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Biological applications of lipoic acid-based polymers: an old material with new promise

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Lipoic acid (LA) is a versatile antioxidant that has been used in the treatment of various oxidation–reduction diseases over the past 70 years. Owing to its large five-membered ring tension, the dynamic disulfide bond of LA is highly active, enabling the formation of poly(lipoic acid) (PLA) via ring-opening polymerization (ROP). Herein, we first summarize disulfide-mediated ROP polymerization strategies, providing basic routes for designing and preparing PLA-based materials. PLA, as a biologically derived, low toxic, and easily modified material, possesses dynamic disulfide bonds and universal non-covalent carboxyl groups. We also shed light on the biomedical applications of PLA-based materials based on their biological and structural features and further divide recent works into six categories: antibacterial, anti-inflammation, anticancer, adhesive, flexible electronics, and 3D-printed tissue scaffolds. Finally, the challenges and future prospects associated with the biomedical applications of PLA are discussed.

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International Edition. Aiming to the urgent needs of the medical device industry, his group is devoted to the whole industry chain layout, ranging from science and technology to industrialization. Several types of infusion and interventional medical devices have been industrialized using their synthesized materials.

Introduction

Lipoic acid (LA), a naturally occurring fatty acid with a five-membered disulfide ring, exhibits both amphiphilicity and antioxidant properties. As a crucial enzyme cofactor in the human body, it is involved in the coordination of mitochondrial energy metabolism, body detoxification, resistance to skin inflammation, and maintaining blood sugar levels.^{1,2} Ever since it was first isolated from hepatocytes in 1951 by Reed *et al.*,³ LA with various biological activities has been widely used in the treatment of many oxidation–reduction (REDOX) diseases, such as diabetes,^{4,5} atherosclerosis,⁶ and cancer therapy.^{7–9} Owing to its unique pharmacological characteristics and biomass sources, the clinical applications and disease treatment mechanisms of LA have been extensively studied for the effective treatment of diabetic peripheral neuropathy (DPN),¹⁰ and its global market demand exceeds 3000 tons annually.

LA is easily activated owing to its high five-membered ring tension and low dynamic disulfide bond energy. A high concentration of LA molecules can undergo dynamic covalent ring-opening polymerization (ROP) triggered by photo/thermal effects. Free radical polymerization initiated by heat is one of the conventional methods to obtain PLA. In the solvent-free molten state, LA can yield cyclic polymers.¹¹ In recent years, there have been significant research studies on achieving the controlled polymerization of LA and its derivatives for the precise structural control of PLA. Among these, the most popular method is to add inert organic bases and mercaptan. It is possible to achieve topological control of PLA through the use of protonic solvents such as thiols, which depend on the

nucleophilicity of thiols.¹² PLA can also introduce non-covalent interactions, such as hydrogen bonds, metal-carboxy coordination, and van der Waals forces, to construct highly ordered self-assembled supramolecular network structures with side chains of carboxyl groups.^{13–15} Additionally, the hydrophilicity and hydrophobicity of PLA can be regulated by modification of the side-chain carboxyl group with chemical reactions such as esterification, amidation, and deprotonation. Dynamic supramolecular polymers mediated by disulfide bonds and side-chain engineering *via* non-covalent crosslinking offer broad range of application prospects for PLA, including sustainable plastics,¹⁶ self-healing hydrogels,^{17–19} cell-penetrating peptides,²⁰ and intelligent biomaterials.^{21,22}

Currently, there have been some works^{23,24} aiming to introduce PLA-based dynamic covalent materials combining self-assembly and reversible crosslinking to provide advanced biocompatible templates. Consequently, these have been extensively studied in biomedical applications, including protein modification,^{25,26} tumor treatment,^{27,28} biosensing,^{29–31} and tissue scaffold 3D-printing.³² Most reviews^{33,34} have primarily focused on the properties and clinical applications of small-molecule LA, while limited attention is given to the synthesis strategies and biological applications of PLA-based systems. In this work, after fully considering the impact of specific structures on biological performances, we highlight the latest advancements of PLA in polymerization methods and biomedical applications, and the prospects for further development in the future (Scheme 1).

Biological features of free LA molecules

LA contains three structural units: a lipophilic five-membered disulfide ring, a hydrophilic/acidic functionalized carboxyl

group, and a hydrophobic alkyl chain. There are two enantiomers of free LA molecules for the chiral carbon atom C6, where the bioactive one is (*R*)-LA.³⁵

LA is described as a “universal antioxidant”, which can reduce lipid peroxidation damage induced by H₂O₂ or Fe²⁺ and Cu²⁺ through direct the scavenging of free radicals⁶ or metal chelation (Fig. 1B).³⁶ LA can be readily reduced into dihydrolipoic acid (DHLA) under oxidizing conditions (Fig. 1A). Both LA and DHLA possess potent antioxidant properties and play a synergistic effect *in vivo*, similar to the relationship between glutathione (GSH) and the oxidized form of glutathione (GSSG). Attributed to its biological origin, good biocompatibility, easy modification, it is widely used in the treatment or prevention of various chronic diseases related to oxidative stress, including anti-aging nutritional supplements,³⁷ and even to suppress the appetite.³⁸

Simultaneously, because of its amphipathic nature, it can be easily absorbed and transported through cell membranes to penetrate tissues, such as the nervous system and the heart, that are mainly composed of fat and water. Disulfide-mediated uptake can be achieved through dynamic covalent exchange of disulfide and mercaptan on the cell surface. Covalent binding to the cell surface may occur *via* several uptake mechanisms, including fusion, endocytosis, or direct translocation into the cytoplasm along the disulfide orbits and micellar pores (Fig. 1C).³⁹ However, the biological applications of free LA molecules are limited due to its rapid metabolism, poor bioavailability, and short biological half-life. Carbone *et al.*⁴⁰ nano-encapsulated LA derivatives and prepared hydrophilic nanocapsules as well as lipophilic-nanostructured lipid carriers to address these issues. However, there are still problems that need to be solved.



