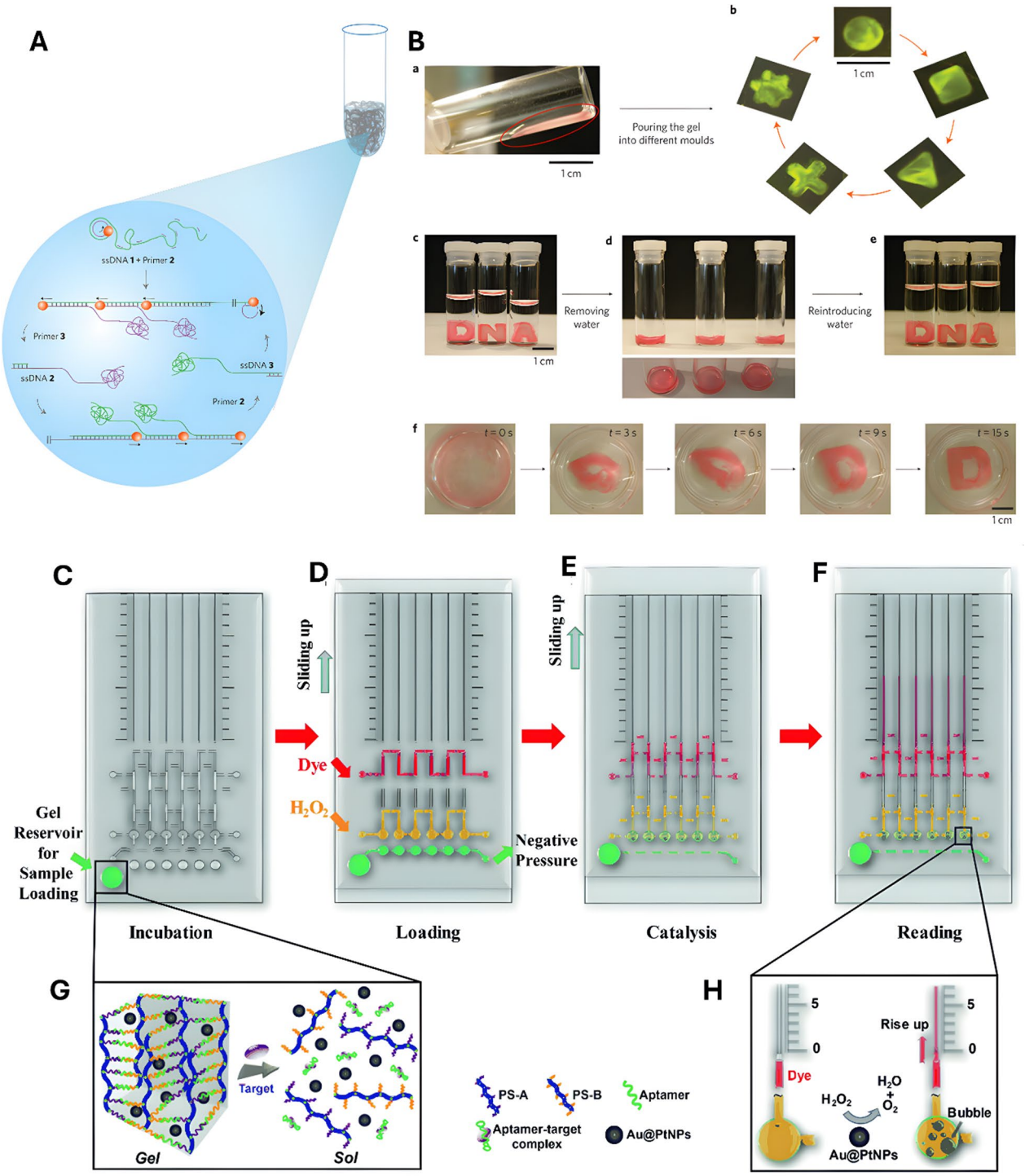


Fig. 5 Pressure-responsive DNA hydrogel formation. **A** Schematic diagram of the stepwise approach for DNA hydrogel synthesis [61]. **B** Liquid- and solid-like properties of the R4M16 hydrogel [36]. **C**–**H**) The working principle of the Au@PtNPs encapsulated target-responsive hydrogel with a volumetric bar-chart chip readout for visual quantitative detection [99]

A-shaped molds. Each hydrogel behaved as a liquid after water removal, adhering to the vial shape. After reintroducing water, the hydrogels recovered to their original morphologies (D, N, and A) within 15 s [36].

Table 1 Stimuli-responsive DNA hydrogel responds to different stimuli according to compositions, such as polymers with other materials and cross-linkers, to show different features of the hydrogel

Types of physical stimuli	Cross-linkers	Name of polymers with other materials	Features of hydrogels	Refs.
Temperature	ssDNA	Polyacrylamide chains/acrylamide monomers/DNA	Unstable, gel dissolution	[41, 52, 59]
Temperature	DNA hybridization, metal ions	DNA, Collagen, hyaluronic acid, chitosan	Biocompatible, biodegradable, tunable mechanical properties	[35, 41, 52, 59]
Temperature	Sticky ends of Y scaffold and linker DNA	DNA	Transition of gel to sol	[38]
Temperature	Enzymatic ligated sticky arm of X-shaped DNA	DNA with AuNPs(nanoparticles) and DOX(drug)	Disassembly of hydrogel and release of AuNPs and DOX	[62]
Temperature	DNA duplex structure	PPO/DNA	Changes in strength can be observed	[63]
Temperature	Complementary ssDNA sequence	Polyacrylamide/DNA with NRs nanoparticles	Transition of gel to sol	[59, 65]
Light	Azobenzene functionalized ssDNA	Polyacrylamide/DNA	Changes volume	[52, 68]
Light	Glucosamine boric acid ester and trans-azobenzene intercalated units	Polyacrylamide/DNA	Cross-linked with low density, low in stiffness, photo-triggered transition is reversible, and finally transition of gel to sol	[69]
Light	Sticky ends of X-shaped DNA motifs	DNA with light-responsive artificial base cnvK	Transition of gel to sol	[70]
Magnetic field responsive	DNA duplex structure (Y scaffold + linker)	DNA with MNPs(nanoparticles)	Transition of gel to sol	[30]
Electrical responsive	Complementary DNA strands	Ferrocene, viologen, polyaniline, polypyrrole, and DNA	Changes in hydrogel properties such as swelling, contracting	[76, 77]

Recently, the Qin group created an attractive multiplexed volumetric bar-chart chip (V-Chip) for quantitative POC diagnostics [97]. Based on Slip Chip technology, the V-Chip employs ink bar charts to show channel pressure changes due to O_2 production from catalase reacting with H_2O_2 for target measurement. V-Chip efficiently converts molecular recognition events into a quantifiable physical metric, oxygen gas volume. Two major issues must be addressed before the design can be extensively utilized in POCT [98]. Conjugating catalase with an antibody takes time and may impact protein structure and function. Second, catalase's finite lifetime in H_2O_2 substrate solution reduces test sensitivity. New signal transduction techniques and catalysts that allow easy sample processing without protein modification and efficiently convert the target recognition event into visually detectable characteristics are needed. The simple and general quantitative POCT method in Fig. 5C, D uses a target-responsive hydrogel for target recognition, Au core/Pt shell nanoparticles (Au@PtNPs) for robust yet efficient signal transduction and amplification, and a volumetric bar-chart chip for visual quantitative readout. The V-Chip gel reservoir holds the prepared gel [99]. Adding target molecules to the gel reservoir creates an aptamer-target complex, dissociating the hydrogel into a sol and releasing Au@PtNPs into the supernatant solution. The top layer of the V-Chip is manually pushed to the loading position, creating three horizontal channels. A pipette is used to provide negative pressure to the bottom connected channel (green) exit to pull supernatant into the channel through the gel reservoir's drilled hole. The V-Chip's top connected channel (red) is then loaded with red ink and the middle channel (yellow) with H2O2. To create six parallel channels in the vertical direction, the V-Chip is slid upward to separate horizontal fluidic paths and bring the supernatant containing Au@PtNPs into contact with H2O, as shown in Fig. 5E, F. Immediately, O2 is produced, and ink is forced into the upper narrower channel (Fig. 5G, H) [99]. Table 1 provides an overview of the various compositions of stimuli-responsive DNA hydrogels and their responses to different stimuli. The table highlights how the incorporation of different polymers, materials, and cross-linkers can influence the hydrogel's properties and functionalities.

DNA-cell interactions: how do physical cues regulate molecular changes?

ECM is a complex network of proteins, glycosaminoglycan, and other biomolecules that provide structural support and biochemical cues for cells in tissues and

organs [28, 100]. It provides a scaffold for cells to attach to and migrate through, and it also regulates cell behavior by presenting biochemical signals that can promote or inhibit cell proliferation, differentiation, and migration. Therefore, the ECM has become a great focus of research in tissue engineering and regenerative medicine, where the goal is to develop functional and biomimetic scaffolds to mimic the natural ECM and promote tissue regeneration [101]. During tissue regeneration, the ECM undergoes dynamic changes essential for repairing and altering damaged tissues. For instance, the ECM can become highly porous in response to injury, allowing cells to enter and migrate into the wound site. It also releases growth factors and cytokines that help stimulate cell proliferation and differentiation and provides mechanical cues that influence cell behavior. In common, ECM platforms are derived from natural sources, such as decellularized tissues or ECM proteins, or they can be synthesized using biomimetic approaches. Decellularized tissues can be obtained by separating cells from native tissues while maintaining the ECM structure and composition [102, 103]. Decellularized tissues provide a natural microenvironment for cells, with native ECM proteins and growth factors that promote cell behavior and tissue regeneration. However, these decellularized tissues may have limited mechanical properties and may not be suitable for all applications. Proteins of the ECM, such as collagen, fibronectin, and laminin, are used to develop ECM platforms [104]. These proteins are filtered from natural sources or synthesized using recombinant DNA technology. ECM proteins coat surfaces, such as cell culture plates or scaffolds, to promote cell adhesion and proliferation. ECM proteins can also be used to develop hydrogels, which provide a 3D microenvironment for cells [105]. Researchers have designed hydrogels to mimic the mechanical properties of native tissues and can be functionalized with ECM proteins or peptides to provide a natural microenvironment for cells. Concerning structural and biochemical functions, the ECM modulates the immune response during tissue regeneration.

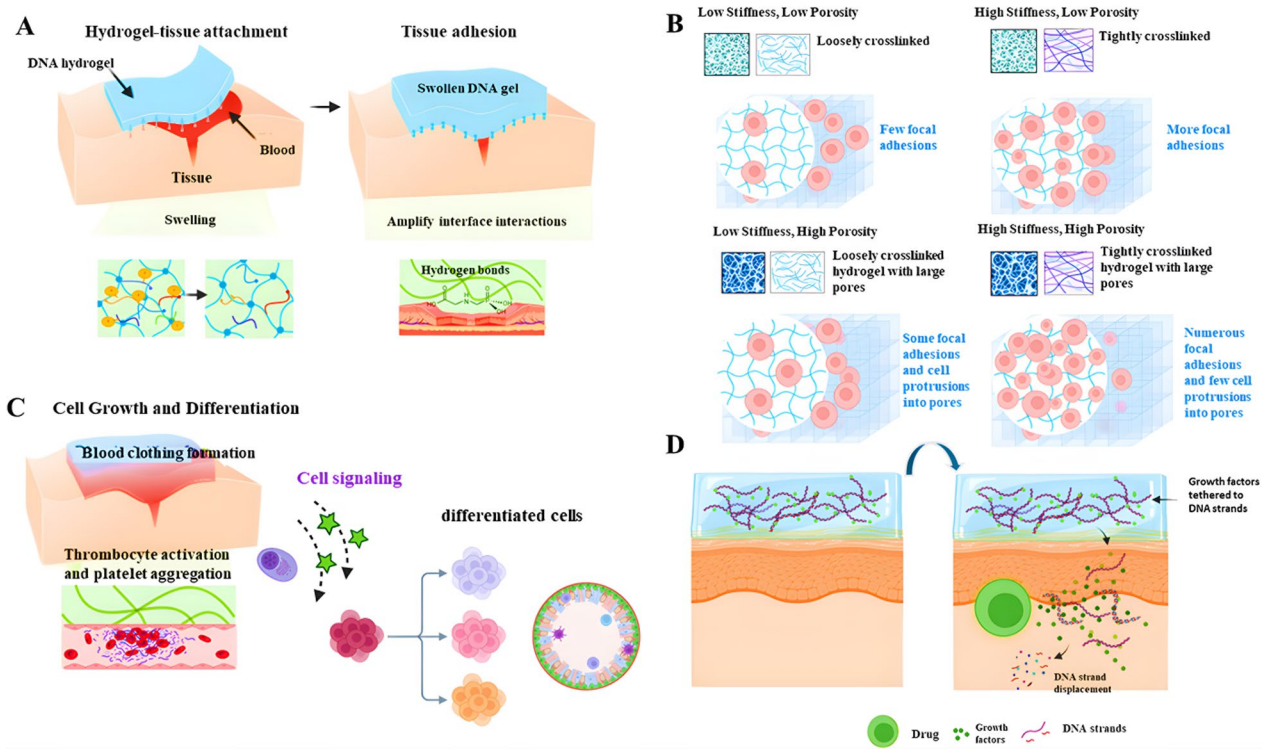


Fig. 6 Schematic representation of DNA Hydrogel Functionalization with ECM platform. **A** Represents DNA hydrogels (swollen) to enhance membrane interaction and tissue adhesion. **B** Effects of DNA Hydrogel Stiffness and Porosity on Cell Adhesion; top-left (low stiffness, low porosity): loosely crosslinked hydrogel with minimal cell spreading and weak adhesion, characterized by few focal adhesions; top-right (high stiffness, low porosity): tightly crosslinked hydrogel with increased cell spreading and stronger adhesion, featuring more focal adhesions. Bottom-left (low stiffness, high porosity): loosely crosslinked hydrogel with large pores, where cells are partially embedded, showing moderate adhesion and some protrusions into pores; bottom-right (high stiffness, high porosity): tightly crosslinked hydrogel with large pores, allowing well-spread cells with strong adhesion and numerous focal adhesions. **C**, **D** Consequence of DNA hydrogels on cellular spreading: Blood clotting formation and cell signaling lead to the differentiation of cells. DNA hydrogels stimulate endocytosis of lipid-binding ligands and release the growth factors(drug) and melting of DNA strands

It acts as a barrier to prevent the infiltration of immune cells into the wound site, and it can also release anti-inflammatory cytokines that help to resolve inflammation and promote tissue repair [106]. Approaches like Biomimetics synthesize ECM platforms that mimic the composition and structure of native ECM. For example, synthetic polymers, such as polyethylene glycol (PEG) or polylactic-co-glycolic acid (PLGA), can be functionalized with ECM peptides to promote cell adhesion and proliferation. This type of synthetic ECM platform is designed to have tunable mechanical properties, degradation rates, and bioactivity [107]. ECM platforms can also be functionalized with bioactive molecules, such as growth factors or cytokines (cytokines are a group of small proteins secreted by immune system cells and other cells responding to various stimuli, such as infection, inflammation, or injury). Cytokines act as signaling molecules that regulate the immune response and other biological processes, such as cell growth, differentiation, and apoptosis). Which enhances tissue regeneration by promoting cell proliferation, differentiation, or migration. For example, ECM platforms can be functionalized with bone morphogenetic protein (BMP) to promote bone regeneration or vascular endothelial growth factor (VEGF) to promote angiogenesis. All in all, we can say that physical stimuli-responsive DNA hydrogels represent a promising platform for creating multifunctional ECM mimics that can provide structural support, biochemical cues, and spatiotemporal control over the cellular microenvironment. By combining these different functions into a single platform, it is possible to create synthetic ECM mimics that can promote tissue regeneration in a variety of contexts like wound healing, bone tissue regeneration, cardiac tissue regeneration, and so on [4, 13, 28].

Functionalization for tailorable bioadhesion

Cell-material interactions play a crucial role in the effectiveness of DNA hydrogels as scaffolds for tissue regeneration. These interactions influence various cellular processes, including adhesion, proliferation, differentiation, and migration, which are essential for successful tissue formation and repair. DNA hydrogels offer unique advantages for cell-material interactions due to their programmability, biocompatibility, and ability to mimic ECM. The nanoscale architecture of DNA hydrogels can be precisely controlled, allowing for the creation of scaffolds that closely resemble the native tissue environment (Fig. 6A) [13, 108, 109]. These interactions between cells and DNA hydrogels are essential for creating functional tissue constructs and promoting tissue regeneration. By tailoring the properties of DNA hydrogels and incorporating bioactive molecules, researchers can develop advanced biomaterials that support cell growth, differentiation, and tissue repair [110]. Cell adhesion and endocytosis were investigated regarding DNA hydrogels. Particularly, X-shaped hydrogel (HGX) improved cell adhesion and spreading by increasing cell area and reorganizing the actin cytoskeleton of the cells. The larger cell size of these hydrogels also increased the absorption of endocytic ligands, such as Cholera toxin B-subunit, Transferrin, and Gal3. Higher concentrations reduced proliferation, whereas HGX hydrogels effectively facilitated cell migration and proliferation in 3D spheroid models between 25 and 50 μM concentrations [110].

One of the key aspects of cell-material interactions in DNA hydrogels is cell adhesion. DNA hydrogels can be functionalized with cell-adhesive peptides such as RGD (arginine-glycine-aspartic acid) to enhance cell attachment. These peptides mimic the binding sites found in natural ECM proteins, promoting cell adhesion and subsequent cellular processes [111]. For example, Le Bao et al. demonstrated that the co-presentation of cell-adhesive peptides (YISGR sequence) and angiogenic functional motifs (VEGF) on DNA hydrogels resulted in enhanced pro-angiogenic properties. The mechanical properties of DNA hydrogels significantly influence cell behavior [112, 113]. By altering the crosslinking density or using dynamic crosslinks, such as i-motif structures, the stiffness, and viscoelasticity of the hydrogels can be tuned to match the target tissue. This mechanical matching is crucial for directing stem cell differentiation and promoting tissue-specific cell functions. DNA hydrogels can also be designed to incorporate bioactive molecules, such as growth factors or small-molecule drugs, which can be released in a controlled manner to guide cell behavior. For instance, encapsulating bone morphogenetic protein-2 (BMP-2) in DNA hydrogels has been shown to enhance the osteogenic differentiation of mesenchymal stem cells for bone tissue engineering applications [114, 115]. The degradation characteristics of DNA hydrogels are another important factor in cell-material interactions. By incorporating enzyme-cleavable sequences, the hydrogels can be designed to degrade in response to cell-secreted enzymes, allowing for cell-mediated remodeling of the scaffold. This dynamic interaction between cells and the scaffold material is crucial for promoting natural tissue ingrowth and integration. Recent studies have also explored the use of aptamer-functionalized DNA hydrogels for selective cell capture and release [116]. This approach allows for the creation of smart scaffolds that can respond to specific cellular cues, enabling more precise control over tissue formation.

Tunable multifunctionality

Discussing the tunability properties of ECM, such as their stiffness, composition, and structure, plays an important role in their interactions with cells for tissue regeneration. Tunability refers to the ability to adjust, or we can say fine-tune, the properties of ECM (like length, stiffness, and composition) to have or achieve desired outcomes regarding cell behaviors and functions. Figure 6B illustrates how variations in DNA hydrogel stiffness and porosity affect cell adhesion. In the low stiffness, low porosity condition (top-left), cells are scattered with minimal clustering, indicating weak adhesion due to insufficient mechanical support and limited surface area for cell attachment. The high stiffness, low porosity scenario (top-right) shows cells more widely distributed, reflecting increased cell spreading and stronger adhesion. This is likely due to the enhanced mechanical cues provided by the stiffer hydrogel, which promotes focal adhesion formation [117, 118]. In the low stiffness, high porosity condition (bottom-left), cells exhibit moderate distribution, suggesting partial embedment within the larger pores and moderate adhesion. The increased porosity allows for some cell protrusions into the hydrogel, facilitating interaction but not as robustly as stiffer matrices. Lastly, the high stiffness, high porosity scenario (bottom-right) displays well-spread cells with strong adhesion and numerous focal adhesions. The combination of mechanical strength and ample pore space supports optimal cell-material interactions, which are crucial for applications in tissue engineering where both structural integrity and nutrient transport are essential [119–121]. This has been made possible by the use of DNA in the artificial ECM, which allows for structural tunability through the engineering of single-stranded domains [122]. Here, the tunability properties of ECM are used to direct cell behavior and functions in several ways, like the stiffness of the ECM, which can affect the differentiation of stem cells into specific cell types, such as bone or muscle cells. The composition of ECM also affects cell behavior, as different ECM proteins can provide different signaling cues that regulate cell regulation, migration, and proliferation.

Furthermore, the dynamic nature of the ECM causes changes in the cellular microenvironment. For example, during tissue repair following an injury, a fibrin and fibronectin-rich provisional matrix deposition offers a temporary scaffold for cell migration and proliferation [123]. Later, the ECM is modified to restore tissue function, resulting in excessive collagen secretion and, ultimately, fibrosis. The ECM environment determines whether tissue is healthy or diseased; more precisely, there is a dynamic interplay involving matrix composition, biochemical molecules (such as growth factors and cytokines), and matrix mechanics, all of which influence cellular activity [28].

Understanding how the use of DNA in the ECM structure can lead to tunable properties, specifically in terms of buffering roles in rheological or stress-endurable, biocompatible aspects, and how DNA hydrogel can adapt to physical stimuli conditions are mentioned here by dividing into the following:

A. The use of DNA in the ECM structure allows for structural tunability via single-stranded domain engineering. By including these domains in the ECM's DNA parts, the scaffold's persistence length and stiffness can be fine-tuned, affecting the outcome of cells' cytoskeletal arrangement and overall shape and the localization of intracellular transcription factors [122].
B. In hydrogel structures, DNA allows for self-assembly, which can be used to create a range of different ECM structures with varying properties. For example, DNA hydrogel can be engineered to self-assemble into specific shapes, such as tubes or spheres, which can be used to create artificial tissues or organs. The use of self-assembling hydrogels in tissue engineering can be particularly useful for creating scaffolds that mimic the structure and function of natural tissues [57].
C. The tunable ECM with DNA hydrogel can interact with cells through shape-memory effects by incorporating the DNA into hydrogels, making it possible to create shape-memory hydrogel. Changes in shape are obtained in response to physical stimuli. As an example, DNA hydrogels can be engineered to change their shape in response to changes in temperature, allowing them to be used in various biomedical applications. Particularly, the use of shape-memory hydrogels in tissue engineering is for creating scaffolds that adapt to the changing needs of the tissue [12].
D. Furthermore, the tunable properties of ECM structure with DNA hydrogel can also provide buffering roles in rheological or stress-endurable biocompatible aspects. The stiffness and composition of the ECM affect cell behavior, as different ECM proteins can provide different signaling cues that regulate cell adhesion, migration, and proliferation. By fine-tuning the properties of ECM with DNA hydrogel, it is possible to create highly biocompatible scaffolds that can withstand the stresses of the surrounding tissues [57].