Scheme 1 Polymerization and depolymerization of LA molecules, and features and biomedical applications of PLAs.



Fig. 1 (A) Mutual transformation of LA and DHLA. (B) Antioxidant mechanism of LA. (C) Scheme of LA promoting cell endocytosis. Reprinted from ref. 39 with permission. Copyright 2020, Wiley.

ROP polymerization of LA molecules

In 1956, Thomas and Reed observed a “viscous liquid” during the oxidation of LA, which was later reported to be PLA.⁴¹ LA exhibits instability under photoconditions with oligomer formation in dilute solutions exposed to long wavelength ultraviolet light. Besides, upon reaching the melting point (59–62 °C) of LA, it rapidly polymerizes to form higher molecular weight polymers. Presently, the most common method of LA polymerization is still free radical polymerization, initiated by ultraviolet light (Fig. 2A) or heat (Fig. 2B). The resulting PLA has a ring interlocking structure.⁴² Interestingly, the total activation energy for this process is very low and is concentration- and temperature-dependent.¹¹ It is challenging to obtain the polymer at below the melting point or under certain concentrations. Conversely, once the temperature exceeds the melting point of LA, polymerization occurs more readily. However, the bulk thermal polymerization process for obtaining PLA without any initiator is reversible, yielding amorphous polymers. When LA molecules are sufficiently close in ethanol at a high concentration, intermolecular disulfide exchange occurs, leading to PLA with alternating disulfide bonds in the main chain with a lower polymerization temperature (Fig. 2C). Nevertheless, the resulting PLA is metastable at room temperature, and after ethanol evaporation, it quickly reverts back to semicrystalline oligomers due to retro-cyclic depolymerization. To address these issues, the controlled thermal polymerization of PLA can be enhanced by leveraging the synergistic effects of both covalent and non-covalent bonds. On the one hand, chemical reactions of the carboxyl group *via* esterification, guanidinylation, amidation, *etc.* can introduce different functional groups and facilitate hydrogen bonds. Zhang *et al.*⁴³ reported a water-soluble ABA triblock copolymer; whereby a hydrophilic poly(ethylene oxide) B block and hydrophobic A block, derived from a carbonate with LA, self-assembled to form a flower-like microgel in water medium. Concentration induction led to physical crosslinking, while the addition of a telechelic dithiol triggered chemical crosslinking. Lu *et al.*²⁵ utilized sulfhydryl protein residues to initiate methoxy oligoethylene glycol-modified LA polymerization, achieving protein-PDS graft synthesis at physiological temperature and reducing the polymerization concentration of LA. Zhang *et al.*¹³ constructed a supramolecular polymer network using three different types of dynamic chemical bonds (chemical crosslinking, hydrogen bonding, and complexation) in a single polymer system. On the other hand, ionic liquid could be added to prevent the depolymerization of PLA. Wang *et al.*⁴⁴ achieved a concentration-induced ring-opening polymerization of LA at room temperature and obtained stable PLA ion sols in air by adding an ionic solution of 1-ethyl-3-methylimidazolium ethyl sulfate. Additionally, the carboxyl group can be complexed with various metal ions (Fe³⁺, Cu²⁺, Zn²⁺, Mn²⁺, *etc.*). These complexations often offer an opportunity to modulate the optical and electrical properties of inorganic surfaces, including quantum dots (QDs), plasma materials, and perovskites.^{45,46}

Although free radical polymerization is convenient, the polymerization of LA often results in a mixture of PLA and a



Fig. 2 Two mechanisms for the ring-opening polymerization for PLA: radical polymerization and thiolate-initiated anionic ROP. (A) Photoinduced ring-opening polymerization for PLA. (B) Thermal polymerization for PLA. (C) Ring-opening polymerization induced by the concentration in ethanol solution. (D) Process of substrate-initiated polymerization: chain initiation, chain growth, and chain termination. Reprinted from ref. 20 with permission. Copyright 2013 Elsevier. (E)–(G) Effect of mercaptan nucleophilicity on the topological structures of PLA. (H) Regioselective ring-opening polymerization for PLA by anion-binding catalysis. Reprinted from ref. 49 with permission. Copyright 2023, Elsevier.