These tunable properties of ECM structure with DNA hydrogel can be adapted under physical stimuli

conditions through self-assembly and engineering single-stranded domains. These properties make DNA hydrogel a promising approach for tissue regeneration in biomedical fields [124].

Enhancing cell growth and differentiation

DNA hydrogels and ECM-based platforms are two promising approaches for multifunctional tissue regeneration. DNA hydrogels are a special type of gel that is composed of DNA molecules that are self-assembled in a 3D pattern using complementary base pairs. These three-dimensional structures are multilayered scaffolds with precise cell patterns, which allow for functional tissue constructs. These gels have a unique feature where their structure and tunable properties can be precisely adjusted. They can react to different physical stimuli like temperature, pH, light, electric fields, and magnetic fields, which can cause changes in their mechanical properties or release helpful bioactive molecules. This ability to respond can be utilized to manage how cells behave, and tissues grow, like cell proliferation, differentiation, or migration. On the other hand, ECM-based platforms are designed to mimic the structure and function of the natural ECM. These platforms can be created to release bioactive molecules that encourage cell adhesion, proliferation, and differentiation. They can also support the growth and differentiation of different cell types, making them perfect for tissue regeneration with multiple functions (Fig. 6C) [31, 125]. Furthermore, ECM-based platforms can be engineered to help form new blood vessels and decrease scar formation, which is important for tissue regeneration. DNA hydrogels are biocompatible because they get along well with living things. DNA is a tiny natural biomolecule found in all living beings, and these DNA hydrogels don't have any toxicity for our bodies or cause any immune responses. These DNA hydrogels have different mechanical properties, like being stiffer, depending on tailoring and the application part of the body. The DNA hydrogels can now be functionalized with ECM proteins or peptides to provide a natural microenvironment for cells. DNA hydrogels functionalized with collagen or fibronectin mimic the composition and structure of native tissues, can enhance cell adhesion, migration, and differentiation, and promote tissue regeneration. In addition, DNA hydrogels are excellent in terms of biocompatibility and biodegradability, with designable mechanical properties and comparable permeability to ECM. They have successfully made artificial ECM engineering techniques such as cell culture, cell encapsulation, and tissue regeneration possible. DNA hydrogels have been used to engineer various tissues, such as skin, bone, neural, cardiac, and blood vessels, etc., and have shown promising results in preclinical studies. DNA hydrogels have an advantage as a base for cells, which is ECM because they can be programmed [126]. This means we can design them to react to physical stimuli like temperature and light, causing changes in how they work or releasing the bioactive molecules. This ability allows us to control cell behavior and how tissues develop, like cell proliferation, differentiation, and migration. For instance, we can create DNA hydrogels that release growth factors or cytokines when specific triggers occur, helping tissues regenerate better. By combining these DNA hydrogels and ECM-based platforms, researchers successfully made multifunctional tissue constructs that can perform multiple functions, such as promoting cell adhesion, proliferation, and differentiation. They also promote the formation of new blood vessels and reduce scar formation. These constructs can be used to repair complex tissue injuries and diseases, such as musculoskeletal injuries and cardiovascular disease. In this way, we can say that the combination of DNA hydrogels and ECM-based platforms presents a promising approach for multifunctional tissue regeneration [23].

Functionalization strategies

Functionalizing DNA hydrogels involves modifying their structure and properties to meet specific biomedical applications [39, 127]. The unique properties of DNA, such as programmable addressability through Watson–Crick base pairing, make it an ideal candidate for creating customizable hydrogels. DNA hydrogels can be classified into hybrid and pure forms. Hybrid DNA hydrogels incorporate synthetic polymers with DNA primarily serving as the crosslinking agent, while pure DNA hydrogels are composed solely of DNA [13, 22].

Several functionalization strategies have been developed to enhance the properties of DNA hydrogels:

1. *Strand Design and Sequence Recognition*: By designing specific DNA sequences, researchers can create hydrogels with tailored properties. For example, the Tan group developed adenosine-responsive hydrogels using aptamers and polyacrylamide, while the Willner group created pH-responsive DNA-based hydrogel microcapsules by modifying polyacrylamide chains with predesigned DNA sequences [128, 129]. Additionally, the branched DNA monomers (BDM) used to form the hydrogel network can be designed with specific sequences and structures to control properties like pore size, mechanical strength, and degradation rate [37]. Therefore, bioactive molecules like growth factors or cell-adhesion peptides can be incorporated directly into the DNA sequences or attached post-gelation to enhance cell–matrix interactions. The enzyme-catalyzed assembly process

allows for in situ encapsulation of cells and drugs under physiological conditions [37]. Furthermore, Y-shaped DNA monomers can produce hydrogels with a fibrous internal structure, as shown. These fibrous networks exhibit fractal-like branching patterns, which could provide an ideal scaffold architecture for cell growth and tissue engineering applications. The ability to control the internal structure through monomer design allows for tailoring of the hydrogel's physical and mechanical properties to match specific tissue types or cellular environments [125].

Enzyme Cleavage: Enzymes can be used to introduce responsiveness to environmental changes. For instance, a study developed a reversible mechanical strength DNA hydrogel by incorporating thermally responsive units into the DNA network [52, 59, 130–132].

2. *Temperature-dependent DNA supramolecule*: Yan et al. employed DNA supramolecular hydrogel as a carrier for BMSCs to treat osteoarthritis (OA) in a severe rabbit model [133]. The hydrogel's protective effects on delivered cells, both in vitro and in vivo, have been investigated, and it was found that it significantly improved BMSC vitality and provided antifriction protection, 3D support, and an optimal microenvironment compared to current clinical strategies at 37 °C. They also validated the molecular mechanism and identified activated pathways related to cartilage development and regeneration, offering insights into potential innovative clinical treatments for OA using MSCs therapy [133]. Additionally, Tang et al. proposed that the DNA hydrogel–based bio-separation system is promising as a powerful biotechnology that will promote the development of extracellular vesicles in nanobiomedicine. The detection mechanism uses molecular beacons (MB) and single probes (SP) integrated into the DNA hydrogel. When exosomes are captured, the MB is displaced and hybridizes with miRNA inside exosomes, generating a fluorescence signal. A matrix showing 100% accuracy in classifying breast cancer patients versus healthy donors based on the DNA hydrogel exosome detection method**.** DNA hydrogel could potentially be modified with photosensitive components to enable controlled release or signal generation upon light exposure, further expanding its capabilities for exosome analysis and manipulation [134].
3. *Chemical and Physical Crosslinking*: DNA hydrogels can be formed using permanent covalent bonds (chemical crosslinking) or noncovalent interactions (physical crosslinking), such as hydrogen bonding and metal–ligand coordination. Chemical crosslinking enhances mechanical strength and environmental resilience, while physical crosslinking provides dynamic and flexible properties [135–138].

These strategies enable the creation of DNA hydrogels with specific mechanical properties, environmental responsiveness, and functional capabilities, making them suitable for various tissue engineering applications.

Incorporation of bioactive molecules

Incorporating bioactive molecules into DNA hydrogels is crucial for enhancing their functionality and promoting tissue regeneration. Bioactive molecules can provide biochemical cues that influence cell behavior, such as proliferation, migration, and differentiation. Figure 6D illustrates the controlled release mechanisms of growth factors from DNA hydrogels, a crucial aspect of regulating cellular behavior in tissue engineering applications. The growth factors tethered to DNA strands within the hydrogel structure showcase two primary release strategies. The first mechanism involves enzymatic cleavage of specific DNA sequences, allowing for targeted release in response to cellular activity or environmental cues [139, 140]. The second method demonstrates DNA strand displacement, where complementary oligonucleotides trigger the release of growth factors. This dual-release system enables precise temporal and spatial control over growth factor delivery, potentially enhancing the efficacy of tissue regeneration strategies by mimicking the dynamic nature of the natural extracellular matrix [141, 142].

1. *Aptamers and responsive units*: DNA aptamers can be integrated into hydrogels to facilitate cell recognition and binding. For example, the Fan group used ATP-responsive DNA hydrogels to capture and release circulating tumor cells (CTCs) by incorporating aptamer-triggered hybridization chain reaction (HCR) units [143, 144].
2. *Growth factors and proteins*: DNA hydrogels can be modified to include growth factors and proteins that promote cell growth and tissue repair. For instance, the incorporation of fibronectin into DNA hydrogels has been shown to enhance neural stem cell (NSC) attachment and differentiation [137].
3. *Enzyme-responsive elements*: Incorporating enzyme-responsive elements allows for controlled degradation and release of encapsulated cells or molecules. For example, DNA hydrogels with i-motif sequences can respond to pH changes, enabling the release of gold nanoparticles or other cargo [145].

By incorporating bioactive molecules, DNA hydrogels can mimic the natural ECM and provide the necessary biochemical signals for tissue regeneration. The interaction between cells and DNA hydrogels is a critical factor in tissue engineering. DNA hydrogels can provide a supportive environment for cell encapsulation, culture, and tissue formation [146]. DNA hydrogels can encapsulate living cells, providing a biocompatible environment that supports cell viability and function. For example, the Yang group successfully captured and released bone marrow mesenchymal stem cells (BMSCs) using DNA hydrogels constructed with a double rolling circle amplification (RCA) technique [36].

DNA hydrogels can mimic the natural ECM by providing a permeable environment that allows for nutrient exchange and cell growth. Jin et al. demonstrated the use of DNA hydrogels to create single-cell envelopes in PDMS microwells, achieving high cell survival rates [130]. These DNA hydrogels can be functionalized with specific ligands to promote selective cell adhesion. For instance, the Mark group showed selective cell adhesion on DNA hydrogels by incorporating cyclic peptide c(RGDfK), which binds to integrins in cancerous cells [132]. Using ssDNA and temperature-stimulated DNA hydrogel application in in vitro also observed that these scaffolds not only serve as cushions for the connected cells but also promote cell spreading and lipid-mediated endocytosis, which in turn enhances cell invasion in a 3D matrix. The initial DNA nanostar models were constructed using AMBERTOOLs based on experimental designs. At high temperatures, the systems behave as unstructured fluids, but as the temperature decreases, nanostars associate through sticky ends to form a polymeric network, altering the system's dynamics and viscosity. The fraction of bonded nanostars, calculated by dividing the number of connected pairs by the total number, follows a two-state model influenced by enthalpy and entropy changes. Radial distribution functions and mean-squared displacement analyses reveal structural and dynamic changes, with diffusion constants decreasing as temperature drops. X-shaped nanostars exhibit slightly lower diffusion due to higher volume fraction, limiting phase space for diffusion [110].

Biomedical applications

Tissue regeneration, the dream of regrowing lost or damaged tissue, is closer than ever with the emergence of physical stimuli-responsive DNA hydrogels. Imagine a delicate mesh woven from DNA strands, cradling water molecules within its intricate web. These aren't just any hydrogels; they're tiny bioengineers programmed to react to changes in their environment like temperature, light, or pressure. This responsiveness makes them ideal for tissue regeneration because they can adapt to the specific needs of each healing process. Think of them as tiny scaffolds that hold cells in place and guide their growth. They can be loaded with growth factors, releasing them in a controlled manner to stimulate cell proliferation and differentiation. Need a stiffer structure to mimic bone? These hydrogels can adjust their mechanical properties on cue and deploy anti-scarring agents to keep the healing process on track. Different stimuli trigger different responses. Temperature-responsive hydrogels are liquid at room temperature for easy application and solidify at body temperature, providing a stable and supportive environment for cells. Light-responsive ones? Imagine shining a specific light to activate growth factor release or guide cell migration with laser-like precision. Even electrical stimulation is possible, mimicking the natural signals orchestrating tissue growth.

Cancer therapy

Stimuli-responsive DNA hydrogels have demonstrated considerable potential in the field of cancer and tumor treatment, specifically in response to physical stimuli. These hydrogels are specifically engineered to undergo alterations in their structure and function when exposed to external physical stimuli such as temperature, light, magnetic fields, and ultrasound. This characteristic makes them extremely adaptable for precise drug delivery and therapeutic purposes.

Physical stimuli-responsive DNA hydrogels have a key application in cancer therapy, namely in photothermal and photodynamic therapy (PTT and PDT). These treatments utilize light to stimulate hydrogel, which can be infused with photosensitizers or photothermal agents. When the hydrogel is exposed to light, it releases these substances, which subsequently produce reactive oxygen species (ROS) or heat to destroy cancer cells. An example of this is the creation of DNA hydrogels that incorporate black phosphorus quantum dots (BPQDs) to increase the responsiveness of tumor cells to PTT and PDT. These hydrogels enhance the ability of therapeutic molecules to enter and move within tumor cells, leading to improved treatment outcomes for solid tumors [147]. A study developed a DNA hydrogel that would undergo natural degradation over some time, especially for the use of photothermal immunotherapy. The process makes the DNA CpG hydrogel of rolling-circle amplification. Subsequently infused, the hydrogel was infused with bis-(3′–5′)-cyclic dimeric guanosine monophosphate (G/DH) and proceeded to coat the formulation with melanin (Mel/G/DH). Mel/G/DH exhibited an increase in temperature upon exposure to NIR light [148]. As a result, the combination of Mel/G/DH and NIR (808 nm) radiation caused the calreticulin protein to be exposed

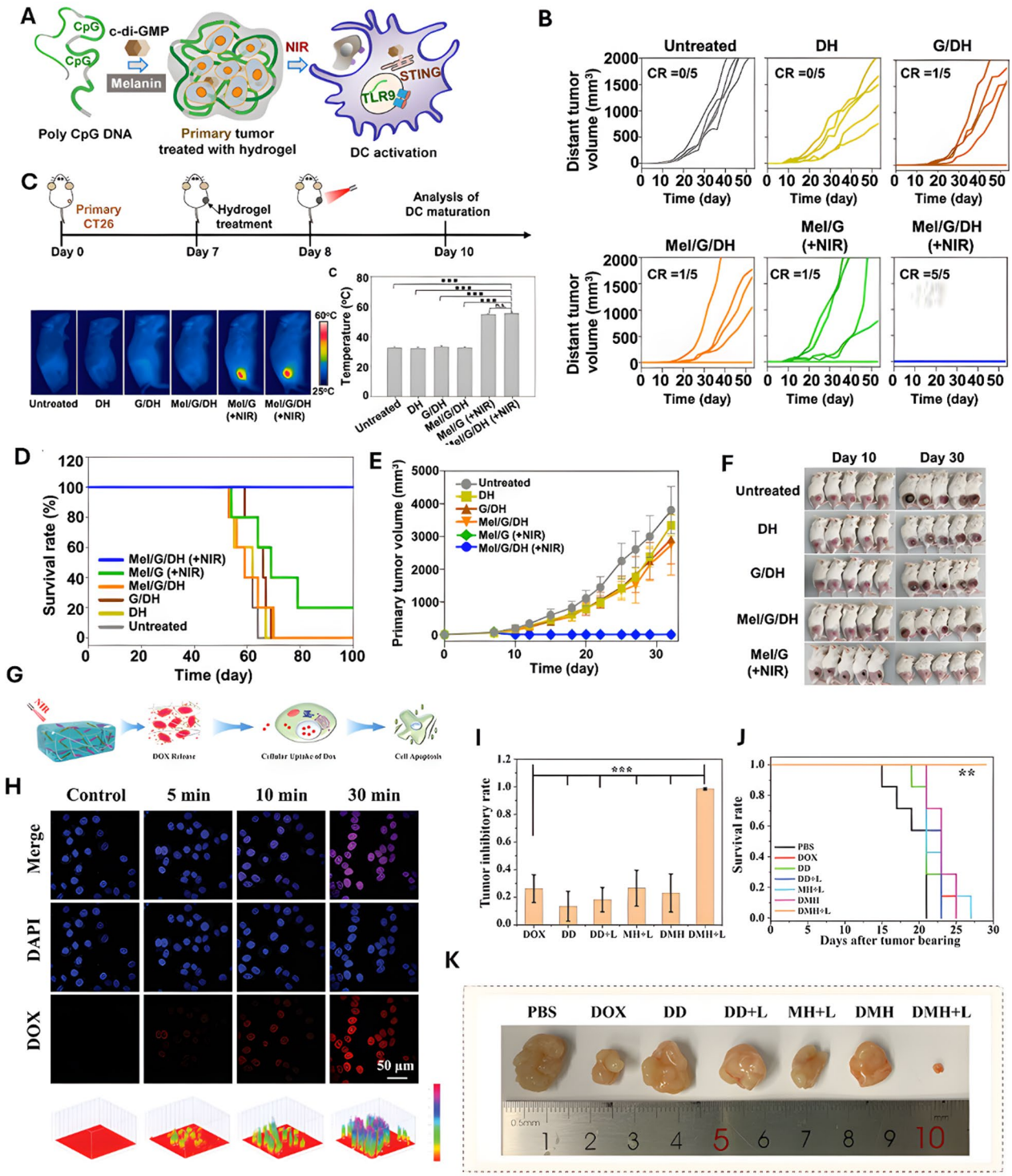


Fig. 7 Physical stimuli-responsive DNA hydrogel in the application of cancer therapy. **A** Action mechanism of melanin-loaded DNA adjuvant hydrogel. **B**, **C** Thermal images and temperature at the tumor site at 5 min post-irradiation, as recorded by an infrared thermal camera (***$p < 0.001$, n.s., not significant) [149]. **D** Survival rate of mice monitored for 100 days. **E** Volumes of primary tumors, measured until day 32 after primary tumor inoculation. **F** The appearance of mice on day 10 and day 30 after primary tumor inoculation. **G** Schematic diagram of the NIR irradiation-triggered DOX release from the DMH hydrogel [66]. **H** CLSM images. **I** The tumor inhibitory rate of HeLa tumor-bearing mice upon different treatments. **J** The survival rate curve of HeLa tumor-bearing mice with days under different treatments. (**$p < 0.01$, ***$p < 0.001$). **K** Photographs of tumor scarified from HeLa tumor-bearing mice under different treatments [66]

to CT26 cancer cells. Consequently, the exposure led to a significant stimulation of dendritic cell (DC) development. Mel/G/DH (+NIR) applied in vivo effectively eradicates primary tumors and promotes DC maturation in lymph nodes. Primary cancer therapy with Mel/G/DH (+NIR) inhibited subsequent tumor growth in another place. Mel/G/DH (+NIR) mice achieved a 100% survival rate, far higher than Mel/G mice. Mel/G/DH (+NIR) treatment affects distant tumor immune microenvironments significantly. Thus, cytotoxic T cells proliferated, and Treg cells decreased, altering the immune response. This study shows that Mel/G/DH can influence the cancer immunological milieu, reducing distant tumor recurrence [149].

Another remarkable application is temperature-responsive DNA hydrogels. These hydrogels may be designed to release their therapeutic payload at precise temperatures, making them very valuable in hyperthermia treatment. Hyperthermia is the process of increasing the temperature of tumor tissues to improve the effectiveness of other therapies, such as chemotherapy and radiation. DNA hydrogels may be engineered to release medications specifically in the tumor microenvironment, triggered by the increased temperatures, so assuring targeted delivery of therapeutic agents [149]. The possibility of DNA hydrogels that respond to magnetic fields for use in cancer treatment. External magnetic fields may be used to alter these hydrogels, enabling precise targeting of specific tumor areas and facilitating controlled and localized medication administration is being investigated. This method reduces the systemic adverse effects commonly linked to traditional chemotherapy and improves the effectiveness of the treatment by focusing it directly on the tumor site [150].

Ultrasound-responsive DNA hydrogels are an example of novel use. Ultrasound waves can activate these hydrogels to release their therapeutic payload. Ultrasound can permeate deep tissues, making it a highly effective stimulus for the release of drugs in tumors that are difficult to access. This technique enables the administration of drugs in a precise and non-intrusive manner, leading to enhanced therapy results for patients with tumors located deep within the body. Physical stimuli-responsive DNA hydrogels provide a versatile method for cancer and tumor treatment. Using external physical stimuli, these hydrogels provide precise and efficient administration of medicinal substances, hence augmenting the efficacy of cancer therapies while mitigating adverse reactions. Continuous research and development in this domain have significant potential for increasing cancer treatment and enhancing patient results. Wu et al. developed a biodegradable DNA CpG hydrogel (DH, created by rolling-circle amplification), filled it with bis-(3′−5′)-cyclic dimeric guanosine monophosphate (G/DH), and coated it with melanin. Mel/G/DH heated under NIR stimulation (Fig. 7A) [149]. NIR irradiation and Mel/G/DH hydrogel treatment removed primary tumors and inhibited distant tumor development. In CT26, intratumorally Mel/G/DH injection and NIR irradiation activated DC in tumor-draining lymph nodes (Fig. 7B). However, Mel/DH (+NIR), Mel/G (+NIR), and Mel/G/DH (+NIR) considerably varied in their capacity to prevent distant tumor growth: 20% of mice in the Mel/DH and Mel/G groups stopped rechallenged tumor growth, whereas 100% of animals in the Mel/G/DH group did. After 5 min of NIR irradiation, tumors treated with Mel/G and Mel/G/DH hydrogel rose from 32.2 ± 1.2 °C to 54.68 ± 0.7 °C and 55.24 ± 1.1 °C, respectively (Fig. 7C). After 32 days, mice treated with Mel/DH, Mel/G, or Mel/G/DH followed by NIR had no primary tumor growth (Fig. 7D–F). At 100 days, mice treated with Mel/G/DH, G/DH, and DH had no survival. Mel/G/DH (+NIR)-treated mice survived 100 days following the original tumor injection [149].

A study developed a thermo-responsive DNA hydrogel platform for photothermal-chemo synergistic treatment [66]. By programming the DNA unit sequence, a DNA hydrogel created by crosslinking polymer chains with duplex DNA structures can have good and predictable thermal-responsive characteristics. The Ti3C2TX-based MXene (MX), with excellent photothermal conversion efficiency and good biocompatibility, and DOX, an anti-cancer drug, were integrated to develop the DNA hydrogel-based photothermal-chemo synergistic therapy. MXene nanosheets efficiently convert NIR irradiation into heat, which disintegrates DNA duplex crosslinking structures in the hydrogel matrix and releases chemotherapeutic agents locally, damages tumor tissues. The DMH hydrogel was tested in vitro for cancer treatment at the cellular level (Fig. 7G). CLSM imaging showed HeLa cell DOX uptake after different treatments, staining the nucleus with 4′,6-diamidino-2-phenylindole. According to Fig. 7H, HeLa cancer cells showed a weak red fluorescent signal of DOX after incubation with DMH hydrogel after 808 nm NIR irradiation (1.44 W cm^{-2}) for 5 min. However, extending the irradiation time to 30 min led to significantly enhanced signals. The tumor inhibitory rate indicates that DMH + L therapy inhibited tumor development well (Fig. 7I). While the same amount of DOX injected into mice caused tumor shrinkage, the DMH + L treatment eliminated the primary tumors upon dissection and significantly improved mouse survival compared to other control groups. The synergistic therapeutic effect of direct photothermal tissue damage, efficient localized DOX delivery, and enhanced tumor tissue DOX uptake under mild hyperthermia temperature upon

NIR irradiation may explain the significantly enhanced therapeutic effect of DMH + L treatment compared to other control groups, including the injection of an equal amount of DOX(Fig. 7J, K) [66].