small number of cyclic monomers. To achieve precise structural control and a low dispersion, researchers have explored living anionic polymerization triggered by mercaptan initiators. Bang *et al.*²⁰ utilized thiolated probes or drugs as initiators for the anion polymerization of guanidinylated LA derivatives at room temperature, with iodoacetamides acting as terminators (Fig. 2D). Notably, PLA is susceptible to reduction and depolymerization,⁴⁷ making it a promising candidate for non-

invasive drug delivery.⁴⁸ Some studies have shown that the structures of PLA depend on the nucleophilicity of the initiators.¹² Alkyl mercaptans tend to yield linear polymers (Fig. 2E), while aryl mercaptans favor ring polymers (Fig. 2F). Linear PLA was successfully obtained using benzyl mercaptan with inert bases (Fig. 2G). What's more, achieving regionally selective PLA remains challenging due to the highly nucleophilic chain ends of PLA, which may react with S-S bond monomers or polymers to induce chain transfer and chemical irregularities. Du *et al.*⁴⁹ addressed this issue using an anion-binding catalyst (Fig. 2H). By regulating the nucleophilicity of mercaptan through hydrogen bond interactions, they reduced the negative charge at the chain ends and improved the regional selectivity of the ROP. However, further investigations are needed to enhance the regional selectivity of PLA synthesis to align with diverse application requirements. As the most controllable way, thiolate-initiated anionic ROP remains problematic in certain applications, such as in inert conditions and non-initiating monomers with active protons. Qu *et al.*⁵⁰ reported an acid-catalyzed cationic ROP, which resulted in high-molecular-weight (over 1000 kDa) poly(disulfide)s under open air without inert protection.

This material exhibits significant potential in the biomedical field due to its interaction between dynamic covalent bonds and dynamic non-covalent sites, as well as its non-toxicity, biological origin, closed-loop recyclability, and other notable characteristics.

Biomedical applications

Antibacterial

So far, most antibacterial polymers lack the capability for reactive degradation. These chemically stable polymers can accumulate in the human body or the environment, potentially leading to significant cytotoxicity and inducing bacterial resistance.⁵¹ The biological origin of PLA enables its biodegradability and biocompatibility *in vivo*, allowing for its rapid degradation by *in vivo* reducing agents. What's more, because of the synergistic effect of dynamic disulfide bonds and dynamic non-covalent sites, as well as the presence of modifiable carboxyl groups, this material holds promise as a responsive degradable antibacterial agent.

Zhao *et al.*⁵² coated poly(TA-Zn) on an alkali heat-treated ZE21B alloy scaffold for enhancing the corrosion resistance and blood compatibility of the scaffold (Fig. 3A). Poly(TA-Zn) scavenged free radicals because the released TA molecules could lower 2,2-diphenyl-1-picrylhydrazyl (DPPH) content by electron transfer or hydrogen. The complex interactions between Zn²⁺ and carboxyl groups replace a part of the weak hydrogen bond interactions, thereby enhancing the network stability. Furthermore, REDV peptide was coupled to the scaffold through amidation reactions to promote the re-endothelialization. PLA rich in guanidine groups can be utilized for efficient drug delivery. The cationic groups facilitate the initial binding of the polymer to the negatively charged bacterial cell membrane,



Fig. 3 (A) Schematic diagram of the material design of poly(TA-Zn)-REDV and the function of its enhanced re-endothelialization and suppressed SMC overgrowth. Reprinted from ref. 52 with permission. Copyright 2023 Elsevier. (B) Synthesis route of Dex-g-PSSn and preventative mechanisms toward bacterial resistance. Reprinted from ref. 53 with permission. Copyright 2023, Wiley.

followed by the entry of lipophilic groups into the membrane to exert their function. Poly(TA-Zn) samples could significantly inhibit the growth of *Staphylococcus aureus*, where the average size of the inhibition ring was 1.56 ± 0.27 mm, and the hemolysis rate of blood cells was less than 5%. Mu *et al.*⁵³ functionalized PLA with guanidine groups and grafted it with dextran to serve as an antibiotic adjuvant (Fig. 3B). The PLAs could be depolymerized *via* GSH, preventing the accumulation of non-degradable polycations *in vivo* and effectively reducing cellular toxicity. This approach reduces bacterial resistance to antibiotics by decreasing the bacterial cell membrane permeability or disrupting the efflux pump functions. In the presence

of Dex-*g*-PSS₃₀, neither MDR-AB nor MRSA cells cultured for more than 30 generations developed resistance to various antibiotics, including levofloxacin and tobramycin.

Anti-inflammation

ROS can oxidize low-density lipoprotein (LDL) to form oxidized LDL, which in turn transforms macrophages into foam cells and induces an inflammatory response.^{54,55} PLA has disulfide bonds, which enable its remarkable electrophilicity and free radical reaction ability, such as the removal of $\cdot\text{OH}$, $\cdot\text{NO}$, $\cdot\text{ONOO}$ *in vivo*, or H_2O_2 and HClO . The tightly crosslinked disulfide bonds and negative carboxylate-rich surface of PLA can effectively prevent blood dilution and serum protein adhesion.⁶ However, the stability and hydrophobicity of PLA limit the release of bioactive LA monomers in water, compromising its biological functions. It has been discovered that LA-Na not only retains the polymerization properties of LA but also rapidly decomposes into small LA sodium salts when exposed to water.⁵⁶

Cui *et al.*⁵⁷ prepared a binary synergistic elastomer adhesive patch made from PLA-Na/PLA by mixing an ethanol solution of PLA with an aqueous solution of PLA-Na, followed by evaporation at 37 °C (Fig. 4). This patch exhibited long-term wet-adhesion and anti-inflammatory properties for treating oral ulcers. The carboxyl groups of PLA could achieve firm tissue adhesion through multiple hydrogen bonds and electrostatic interactions with the amino groups on the tissue surface. Additionally, the hydrophobicity of PLA limited water absorption by the patch, and disrupted the orderly arrangement of PLA-Na. The introduction of PLA *via* multi-component $-\text{COOH}\cdots\text{O}=\text{C}-$ hydrogen bonds reduce the dissociation rate of PLA-Na, enabling a slow and sustained release of LA-Na. Immunohistochemical staining further demonstrated that the patch significantly

downregulated the expressions of pro-inflammatory cytokines IL-6 and TNF- α in mini-pig oral mucosa tissue.