Bone tissue regeneration

Our bones have a special material around them, ECM, which is made of different stuff like inorganic hydroxyapatite, organic constituents like type I collagen, water, lipids, and proteins like growth factors. When our bones have issues, like fractures, we need treatments to help them heal [110]. In the past, we used methods like using our bone (autografts), using bone from others (allografts), or using bone from different species (xenografts). However, these methods have problems like infection risk, donor site issues, and complications with tissue union and immune response. To solve these problems, scientists have been working on bone tissue engineering. They use certain materials and factors to help bones heal better and faster. One material they use is hydrogels, which act like bone ECM and deliver growth factors, as well as other substances that aid in healing the process [151]. The ideal hydrogel for bone regeneration should have specific properties like promoting bone growth, being compatible with bone tissue, and not causing inflammation. Mechanical strength is also important for bone hydrogel scaffolds, as they need to withstand the pressure and stress in the bones. Some new hydrogels have been developed with a strong double-network structure that can self-heal. Scientists have also added nanoparticles and synthetic and natural polymers to enhance hydrogel properties. Creating the right pore size and interconnected porosity in hydrogels is crucial, as it helps control drug release and allows for the exchange of oxygen and nutrients. Scientists are exploring natural and synthetic hydrogels that can be injected or 3D printed, as they have a high potential for self-healing. Crosslinking the hydrogel is important in improving its mechanical properties and stability. Researchers are using various polymer sources and crosslinking technologies to construct bone tissue engineering hydrogels, each with its advantages and limitations for bone repair [152].

Low bone mass disease osteoporosis causes bone tissue degeneration and high bone fracture rates [153]. Osteoporosis rates rise with age, and systemic therapies can have serious negative effects [153]. The selective targeting of bone-resorbing osteoclast cells by Rho-inhibiting C3 toxins causes brittle bones and increased fracture risk. Local administration and release of the C3 protein toxin from a polypeptide-DNA hybrid hydrogel appears promising for spatial and selective regulation of bone-degrading osteoclast cells [154]. A polypeptide backbone derived from human serum albumin with grafted ssDNA sequences and dendritic DNA with four sticky ends, two of which are complementary to the grafted sequences for cross-linking and the other two hybridize the DNA-tagged C3 proteins, made up of the hydrogel [155]. Self-healing DNA hybrid hydrogels allowed injection-based local placement and controlled destruction by proteases and nucleases to release C3. It inhibited osteoclast migration and resorption in vitro without altering bone-forming osteoblast activity, viability, or proliferation. Thus, the hydrogel has considerable promise for local bone quality improvement in osteoporotic bone and patient-specific preventive therapy of high-risk bones [61, 156–158].

Skin tissue regeneration

Chronic wounds are a major challenge for healthcare, affecting millions of people worldwide. Traditional wound dressings often fall short, providing suboptimal healing environments and failing to address the complex needs of chronic wounds. In recent years, physical stimuli-responsive DNA hydrogels have emerged as a promising new approach to wound healing, offering a number of advantages over conventional dressings [3, 159]. The hydrogel can be loaded with drugs or growth factors and released in a controlled manner in response to a specific stimulus, such as a change in temperature or pH. This targeted delivery can improve the efficacy of the treatment and reduce side effects. It can provide a moist environment that promotes cell proliferation and migration, thereby accelerating wound healing. It can be designed to release anti-inflammatory agents in response to inflammation, helping to reduce pain and swelling, and it can be incorporated with antibacterial agents to prevent wound infections. DNA hydrogels are generally biocompatible and can be designed to degrade over time, eliminating the need for removal [160]. These hydrogels can gel at body temperature and remain liquid at lower temperatures, making them easy to apply to the wound. They can be loaded with drugs or growth factors released as the hydrogel warms up to body temperature, activated by light, allowing for controlled drug delivery or wound disinfection on demand. Also used to deliver drugs or growth factors deep into the wound tissue using ultrasound waves [161].

Nam and the group reported a breathing therapeutic matrix by covalently assembling a DNA micro scaffold (DNA microscaf) with therapeutic mammalian cells. Our method uses metabolically modified cells as active building blocks and medicinal agents. A DNA micro scaffold with a pre-assigned clickable moiety stores biological functionalities for in vivo localization. Ultra-soft mechanical qualities allow the insertion of a complete therapeutic matrix without surgery in the final build. As cells multiply, scaffold-cell active connections

weaken, permitting cell dislodging. Replacement of injured tissue is also possible when the cellular DNA hydrogel slowly disintegrates [161, 162]. Microstructure a DNA hydrogel integrating DBCO-modified monomers (5-dibenzylcyclooctyl-PEG4-deoxycytidine-5′-triphosphate; 5-DBCO-PEG4-dCTP) in isothermal enzymatic replication of DNA, used to create DNA micro scaffolds that display DBCO. The scaffolds were efficiently cross-linked to AzPCs (Unit A) thanks to the preparation of DNA microscafs, which improved the surface area for multivalent conjugation. At physiological temperature, biorthogonal click chemistry between the AzPCs and DNA microscaf units allowed for the fast synthesis of cellular DNA hydrogels (about 30 min). Controlling the final cell densities with the unit assembly technique, including numerous cell kinds, is a breeze, all while efficiently trapping the cells. Hypodermic needles (31G; Ø int. 0.9–1 mm) can be used to inject the matrix due to the viscoelasticity of rCDH. In addition, rCDH can adapt to a complicated surrounding region, which is attributed to its high level of flexibility.

Despite the promising potential of physical stimuli-responsive DNA hydrogels, some challenges still need to be addressed before they can be widely used in clinical settings. These challenges include the high cost of production, the need for further research on the long-term safety and efficacy of these hydrogels, and the development of methods for large-scale manufacturing. In vivo tests have been done using injectable rCDH adaptability in normal mice liver puncture wound model. An in vivo imaging system captured the injected location after injecting cy5-labeled rCDH into an 8 mm wound. These results verified fine rCDH injection into a particular location and volume manipulation. On days 0, 3, and 6 following skin excision, PBS, DNA hydrogel, and rCDH were administered three times at the wound site to monitor wound healing. Compared to PBS, co-culturing with rCDH accelerated wound closure on day 8. The DNA hydrogel enhanced regeneration, although less than rCDH, demonstrating the therapeutic effectiveness of cells as matrix building blocks. H&E examination of tissue slices showed that the co-culture rCDH-treated lesion had more granulation tissue and thicker dermal layers, indicating full regeneration. Masson's trichrome (MT) staining showed that the co-culture rCDH-treated group formed a collagen layer more clearly. The co-culture of rCDH enhances angiogenesis, cell proliferation, and migration, accelerating tissue healing by enabling intercellular communication and therapeutic cell transport [161]. Diabetic wounds are a major healthcare challenge, affecting millions worldwide. These wounds are notoriously slow due to several factors, including poor blood circulation, high blood sugar levels, and impaired immune function. Traditional wound dressings often fall short, providing suboptimal healing environments and

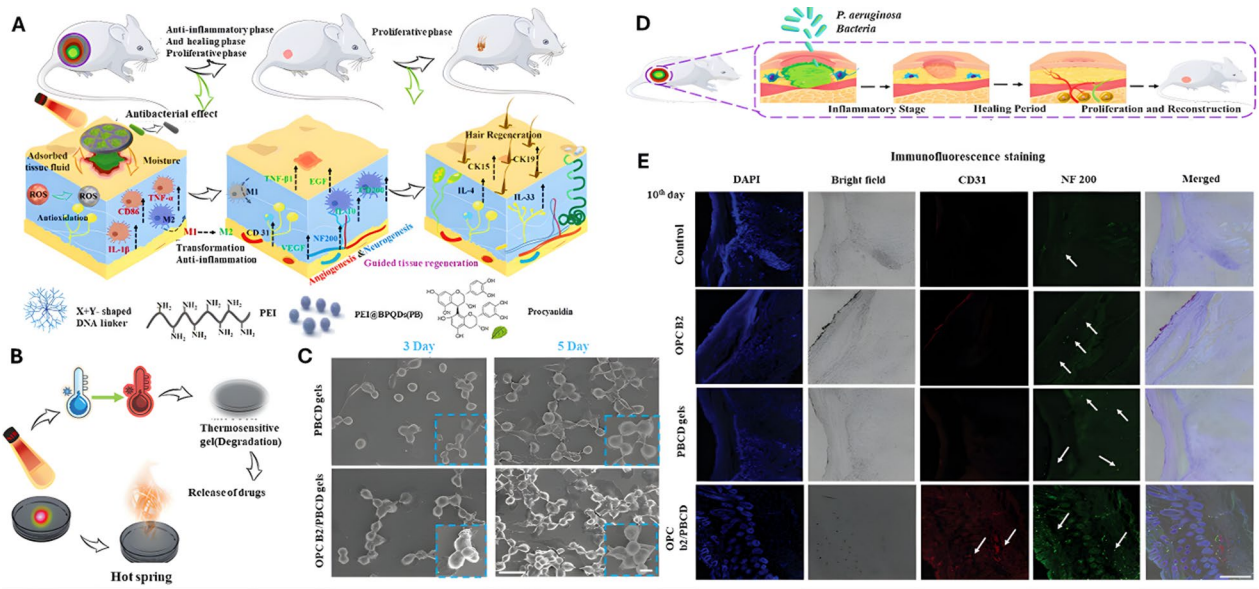


Fig. 8 Physical stimuli-responsive DNA hydrogel in the application of Nerve tissue regeneration. **A** Schematic diagram of the formation of multi-functions OPC B2/PBCD gels. **B** Photothermal properties of gel with "hot spring" characteristics, a schematic diagram illustrating the amount of OPC drug in PBCD gels. **C**, **D** Drug loading rate of PBCD gel. **E** Representative SEM images showed the increase of NIH 3T3 and Schwann cells after 3 and 5 days (bar: 500 nm, 1000 nm)

failing to address the complex needs of diabetic wounds [163].

Regenerative medicine still has significant difficulty in healing wounds in individuals with infections and diabetes. The reason is that standard medical dressings are deficient in key areas. These characteristics include extensive fluid absorption, shape ability, self-healing capabilities, correct tissue regeneration assistance, and normalization of bodily functioning. DNA hydrogel, which incorporates a physical stimulus-responsive element into its architecture, has been developed by scientists to tackle this issue. One type of dressing used in wound healing is photothermal sensitivity, which allows it to change its temperature in response to light. To speed up the healing process, this quality is probably put to use by applying targeted heat to the affected area of the skin. The multifunctional DNA hydrogel dressing discussed here has physical stimuli-responsive properties, one of which is photothermal responsiveness. In a study, a DNA hydrogel dressing is created by cross-linking a cationic polymer called polyethyleneimine (PEI) with BPQDs (abbreviation for BPQDs-doped cationic polymer) to make physiologically active DNA hydrogels (abbreviated as PBCD gels) [164]. These gels are capable of loading the wound-healing nutrient procyanidin B2 (abbreviated as OPC B2) and are referred to as OPC B2/PBCD gels, where the thorough assessment of the many performance characteristics of the DNA hydrogel as a dressing [165].

Nerve tissue regeneration

Nervous system injuries can be devastating, often leading to permanent functional deficits. Regenerating damaged nerves is a complex challenge, and current approaches often fall short [166]. However, physical stimuli-responsive DNA hydrogels are emerging as a promising new strategy for nerve tissue regeneration, offering unique advantages over traditional methods [167]. Delivery of growth factors under controlled conditions involves the hydrogel being loaded with growth factors and then releasing them in response to a change in temperature. Because of this focused distribution, the appropriate factors are guaranteed to arrive at the appropriate location at the appropriate time, thus increasing their efficiency [168]. The hydrogel may have stiffness and mechanical qualities comparable to the surrounding nerve tissue, providing a supporting scaffold for nerve cell development and guidance. This mimics the natural environment of the nerve to mimic the natural neural environment. Specific molecules on the hydrogel can promote nerve cell adhesion and migration, directing nerve cells along the appropriate path for regeneration can be accomplished by promoting nerve cell adhesion and migration. Reducing the production of scar tissue can be detrimental to the growth of nerves. The hydrogel can release anti-scarring chemicals. These hydrogels are temperature-responsive, meaning they are liquid at ambient temperature, making them easy to apply, and they solidify at body temperature, creating a stable environment. In the case of light-responsive hydrogels, one might imagine flashing a particular light to trigger the release of growth factors or drive the migration of nerve cells. Electroactive hydrogels are gels that will react to electrical stimulation and replicate the natural signals responsible for directing nerve cells' development and function [169].

In a study, Zhou et al. obtained a multifunctional DNA hydrogel by grafting DNA units, polyethyleneimine dynamic cross-linking, and doped heating function black phosphorus quantum dots and photostimulated (Fig. 8A) [165]. The DNA hydrogel has good exudate absorption, tunable heating, mechanical behavior, self-healing, writability, tissue adhesion, and antimicrobial characteristics. DNA hydrogels containing procyanidin B2 (OPC B2) have antioxidant and free radical-scavenging capabilities. The DNA hydrogel dressing can also convert pro-inflammatory M1 macrophages into healing M2 ones, stabilizing the wound. Remarkably, DNA hydrogel coating activates neurons to mend, speeding skin nerve regeneration and angiogenesis [170]. Hot spring baths can treat many ailments owing to their thermal stimulation temperature, trace elements, and nutrient-rich water. Figure 8B represents the PBCD gel disintegration and release process. Under NIR, PBCD gel thermally releases nutrients. Natural vitamin OPC B2 resists oxidation and free radicals and promotes cell growth and angiogenesis. OPC B2, a popular skin vitamin, was first incorporated into PBCD gels. PBCD gels attained drug loading saturation when the ratio reached 3.6 and 4.2 since the highest absorption peak was virtually the same height. The work examines the photothermal performance of BPQDs in PBCD gels at 25, 50, and 75 μg mL^{-1}. Multiple concentration materials were irradiated with an 808 nm laser (0.5 Wcm^{-2}) for 10 min, and the temperature change was monitored in real-time. Temperatures of 25, 50, and 75 μg mL^{-1} quickly climbed to 43.1, 54.3, and 62 °C within 3 min at room temperature. Optimal concentration PBCD gels (5 mm, BPQDs 25 μg mL^{-1}) were chosen for biological function study, considering mechanical and photothermal characteristics. CCK8 test kit is employed to determine substance toxicity and optimal concentration. At a dosage of 12.5 mg mL^{-1}, four materials provided a cell survival rate of over 94% after 24 h (Fig. 8D, E). Skin wound healing requires fibroblast and nerve cell proliferation and migration. SEM also indicated that the two cell types proliferated more on the 5th day than on

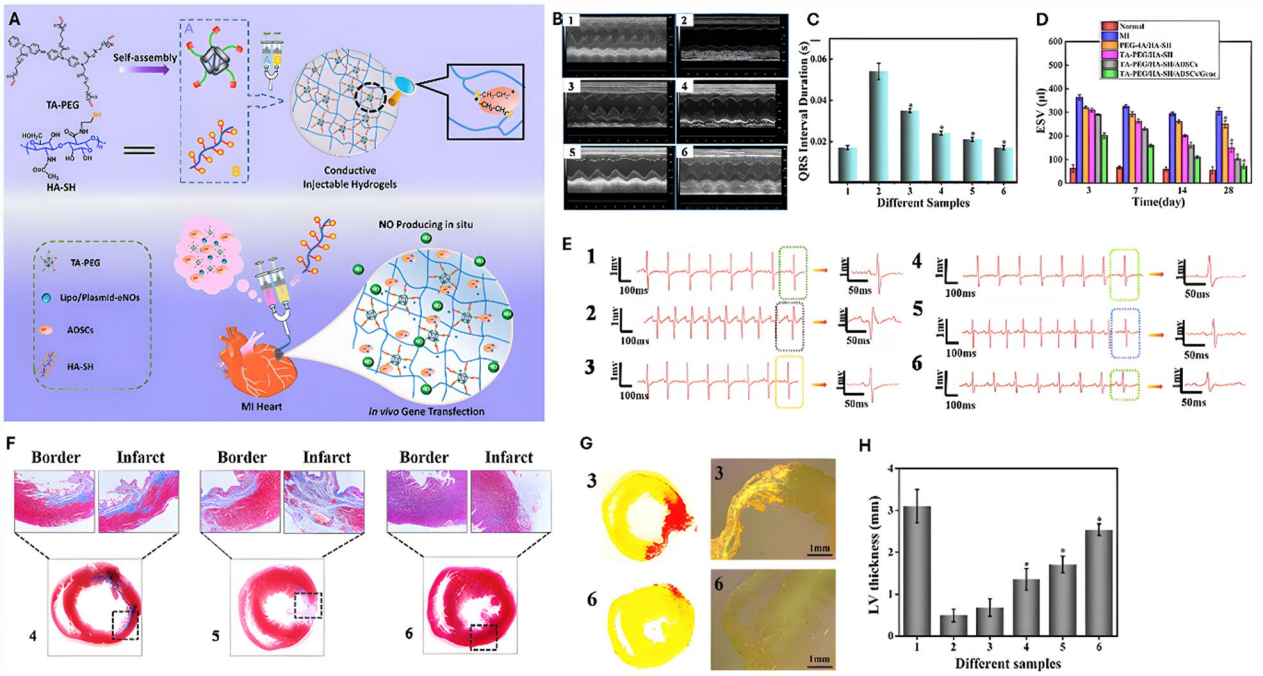


Fig. 9 Physical stimuli-responsive DNA hydrogel in the application of cardiac tissue regeneration. **A** Schematic representation of conductive DNA hydrogel formation. **B** Evaluation of heart functions (4 weeks), representative ECHO images. **C** QRS intervals observed by ECG. **D** Quantitive analysis of Changes of EF, FS, ESV, and EDV evaluated by ECHO. **E** Representative ECG of various groups. **F** Evaluation of cardiac structure by Masson's trichrome staining. **G** Sirius red staining (UP and Down) of the left ventricle (28 days), after treatments, and the representative immunofluorescent staining images of α-Actinin. **H** Quantitative analysis of LV thickness [177]

the 3rd (Fig. 8C). The two cell types also migrated on the 5th day compared to the 3rd [165].

Consequently, from the outset, a rise in cellular proliferation resulted in elongated neurite processes. Furthermore, it is feasible to observe the dividing cells that participate in the segregation of chromosomes during cell division by employing immunostaining with an alpha-tubulin antibody. The XP matrix demonstrated a significant augmentation in the proliferation rate and the number of neuroblastoma cells. Notably, our utilization of the ImageJ program was completely dedicated to quantifying the number of dividing cells (inset). Surprisingly, we found that there were 32 cells undergoing division in the XP matrix within the studied region, compared to 24 in HGX and 12 in CS. The XP matrix's potential for brain tissue engineering strongly supports our argument and has a substantial impact [171].

There are still obstacles to overcome, such as high production costs and the need for more studies on efficacy and safety over the long term. The field, on the other hand, is undergoing rapid development, which has the potential to change the process of nerve tissue regeneration and provide hope for people who have suffered nerve injuries to regain their function. VEGF and EGF expression were assessed to assess the effectiveness of the DNA dressing in treating infected wounds. Western blot analysis revealed that the protein was highest expressed in the OPC-B2/PBCD gels + laser group. According to reports, endogenously driven neuron dedifferentiation has transformed into a healing state, creating angiogenesis and wound repair elements. New neurons and blood vessels formed on the region were designated as NF200 (green) and CD31 (red). CD31 expression was eliminated in the control, OPC B2, and PBCD gel groups, although NF200 protein expression increased. Only the OPC B2/PBCD gels + Laser group produced high amounts of CD31 protein, showing that our bioactive dressing may stimulate mature neurons into repair mode, drive neural ingrowth to the wound site, expedite blood vessel creation, and ensure wound healing. Stimulation increased the expression of GAP-43 in the cultured neural cells [172]. This protein also played a vital role in axonal regeneration and synaptic plasticity, and the increase in GAP-43 expression was dependent on the frequency and amplitude of the applied electrical field, with higher frequencies and amplitudes leading to a more substantial increase [27]. The results of these studies suggest that magnetic and electric field-responsive DNA hydrogels have promising applications as substrates for promoting CNS tissue regeneration. By providing a means to enhance

neurite outgrowth and axonal regeneration in neural cells through controlled physical stimulation, these substrates offer exciting possibilities for future therapeutic applications in the field of neural tissue regeneration [27, 172].

Cardiac tissue regeneration

The regulated delivery of growth factors and other bioactive compounds is one of the main uses of these hydrogels in cardiac tissue regeneration. To encourage angiogenesis and the development of new blood vessels at the site of myocardial infarction (MI), temperature-responsive DNA hydrogels, for example, can be engineered to release angiogenic growth factors, such as vascular endothelial growth factor (VEGF), at particular temperatures [173, 174]. By precisely delivering the therapeutic substances when and where they are needed, this regulated release system promotes heart function and the regeneration process. A myocardial infarction (MI) causes mass cardiomyocyte death, an unfavorable microenvironment, a fibrosis scar without electrical connections, and a shortage of blood flow [175, 176]. Clinic MI treatment efficacy is conservative since the three elements are interconnected. A comprehensive strategy focused on these three essential elements would repair cardiac function, Using in situ Michael addition reaction, multi-armed conductive crosslinker tetra aniline-polyethylene glycol diacrylate (TA-PEG), and thiolated hyaluronic acid to obtain an injectable conductive hydrogel. A soft conductive hydrogel with similar cardiac conductivity and anti-fatigue efficacy was loaded with plasmid DNA encoding eNOs nanocomplexes and ADSCs for MI treatment. The comprehensive TA-PEG/HA-SH/ADSCs/Gene hydrogel system was injected into SD rats' infarcted myocardium(Fig. 9A) [177]. Proangiogenic growth factors, myocardium-related mRNA, and eNOs expression increased in cardiac tissue, as did nitrite levels. Electrocardiography(Fig. 9B), cardiogram, and histological study (Fig. 9C–E) showed a considerable improvement in cardiac functions, including a higher EF, shorter QRS interval, smaller infarction size, reduced fibrosis area, and higher vascular density. As shown in Fig. 9F Masson's-trichrome staining of images, picrosirius red