Antitumor

The tumor microenvironment plays a crucial role in the initiation, proliferation, invasion, and metastasis of tumor cells. Numerous studies have been conducted to develop therapeutic strategies targeting the acidic environment (with a higher GSH concentration than normal cells), H_2O_2 overexpression, hypoxia, and severe inflammation.²⁷ Disulfide bonds exhibit minimal cytotoxicity toward tumor cells. PLA can undergo rapid *in vivo* biodegradation by reducing agents such as GSH,^{20,58} resulting in the formation of small molecules possessing antioxidant properties, and it exhibits non-conventional thiol-mediated covalent translocation with the cell membrane.³⁹ Therefore, incorporating disulfide bonds into prodrugs allows for their reduction to sulfhydryl groups within the tumor cytoplasm at high GSH concentrations.^{28,59} This enhances the hydrophilicity of the drug system, promotes adjacent chemical bond hydrolysis (such as ester bonds), and facilitates drug release.

Zhu *et al.*⁶⁰ utilized amidation to attach guanidylated lysine derivatives to LA and then polymerized them with methoxypolyethylene glycol mercaptan to obtain bioreducible cell-penetrating polyguanidine (mPEG₂₂₅-*b*-PSS_{*n*}) for delivering plasmids KillerRed-p53 (PSR-p53) in antitumor therapy (Fig. 5). Among them, the agarose gel electrophoresis results showed that disulfide bonds within mPEG₂₂₅-*b*-PSS₂₆ framework were decomposed in the presence of 8 mmol L⁻¹ GSH and greatly facilitated DNA release. The immunohistochemical staining results indicated that the complex formed with pKR-p53 could enter tumor cells through mercaptan mediation and successfully express KillerRed and p53 proteins upon GSH-mediated plasmid release.

Adhesion

Polymer-based biomedical tissue adhesives have attracted widespread attention in the medical field due to their excellent performances. They are suitable for closing and regenerating various tissue injuries. However, their applications are somewhat restricted due to their weak wet adhesive and lack of biological functionality. Adhesive hydrogels have been extensively utilized in biomedical applications,^{61–63} such as wound dressings, sutures, and strain sensors, owing to their high-water content and resemblance to biological soft tissues. Self-healing hydrogels are typically engineered by incorporating dynamic covalent or reversible non-covalent bonds, including disulfide bonds, acylhydrazones, hydrogen bonds, coordination bonds, guest–host interactions, and hydrophobic interactions. PLA possesses disulfide bonds that can strongly bind to tissues, and can introduce a variety of dynamic bonds to fulfill the requirements of adhesive hydrogels.⁶⁴ The natural biocompatible polymer skeleton also facilitates its biological applications.

Chai *et al.*⁶¹ achieved covalent bonding between polydopamine (PDA) and PLA through a Michael addition (reaction between the catechol groups of PDA and the thiols of PLA), while also establishing non-covalent bonding through



Fig. 4 Schematic diagram of the preparation and application of a PLA-based binary synergistic elastomer adhesive patch. Reprinted from ref. 57 with permission. Copyright 2023, Springer.



Fig. 5 Schematic diagram for the preparation and antitumor therapy application of mPEG₂₂₅-b-PSS_n. Reprinted from ref. 60 with permission. Copyright 2022, Wiley.

hydrogen bonds. By introducing PLA as a natural biocompatible skeleton and utilizing PDA as a crosslinking agent, they demonstrated an enhanced adhesion capability and anti-swelling ability. The synergistic effects resulting from the combination of covalent bonds and non-covalent interactions between PLA and PDA imparted a high stretchability, self-healing ability, and toughness to the hydrogels. These hydrogels could be employed as wound dressings for skin defects. Additionally, the presence of PLA in the hydrogel provides antioxidant properties that help eliminate free radicals and inhibit inflammation. Furthermore, PDA's catechol groups can establish strong contact with proteins and cells, thereby promoting cell attachment and proliferation. Cui *et al.*⁶⁵ created a self-stabilized deep eutectic supramolecular polymer adhesive by heating a mixture of LA and LA-Na (*S*-, *R*-isomer, racemate) with LA in one step. When the LA/LA-Na ratio was 2, the adhesion strength of the adhesive was increased significantly in water, from 137 kPa to 1.07 Mpa after soaking in water for 1 day. As a tissue sealant, the adhesive could replace surgical sutures and enhanced wound healing of a rat skin incision.

Flexible electronics

Flexible electronic materials possess both high electrical conductivity and material flexibility, making them highly promising for applications in wearable devices and flexible energy-storage systems. Additionally, to meet the diverse requirements of biomedicine, flexible electronic devices need to exhibit specific properties, such as surface adhesion, permeability, biocompatibility, and long-term stability. Various ionic conductive gel materials, including ionic gels and hydrogels, have been employed for the development of flexible sensors.⁶⁶ Hydrogels offer adjustable mechanical properties and extensibility, but their practical application is often limited due to their poor environmental stability. Ionic gels demonstrate a

non-combustible property with low volatility along with excellent chemical, thermal, and electrochemical stability, which makes them highly suitable for sensing, energy conversion, and separation.^{67–69} Nevertheless, most ionic gels suffer from inadequate mechanical properties. By strategically combining soft segments with hard segments possessing different chemical structures, as well as arranging specific covalent bonds or intermolecular forces to form various concentrated state structures, the overall material properties can effectively be regulated.

In addition to permanent covalent crosslinkers, PLA polymer networks can also introduce disulfide bonds, ionic crosslinks, electrostatic interactions, and hydrogen bonds to provide the desired flexibility, adhesion, and self-healing characteristics required for ideal wearable electronic devices. Moreover, the presence of disulfide bonds within the main chain imparts additional thermal/photosensitivity while promoting remoldability and recyclability. Wang *et al.*⁷⁰ incorporated an ionic liquid into ethanol-induced polymerized PLA to fabricate an ionic gel, which could then serve as a flexible sensor for monitoring the physiological temperature signals of firefighters. The weak hydrogen bond interactions between the ions and the carboxyl groups on the molecular chain promoted molecular chain deformation, facilitating the movement of positive/negative ions within the polymer composite under strain. Consequently, this led to obvious changes in resistance within the composite material. Pei *et al.*⁷¹ synthesized a zwitterionic dynamic elastomer from the copolymerization of LA, 1-ethyl-3-methylimidazo-lipoic acid ([Emim][LA]), and 1-ethyl-3-methylimidazole acetate ([Evim][Ac]) to detect weak physiological activity in the wrist. Dang *et al.*⁷² added acrylic acid (AA), choline chloride (CCL), and Fe³⁺ during the heating polymerization of PLA to obtain a novel LA-based ionic conductor for monitoring human joint activity. In addition to adding iron ions to PLA to introduce coordination bonds to provide good stretchability, Zhang *et al.*³¹ also introduced a mussel-like activity to ensure that the material had high intrinsic adhesion on both wet and dry skin surfaces. The material could be used to prepare breathable skin electrode arrays for stable 12-lead clinical electrocardiography (ECG) monitoring. Khan *et al.*⁷³ prepared an entirely gel-based triboelectric nanogenerator (TEENG) with high stretchability, self-healing, and anti-freezing capabilities to harvest energy by sandwiching a conductive self-healable organohydrogel (CSO) as an electrode between two symmetric non-conductive self-healable organogel (NSO)-based piezoelectric layers (Fig. 6). Besides adding an ionic liquid into the matrix for electrical conductivity, Qu *et al.*⁷⁴ reported an intrinsically conductive assembled network combining reversible disulfide with β -sheet-inspired H-bonding. The amphiphathic LA-derived small molecular system could assemble into an ordered network *via* an evaporation-induced polymerization and exhibited high efficiency in ion transport ($3.56 \times 10^{-3} \text{ S cm}^{-1}$).

Tissue scaffold 3D-printing

3D printing technology holds great potential in tissue engineering and regenerative medicine. It allows for the precise fabrication of personalized scaffold structures, tailored to match

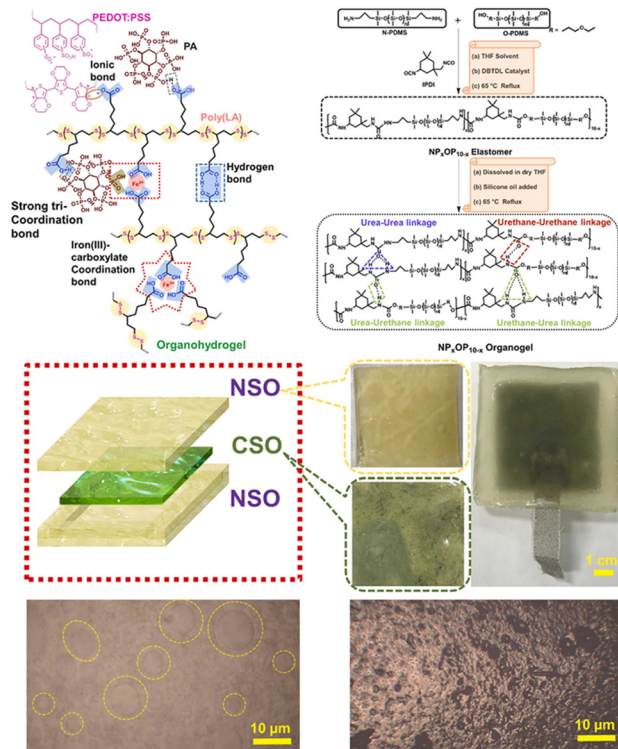


Fig. 6 Synthetic structures of organohydrogels and organogels and diagram of a TENG device. Reprinted from ref. 73 with permission. Copyright 2023, Elsevier.

specific shapes, sizes, pore structures, and porosities.⁷⁵ In tissue engineering, 3D printing scaffolds must provide structural support and create a suitable microenvironment to support cellular behavior. In recent years, the 3D printing of supramolecular materials with hydrogels as the main object has been realized. Cai *et al.*⁷⁶ found that PLA obtained by LA thermally activated dynamic polymerization exhibited a unique time-dependent self-reinforcing pattern (after 3D printing, the mechanical capabilities and stiffness of PLA and LA continued to increase over time). Therefore, LA can be used as an initial material for 3D-fused deposition modeling (3D-FDM).