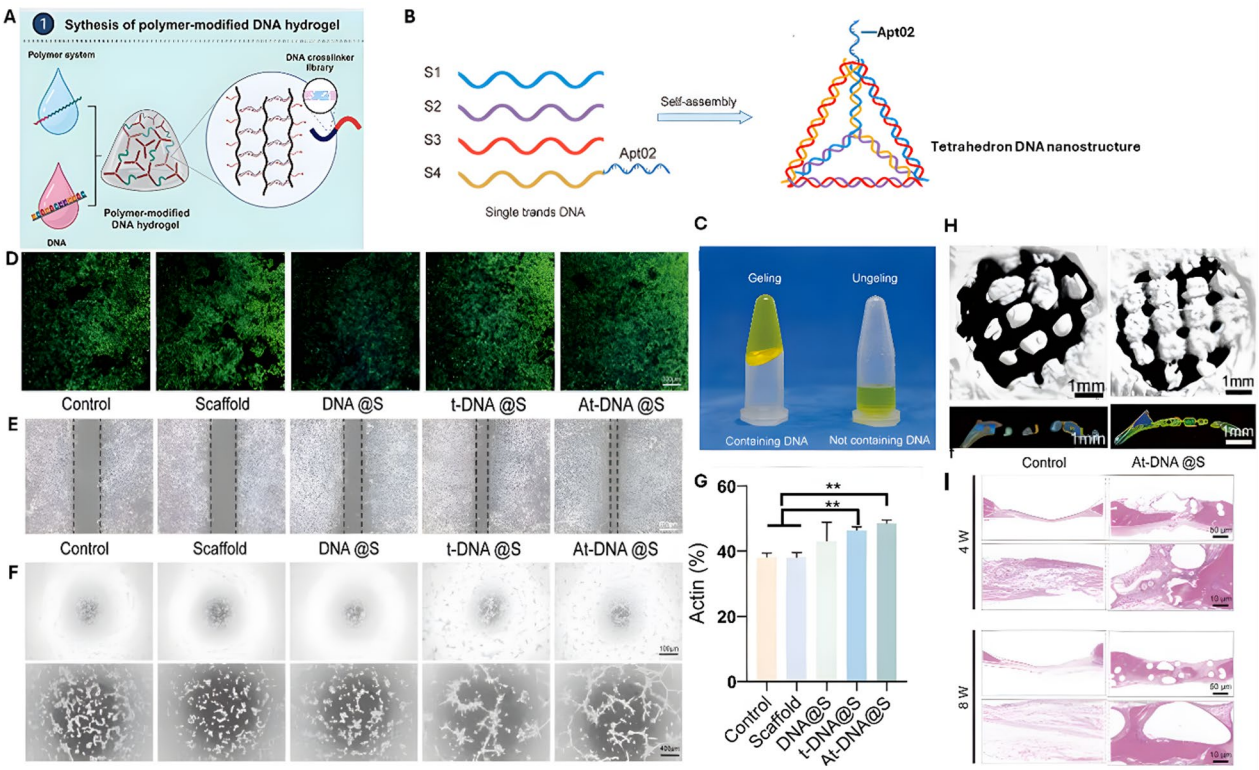


Fig. 10 Physical stimuli-responsive DNA hydrogel in the application of Vascular tissue regeneration. **A** Schematic representation of the construction of DNA hydrogel composite material. **B** Schematic diagram of DNA framework and synthesis. **C** Image of DNA hydrogel **D** DNA hydrogel scaffold enhances in vitro angiogenesis, the cytoskeleton of HUVECs (48 h). **E** Cell scratch experiment to verify the migration ability of HUVECs (24 h). **F** Optical images of the capillary-like network formed by HUVECs (8 h) on Matrix gel with the specimen-conditioned medium. **G** Quantitative analysis of the cellular cytoskeleton. **H** Micro-CT images depict cranial defects treated with constructs, encompassing both cross-sectional and longitudinal views. **I** Histological images stained with H&E exhibit cranial defects following the implantation of scaffold constructs for 4 and 8 weeks. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ [95]

staining slices showed the infarcted myocardium stained with high-contrast red. As seen in Fig. 9G red colour was greatly decreased. This combo technique uses conductive injectable hydrogels filled with stem cells and gene-encoding eNO nanoparticles to cure MI effectively (Fig. 9H) [177].

Light-responsive DNA hydrogels provide an additional encouraging strategy. Certain light wavelengths can activate these hydrogels and cause the release of encapsulated growth factors or cells. The results of heart tissue regeneration can be greatly enhanced by this technique, which enables precise and non-invasive control over the release of therapeutic substances. For instance, light-triggered hydrogels containing cardiomyocytes or stem cells can release these cells at the infarcted region, encouraging the growth of new cardiac tissue and enhancing heart function. The potential of magnetic field-responsive DNA hydrogels in cardiac tissue engineering is also being investigated. External magnetic fields can be used to modify these hydrogels and target particular regions of the heart. By delivering therapeutic molecules locally, this focused strategy lowers systemic adverse effects and increases treatment success. To encourage tissue repair and regeneration, magnetic nanoparticles encapsulated in the hydrogel, for instance, can be guided to the site of heart damage and release growth factors or other bioactive molecules. Another novel use is for DNA hydrogels that respond to ultrasounds. Ultrasound vibrations can cause these hydrogels to release their therapeutic contents. Due to its deep tissue penetration, ultrasound is a useful trigger for medication release in heart tissue regeneration [178]. Patients with heart injuries can receive more effective therapy thanks to this non-invasive approach that precisely controls the release of therapeutic agents [85]. Lastly, DNA hydrogels that respond to physical stimuli provide a flexible and efficient platform for the regeneration of heart tissue. These hydrogels enhance the regeneration process and improve patient outcomes by delivering therapeutic chemicals in a regulated, targeted, and efficient manner by utilizing external physical triggers. The field's current research and development have enormous potential to advance regenerative medicine and cardiac tissue engineering.

Vascular tissue regeneration

Physical stimuli-responsive DNA hydrogels have developed as novel materials for vascular tissue regeneration, taking advantage of their capacity to respond to external physical triggers such as temperature, light, magnetic fields, and ultrasound. These hydrogels may be created to release therapeutic drugs in a regulated manner, promote cell proliferation, and imitate the natural ECM, all of which are required for optimal vascular tissue repair and regeneration [179, 180]. One of the key uses of these hydrogels in vascular tissue regeneration is to distribute growth factors and other bioactive compounds in a regulated manner. For example, temperature-responsive DNA hydrogels may be constructed to release VEGF at specified temperatures, stimulating angiogenesis and the development of new blood vessels at the site of injury [181, 182]. This regulated release system guarantees that therapeutic substances are supplied exactly when and where they are required, therefore improving the regeneration process [183]. Light-responsive DNA hydrogels are another potential option. Specific light wavelengths can activate these hydrogels, causing the release of contained growth factors or cells. This technology enables non-invasive and exact control over the delivery of therapeutic substances, which can considerably improve the results of vascular tissue regeneration. For example, light can cause hydrogels containing endothelial cells to be released at the damaged site, stimulating the development of new blood vessels and increasing blood flow [5]. However, high gelation temperatures (about 46 °C) and expensive fabrication costs restrict cell encapsulation and applicability. In a recent study, Han et al. revealed a new polymer-modified DNA hydrogel produced utilizing nucleic acid nanotechnology, which gels at a more biocompatible temperature of 37 °C and is inexpensive (Fig. 10A) [95]. This hydrogel then contains tetrahedral Framework Nucleic Acid (tFNA), which improves osteogenic mineralization, furthermore given the adaptability of tFNA. And changed its chains using Aptamer02 (Apt02), an aptamer known to promote angiogenesis. This dual method has been shown to considerably accelerate osteogenic differentiation in bone marrow stromal cells (BMSCs) and angiogenesis in human umbilical vein endothelial cells (HUVECs), with cell sequencing supporting its efficiency [184]. In in vivo investigations in rats with critical-size cranial bone defects show that they are efficient in promoting new bone growth. This invention not only provides a realistic approach for healing segmental bone abnormalities, but it also paves the way for future breakthroughs in bone organoid assembly, representing a huge step forward in tissue engineering and regenerative medicine. In this work, anchor DNA strands were dispersed in acrylamide and polymerized to generate a polymer with numerous DNA strands as the main chain [95]. A DNA hydrogel was created by connecting acrylamide polymers with DNA crosslinkers that may complement the anchor DNA via Watson–Crick base pairing. For base pairing with anchor DNA, 64 crosslinkers were utilized to make the DNA hydrogel homogenous. Heat-activated blocking strands connect with crosslinkers by complementary base pairing at 4 °C, limiting DNA hydrogel formation. The DNA hydrogel

forms when these strands separate from the crosslinkers at 37 °C. Its temperature-responsive self-assembly makes it ideal for humans (Fig. 10C). PIPAM, PEG, and PDMS are thermoresponsive materials that change their physical or chemical characteristics at specified temperatures [95]. Successful vascular bone repair requires osteogenesis promotion. tFNA, made from four specially designed oligonucleotide strands (S1, S2, S3, S4) [183] activates stem cell development into osteoblasts (Fig. 10B). The study has five groups: Control, Scaffold, DNA hydrogel + scaffold (DNA@S), t-DNA@S, and Apt-tFNA@S. Figure 10D–G shows that all groups had normal cytoskeletal shapes, indicating that the components did not harm cellular viability. This implies that DNA hydrogel extracts at this concentration were cell-safe. Additionally, Apt02 promotes angiogenesis like VEGF, therefore, we examined how each group affected blood vessel creation. Wound healing tests to evaluate HUVEC migration. Group effects on HUVEC tube development were also examined. These experiments show that At-DNA@S composite material actively promotes angiogenesis [95, 185].

The final phase of investigation includes in vivo animal tests utilizing the Sprague Dawley (SD) rat cranial defect model to evaluate DNA hydrogel-scaffold regenerating capacities. Evaluated cranial healing 4 and 8 weeks after damage. To evaluate bone regeneration at defect areas, μCT reconstruction was used (Fig. 10H). The scaffold holes and defect margins showed new bone development. New bone development was assessed by H&E staining (Fig. 10I) [95]. Scaffolds offered a porous structure, and DNA hydrogel had some osteogenic impact, but bone growth was restricted. The Control group had fibrous tissue without new bone development [95, 186].

Magnetic field-responsive DNA hydrogels are also being investigated for their application in vascular tissue engineering [187]. These hydrogels may be adjusted using external magnetic fields to target particular parts of the vascular system. This focused method enables the localized distribution of medicinal substances, decreasing systemic adverse effects and increasing treatment success. Magnetic nanoparticles contained in the hydrogel, for example, can be guided to the site of vascular damage and release growth factors or other bioactive compounds that stimulate tissue repair [188]. DNA hydrogels that respond to ultrasound are another novel use. Ultrasound waves can cause these hydrogels to release therapeutic payloads. Ultrasound may penetrate deep tissues, making it a potent trigger for drug release during vascular tissue regeneration [189, 190]. This approach provides non-invasive and precise control over the distribution of therapeutic drugs, hence enhancing treatment results for patients with vascular injuries.

In biosensing, these hydrogels can be integrated with various detection platforms to create highly sensitive and specific sensors. For example, DNA hydrogels combined with optical or electrochemical sensors can detect the presence of metal ions, nucleic acids, proteins, and other biomolecules by undergoing conformational changes that alter their physical properties, thereby generating measurable signals [14, 191]. The programmability and biocompatibility of DNA hydrogels make them ideal for constructing biosensors that can respond to a variety of stimuli, such as pH, temperature, and light, enabling precise and reliable detection of target analytes in complex biological environments [192]. In cancer therapy, pressure, and shear stress-responsive DNA hydrogels can be utilized for localized and controlled drug delivery [193]. These hydrogels can encapsulate chemotherapeutic agents and release them in response to mechanical stimuli present in the tumor microenvironment. This targeted release mechanism minimizes systemic toxicity and enhances the therapeutic efficacy of the drugs [194]. Additionally, the mechanical properties of these hydrogels can be tailored to match the specific requirements of different cancer types, providing a versatile platform for personalized cancer treatment [13, 195].

For tissue regeneration, pressure and shear stress-responsive DNA hydrogels offer several advantages. Their ability to respond to mechanical cues makes them ideal for dynamic tissue environments where mechanical forces play a crucial role in tissue development and healing. These hydrogels can be engineered to release growth factors, cytokines, or other bioactive molecules in response to mechanical stimuli, promoting cell proliferation, differentiation, and tissue repair [196]. For instance, the shear-thinning properties of DNA hydrogels enhance their injectability, allowing for minimally invasive delivery to target sites, where they can support tissue regeneration by providing a conducive environment for cell growth and matrix formation [136, 197]. As shown in Table 2, Physical stimuli-responsive DNA hydrogels combine with other materials like growth factors, polymers, and drugs, which all together are a response to or triggered by different stimuli that present their main objectives in multifunctional tissue regenerations.

Advantages of physical stimuli-responsive DNA hydrogel

Enhancing bioactivity and multi-modal functionality

Physical stimuli-responsive DNA hydrogels have various advantages for multifunctional tissue regeneration. The mechanical characteristics of these hydrogels may change, or bioactive molecules may be released when exposed to certain physical stimuli, such as changes in

Table 2 Physical stimuli-responsive DNA hydrogels with material conjugation and their role in application

Tissue regeneration	DNA functionalization	Types of physical stimuli	Model for study	Objectives	Refs.
Bone regeneration	DNA hydrogel scaffold loaded with growth factors and hydroxyapatite nanoparticles DNA self-assembles into hydrogel. Combine the VEGF-filled DNA hydrogel with a 3D-printed PCL scaffold	Temperature	Rat calvaria defect model	To promote bone regeneration and repair	[23]
Wound healing	DNA hydrogel developed by integrating DBCO modified monomers into the DNA structure, enabling effective cross-linking with Azide modified cells and forming a scaffold	Temperature	In-vivo mouse model (liver) And in-vitro (skin)	aims to improve wound healing by creating a supportive and adaptable environment that promotes tissue regeneration and speeds up the healing process	[161, 198]
Diabetic wound healing	Base pair sequences are used to form DNA hydrogel. PB and PEI were added into the DNA hydrogel to create a 3D structure. loaded with BPQDs to generate heat while exposed to light	Light and temperature	Mouse model (diabetic infected)	A cutting-edge wound dressing that effectively tackles the unique challenges of healing wounds in diabetes, guiding tissue regeneration in diabetic patients	[165]
Cartilage regeneration	DNA hydrogel scaffold functionalized with chondroitin sulfate and growth factors	Light, and temperature	Rabbit cartilage defect model	The objective is to create a type of DNA hydrogel that can change its properties in response to physical stimuli like light or temperature	[17]
Nervous system tissue regeneration (peripheral nerve)	DNA hydrogel scaffold functionalized with laminin and growth factors	Electrical stimulation, temperature	Rat sciatic nerve injury model	To promote nerve regeneration and repair	[199]

pH, temperature, light, magnetic fields, or electric fields. Using this responsiveness it can control the cell's behavior and tissue growth, for example, by promoting cell proliferation, migration, and differentiation [200, 201]. DNA hydrogels that respond to physical stimuli have several advantages for multifunctional tissue regeneration, including:

1. *Precise control over cell behavior*: Physical stimuli-responsive DNA hydrogels are designed to release bioactive molecules, such as growth factors or therapeutic agents, in response to specific physical stimuli. This can provide precise control over cell behavior and tissue development by promoting cell proliferation, differentiation, or migration. For instance, DNA hydrogels can be designed to release growth factors in response to changes in temperature, which can enhance tissue regeneration. This molecular precision of DNA allows for control over hydrogel properties at the nanoscale, which is challenging to achieve with many polymer or natural hydrogels [173, 202, 203].
2. *Mechanical properties tailoring*: Physically stimulated DNA hydrogels can be designed to have tunable mechanical properties, like stiffness or elasticity, that have been tailored to specific tissue types or applications. This provides a natural microenvironment for cells and promotes tissue regeneration. As an example, DNA hydrogels can be designed to mimic the mechanical properties of native tissues, like we can say for bone, to enhance cell adhesion, migration, and differentiation [204–206].
3. *Biocompatibility*: While many natural hydrogels are biocompatible, DNA hydrogels have the advantage of being composed of a naturally occurring biological molecule, potentially reducing immunogenicity compared to synthetic polymer hydrogels. This characteristic makes them an ideal material for tissue engineering and regenerative medicine applications [207, 208].
4. *Biodegradability*: DNA hydrogels can be designed to degrade in response to specific enzymes or conditions, offering more precise control over degradation compared to many polymer hydrogels.
5. *Non-invasive*: DNA hydrogels can be delivered to the target tissue non-invasively, like through injection or topical application, reducing the risk of complications and improving the patient's comfort [207, 208].
6. *Versatile and programmable*: Unlike polymer or natural hydrogels, DNA hydrogels can be precisely engineered at the molecular level due to the specificity of base pairing. This allows for unparalleled control over structure and function. DNA hydrogels that respond to physical stimuli are versatile and programmable, enabling exact control over their properties and functions. They have been designed to modify their mechanical properties or release bioactive molecules in response to different physical stimuli, such as changes in pH, temperature, light, magnetic fields, electric fields, etc. This responsiveness can control cell behavior and tissue growth, for example, by promoting cell proliferation, differentiation, and migration [31, 57, 209].
7. *Multifunctionality*: DNA hydrogels can incorporate multiple stimuli-responsive elements and functional domains more easily than traditional hydrogels, enabling more complex and precise control over material properties and bioactive molecule release.
8. *Enhancing tissue regeneration*: Nowadays, physical stimuli-responsive DNA hydrogels have shown promising outcomes in preclinical studies for tissue regeneration. They have been used to engineer various tissues, such as bone, cardiac, skin, and neural systems, and have shown enhanced tissue regeneration compared to traditional hydrogels and materials. This makes them a promising material, potentially enhancing tissue regeneration therapies by providing a versatile and programmable biocompatible platform for the controlled release of growth factors and other molecules. However, further research is needed to fully understand the mechanisms underlying these physical stimuli and optimize the design of DNA hydrogels for specific tissue regeneration applications [46, 210].

Potential side effects and risk mitigation

While DNA hydrogels show great promise, potential side effects, and risks should be considered:

1. *Immunogenicity*: Although DNA is generally biocompatible, certain sequences or modifications could potentially trigger immune responses [195, 211]. This risk can be mitigated by careful sequence design and screening for immunostimulatory motifs.
2. *Unintended gene regulation*: DNA sequences in hydrogels could potentially interact with cellular machinery, leading to unintended gene regulation [212]. This can be addressed by avoiding sequences with known regulatory functions and testing for off-target effects.
3. *Degradation products*: The breakdown of DNA hydrogels could potentially release fragments that interfere with cellular processes [13]. Using naturally

occurring DNA bases and avoiding synthetic modifications can help ensure safe degradation.

4. *Nanomaterial toxicity*: Like other nanomaterials, DNA nanostructures in hydrogels could potentially accumulate in organs or cause cellular stress [13]. Thorough biodistribution and toxicity studies are crucial to assess and mitigate these risks.
5. *Stability in physiological conditions*: DNA hydrogels may be susceptible to enzymatic degradation or structural changes in vivo. Incorporating stabilizing modifications or protective coatings can enhance their stability and performance [7, 12]. To minimize these risks, researchers should focus on: rigorous in vitro, in vivo safety testing and careful sequence design to avoid potentially harmful motifs, development of strategies to enhance stability, control degradation, comprehensive characterization of degradation products, their effects, and long-term studies to assess potential cumulative effects.

Challenges and limitations

Physical stimuli-responsive DNA hydrogels, while promising for tissue regeneration application, face significant challenges that limit their widespread adoption and clinical translation. The high cost of synthesis, requiring specialized equipment and reagents, poses a major economic hurdle [213]. Additionally, the complex design process, involving careful selection of DNA sequences and optimization of crosslinking density and mechanical properties, can be time-consuming and challenging. These factors, combined with the limited range of applicable stimuli in vivo and high sensitivity to environmental changes, restrict the practical use of these hydrogels in dynamic biological settings.

Scaling up production for large-scale applications remains a significant obstacle, with time-consuming and expensive DNA synthesis and purification processes hindering commercial viability [14]. Moreover, despite DNA being a natural polymer, these hydrogels can still elicit immune responses in vivo, potentially causing inflammatory reactions or rejection by the host organism. This immunogenicity, along with regulatory and safety concerns surrounding the use of synthetic DNA materials in medical applications, presents additional barriers to clinical implementation.

The long-term effects of DNA hydrogel degradation products on cellular and tissue environments are not fully understood, adding another layer of complexity to their development and use. Addressing these challenges will be crucial for advancing the field of physical stimuli-responsive DNA hydrogels and realizing their full potential in tissue regeneration and other biomedical applications. Future research must focus on overcoming these limitations to enable the translation of these promising materials from laboratory settings to clinical use, balancing their innovative potential with practical considerations of cost, scalability, and safety.

Conclusion

DNA as a natural polymer in hydrogels has several advantages over traditional gels, such as their biocompatibility, tunable mechanical properties, and programmability. They can provide a natural microenvironment for cells while also responding to specific physical stimuli to control cell behavior. By applying the effective experiment, researchers are finally introducing the incorporation of physical stimuli into the DNA hydrogel, followed by the all-DNA hydrogel or Hybrid DNA hydrogel method, and successfully showing the formation of physical stimuli-responsive DNA hydrogels. Physical stimuli-responsive DNA hydrogels offer a promising platform for multifunctional tissue regeneration by providing a tunable and dynamic ECM mimic that can precisely position bio-signals and cells themselves and facilitate nutrient transport and cellular attachment and differentiation. These hydrogels can be designed to respond to specific physical stimuli, such as pH, temperature, light, magnetic or electric fields, pressure, ultrasound, and so on, which can trigger changes in their mechanical properties or the release of bioactive molecules. This responsiveness allows for precise control over the release of therapeutic agents or growth factors and enables targeted delivery to specific cells or tissues for their development, such as promoting cell proliferation, differentiation, and migration. Physically stimulated DNA hydrogels mimic the ECM and provide a natural microenvironment for cells. These approaches can enhance tissue regeneration and have shown promising results in preclinical studies for tissue engineering. In numerous trials, such as those involving wound healing, bone tissue regeneration, cardiac tissue regeneration, and neural tissue systems, these hydrogels (sometimes as scaffolds) have been successfully employed to treat the injured tissue and grow new tissue in the damaged location. However, despite the challenges associated with their development and translation into clinical applications, physical stimuli-responsive DNA hydrogels offer great potential for tissue regeneration. By addressing the limitations and challenges, various strategies can be implemented to circumvent the constraints of DNA hydrogels. Incorporating hybrid materials or increasing crosslinking density can be used to improve mechanical strength. Chemical modifications and protective encapsulation can be implemented to enhance the stability and

durability of the hydrogels, optimizing the design of the DNA sequences, ensuring long-term biocompatibility degradability, reducing their production cost and Scalability, and obtaining regulatory approval.

Moreover, the body's immune system might react to foreign materials like hydrogel. Strategies to minimize this risk need to be developed. DNA hydrogels can be further developed as an ECM platform for multifunctional tissue regeneration by performing appropriate research to address these limitations and challenges and ensure the safety and efficacy of DNA hydrogels for clinical use. Overall, physical stimuli-responsive DNA hydrogels have great potential as an ECM platform for multifunctional tissue regeneration, and further research and development in this field can lead to new and innovative approaches for tissue regeneration and regenerative medicine.

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Author contributions
Authorship Statement R.A. contributed to the original draft, formal analysis, resources, and conceptualization. S.D.D. was responsible for writing and reviewing methodology, formal analysis, and resources. H.M. handled visualization, review, and editing. T.V.P. participated in formal analysis, review, and editing. K.G. is involved in conceptualization, data curation, and supervision. A.R. focused on investigation, supervision, and review. H.K. took charge of validation, review, and editing. J.L. worked on data curation, review, and editing. Hyeonseo Park was involved in the review and editing. C.M. Funding acquisition, supervision. Ki-Taek Lim contributed to conceptualization, data curation, formal analysis, funding acquisition, and supervision.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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