To achieve biomedical and tissue engineering applications, 3D printing materials need to be biodegradable, biocompatible, and self-repairable, but also need to have sufficient printing capacity, stent reliability, and mechanical properties. Tran *et al.*⁷⁷ found that a poly(malate-co-propylene oxide) copolymer capped with LA and grafted by UV irradiation of the LA-capped backbone polymer could control the printable, mechanical, and cell adhesion of the gel, as well as the reliability of the printed scaffold during 3D printing to obtain a printing scaffold with high resolution and mechanical properties. After that, they then found that adding cell adhesion proteins, such as gelatin and albumin, to the gel made it possible to print biologically functional 3D scaffolds (Fig. 7).³² In *in vivo* studies, four weeks after implantation in mice, protein-loaded scaffolds showed great biocompatibility and increased angiogenesis without an inflammatory response, demonstrating the promise as a printable tough hydrogel ink for tissue engineering.

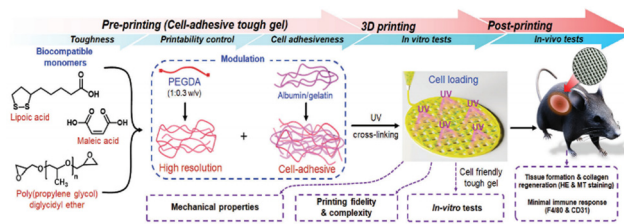


Fig. 7 Process and application scenario of a 3D printing biocompatible protein support scaffold. Reprinted from ref. 32 with permission. Copyright 2023, Wiley.

Conclusions and perspective

In summary, PLA polymerization possesses several advantages, including a simple preparation process, mild conditions, low cost, and easy modification. Polymer materials with dynamic disulfide bonds have exhibited good compatibility, degradation, flexibility, rapid self-healing, and reversible adhesion. Additionally, PLA has demonstrated an antioxidant capacity and can be applied in REDOX diseases, such as for its anti-tumor effects, antibacterial properties, and anti-inflammatory effects.

This review summarized the polymerization strategies of LA, particularly focusing on two polymerization methods: free radical polymerization and thiol-initiated anionic polymerization. Additionally, this work summarized the use of modifiable carboxyl groups to introduce functional moieties or supramolecular interactions for achieving the ordered self-assembly of polymers. Furthermore, leveraging the dynamic disulfide bonds and non-covalent binding sites in PLA can promote enhanced performance and broaden its applications. We also highlight recent advancements in biomedical applications (Table 1), especially in emerging fields, such as hydrogels, biosensors, and 3D-printed tissue scaffolds. Moreover, due to the low toxicity and ease of depolymerization, PLA can serve as a suitable polymer substrate for constructing functional microenvironments. For instance, PLA-based nanoparticles can facilitate the transfer of active molecules, such as mRNA, or construct vesicles for drug-delivery purposes.^{6,25,78} It also offers a viable approach for molecular engineering hybrid materials by regulating optical/electrical properties on inorganic surfaces, like QDs, plasma materials, and perovskites.⁷⁹

However, there are still many challenges in the design and synthesis of PLA-based biomaterials, including around the following themes:

(1) Polymerization methods and mechanisms: currently, the main methods for PLA are still free radical polymerization and thiol-initiated anionic polymerization. However, more diverse polymerization approaches are needed to achieve controlled PLA polymerization in both equilibrium-controlled and non-equilibrium systems. The dynamic properties of this material, such as self-healing ability, stimulus responsiveness, and recyclability, are mostly governed by thermodynamic equilibrium control.

(2) Precise molecular control: achieving structurally controlled PLA relies on a thiol-initiated anionic ROP. However,

Table 1 Summary of the biological applications of PLAs and material characteristics

Bio-applications	Components	Polymeric mechanism	Material properties	Ref.
Antibacterial	Poly(LA-Zn); ZE21B Dex-g-PSS ₁₆ ; rifampicin	Thermal ROP Thiolate-mediated ROP	Self-healing; anti-coagulant; hemocompatibility; promoting re-endothelialization Preventing bacterial resistance; biological absorption; degradation	52 53
Anti-inflammation	PLL hydrogels; NaHCO ₃ ; LiCl Poly LA-Na/poly LA	Concentration-induced ROP Concentration-induced ROP	Facile injectability; adequate adhesiveness; self-healing; conductivity Flexibility; adhesion; wet-resistance	56 57
Antitumor	mPEG _{22.5} -b-PSS ₂₆ ; pKR-p53 or pEGF	Thiolate-mediated ROP	Biocompatibility; degradability; transfection	60
Adhesives	PPA-PLA hydrogel	Thermal ROP	Stretchability; resilience; self-healing; repeatable adhesion; capacity; cell affinity; biodegradation	61
Flexible electronics	PLA/PLA-Na P(LA-co-AM)/[EE] ionogel PLTA-Fe ³⁺ ; SEBS/Au P(LA-co-[Emim][LA]-co-[Evim][Ac]; AlCl ₃)	Thermal ROP Thiolate-mediated ROP Thermal ROP Thermal ROP	Wet-adhesion; biocompatibility; oxidation resistance; antibacterial Tensile strength; toughness; stretchability; transparency, shape-memory designability Stretchability; adhesion; water and air permeability Transparency; adhesion; self-healing; remoldability; recyclability; stretchability; sensing durability; conductivity Self-healability; stretchability; anti-freezing; energy-harvesting	65 70 31 71 73
3D printing	Electrode(PLA; PA; FeCl ₃ ·H ₂ O; PEDOT:PSS); tribolayers(NP ₂ OP _{10-x} organogel) PLA; AA; CCl ₄ ; Fe ³⁺ Poly(STGly- <i>n</i>) PLA/LA MPLE gel; PEDGA; gelatin; albumin PEDGA(LA-capped); MPLE gel	Thiolate-mediated ROP Thermal ROP Thermal ROP Thermal ROP Condensation polymerization; radical graft polymerization Condensation polymerization; radical graft polymerization	Transparency; stretchability; sensing; recyclability Efficient cation conductivity; chemical recyclability Self-reinforcing; time-dependent assembly Bioactivity; biofunctionality; cytocompatibility; bioadhesion; cell spreading; print-ability; long-term stability 3D printability; biocompatibility; adhesiveness; self-healing; tunable swell-ability; degradation	72 74 76 32 77

PXBP: Dex-g-PSS₁₆; dextran-graft-poly(5-(1,2-dithiolan-3-yl)-N-(2-guanidinoethyl)pentanamide). PLL: poly(lipoic acid-co-sodium lipoate). SEBS: styrene ethylene butylene styrene. Emim: 1-ethyl-3-methylimidazole. Evim: 1-ethyl-3-vinylimidazole. PEDOT:PSS: poly(3,4-ethylenedioxythiophene) polystyrene sulfonate. MPLE: poly(maleate-propylene oxide)-lipoatepoly(ethylene oxide). PEGDA: poly(ethylene glycol) diacrylate.

most initiators, such as bases and thiols, need to remain inert. Achieving structural control, such as controlling the relative molecular weight and distribution, is crucial for enhancing the material performance. For example, in tissue engineering, 3D-printing tissue scaffolds require materials with excellent mechanical properties and specific biological functionality. Additionally, synthesizing stereoselective polymers remains challenging.

(3) Clinical translation and industrial-scale synthesis: current research on PLA-based materials is primarily at the laboratory stage. Before clinical adoption, a series of biocompatibility assessments and *in vitro/vivo* tests are necessary. The large-scale production of PLA still faces challenges, especially considering its depolymerization reversibility, which complicates storage.

(4) New applications: with technological advancements, the synergistic use of various tools and materials can broaden the material scales to match different application needs. For instance, simulating and screening synthetic pathways and mechanisms using big data tools, like artificial intelligence, can accelerate material development rates and reduce costs.

LA, an endogenous small molecule, has been widely used in clinical treatments for conditions such as diabetes, Alzheimer's disease, and cancer over the past seventy years.³³ In the latest 10 years, because of its strong antioxidant and rich biological sources, it has attracted widespread attention and experienced rapid development. Despite the remaining unresolved problems and the fact there is a long way to go in terms of clinical applications, further optimization through various of scientific tools is expected to stimulate its application in material design and other cutting-edge fields.

Abbreviations

DHLA	Dihydrolipoic acid
GSH	Glutathione
GSSG	Oxidized glutathione
REDOX	Oxidation-reduction
DPN	Diabetic peripheral neuropathy
QDs	Quantum dots
PDS	Polydimethylsiloxane
LDL	Low-density lipoprotein
Emim	1-Ethyl-3-methylimidazole
Evim	1-Ethyl-3-methylimidazole
ECG	Electrocardiography
TENG	Triboelectric nanogenerator
CSO	Conductive self-healable organohydrogel
NSO	Non-conductive self-healable organogels
3D-FDM	3D-fused deposition modeling

Author contributions

Qing Yu: investigation, methodology, formal analysis, writing – original draft. Zhiyue Fang: writing – review & editing, formal analysis. Shifang Luan: writing – review & editing, conceptualization.

Lei Wang: investigation, methodology, project administration, supervision, funding acquisition, writing – review & editing. Hengchong Shi: supervision, funding acquisition, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- M. D. Spalding and S. T. Prigge, *Microbiol. Mol. Biol. Rev.*, 2010, **74**, 200–228.
- K. Kofuji, T. Isobe and Y. Murata, *Food Chem.*, 2008, **115**, 483–487.
- J. L. Reed, G. B. DeBusk, C. I. Gunsalus and S. C. Hornberger, *Science*, 1951, **114**, 93–94.
- J. M. May, Z.-c Qu and D. J. Nelson, *Clin. Biochem.*, 2007, **40**, 1135–1142.
- S. K. Azzam, H. Jang, M. C. Choi, H. Alsafar, S. Lukman and S. Lee, *Mol. Pharmaceutics*, 2018, **15**, 2098–2106.
- X. Lu, Z. He, X. Xiao, X. Wei, X. Song and S. Zhang, *Small*, 2023, **19**, 2303459.
- M. J. Jeon, W. G. Kim, S. Lim, H.-J. Choi, S. Sim, T. Y. Kim, Y. K. Shong and W. B. Kim, *Mol. Cell. Endocrinol.*, 2015, **419**, 113–123.
- P. P. Phiboonchaiyanan and P. Chanvorachote, *Cell. Oncol.*, 2017, **40**, 497–510.
- J. Tripathy, A. Tripathy, M. Thangaraju, M. Suar and S. Elangovan, *Life Sci.*, 2018, **207**, 15–22.
- D. Ziegler, M. Reljanovic, H. Mehnert and F. A. Gries, *Exp. Clin. Endocrinol. Diabetes*, 1999, **107**, 421–430.
- A. Kisanuki, Y. Kimpara, Y. Oikado, N. Kado, M. Matsumoto and K. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 2010, **48**, 5247–5253.
- Y. Liu, Y. Jia, Q. Wu and J. S. Moore, *J. Am. Chem. Soc.*, 2019, **141**, 17075–17080.
- Q. Zhang, C.-Y. Shi, D.-H. Qu, Y.-T. Long, B. L. Feringa and H. Tian, *Sci. Adv.*, 2018, **4**, eaat8192.
- Y. Deng, Q. Zhang, C. Shi, R. Toyoda, D.-H. Qu, H. Tian and B. L. Feringa, *Sci. Adv.*, 2022, **8**, eabk3286.
- C.-Y. Shi, D.-D. He, B.-S. Wang, Q. Zhang, H. Tian and D.-H. Qu, *Angew. Chem., Int. Ed.*, 2022, **62**, e202214422.
- K. R. Albanese, P. T. Morris, J. Read de Alaniz, C. M. Bates and C. J. Hawker, *J. Am. Chem. Soc.*, 2023, **145**, 22728–22734.
- J. Gao, Q. Zhang, B. Wu, X. Gao, Z. Liu, H. Yang, J. Yuan and J. Huang, *Small*, 2023, **19**, 2207334.
- K. X. Hou, S. P. Zhao, D. P. Wang, P. C. Zhao, C. H. Li and J. L. Zuo, *Adv. Funct. Mater.*, 2021, **31**, 2107006.
- D. Chao, S. Changyou, L. Hongchen, C. Yian and Q. Haisong, *Nano Energy*, 2021, **90**, 106619.
- E.-K. Bang, G. Gasparini, G. Molinard, A. Roux, N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2013, **135**, 2088–2091.
- S. Huang, Y. Shen, H. K. Bisoyi, Y. Tao, Z. Liu, M. Wang, H. Yang and Q. Li, *J. Am. Chem. Soc.*, 2021, **143**, 12543–12551.
- C. Dang, F. Zhang, Y. Li, Z. Jin, Y. Cheng, Y. Feng, X. Wang, C. Zhang, Y. Chen, C. Shao, Q. Zheng and H. Qi, *Small*, 2022, **18**, 2200421.
- Q. Zhang, D.-H. Qu, B. L. Feringa and H. Tian, *J. Am. Chem. Soc.*, 2022, **144**, 2022–2033.
- C. D. Vo, G. Kilcher and N. Tirelli, *Macromol. Rapid Commun.*, 2009, **30**, 299–315.
- J. Lu, Z. Xu, H. Fu, Y. Lin, H. Wang and H. Lu, *J. Am. Chem. Soc.*, 2022, **144**, 15709–15717.
- M. C. Montasell, P. Monge, S. Carmali, L. M. Dias Loiola, D. G. Andersen, K. B. Løvschall, A. B. Søgaard, M. M. Kristensen, J. M. Pütz and A. N. Zelikin, *Nat. Commun.*, 2022, **13**, 4861.
- Z. Zhang, Q. Cao, Y. Xia, C. Cui, Y. Qi, Q. Zhang, Y. Wu, J. Liu and W. Liu, *Bioact. Mater.*, 2023, **31**, 408–421.
- D. Zhong, H. Wu, Y. Wu, Y. Li, X. Xu, J. Yang and Z. Gu, *Nanoscale*, 2019, **11**, 15091–15103.
- D. Pei, S. Yu, P. Liu, Y. Wu, X. Zhang, Y. Chen, M. Li and C. Li, *Adv. Funct. Mater.*, 2022, **35**, 2204257.
- Y. Wang, Y. Zhang, Z. Zhang, T. Li, J. Jiang, X. Zhang, T. Liu, J. Qiao, J. Huang and W. Dong, *ACS Nano*, 2022, **16**, 3394–3403.
- Z. Jun, H. Mingshuang, Z. Xinran, W. Huiquan, L. Xiangxiang, Z. Xiaoying and Y. Hui, *Adv. Mater. Technol.*, 2023, **8**, 2300243.
- H. N. Tran, I. G. Kim, J. H. Kim, A. Bhattacharyya, E.-J. Chung and I. Noh, *Macromol. Biosci.*, 2023, **23**, 2300316.
- S.-Y. Lv, S. He, X.-L. Ling, Y.-Q. Wang, C. Huang, J.-R. Long, J.-Q. Wang, Y. Qin, H. Wei and C.-Y. Yu, *Int. J. Pharm.*, 2022, **627**, 122201.
- M. D. M. Viana, P. S. S. Lauria, A. A. D. Lima, L. C. F. Opretzka, H. R. Marcelino and C. F. Villarreal, *Antioxidants*, 2022, **11**, 2420.
- M. H. Brookes, B. T. Golding, D. A. Howes and A. T. Hudson, *J. Chem. Soc., Chem. Commun.*, 1983, 1051–1053.
- L. M. Gaetke and C. K. Chow, *Toxicology*, 2003, **189**, 147–163.
- T. M. Hagen, R. T. Ingersoll, J. Lykkesfeldt, J. Liu, C. M. Wehr, V. Vinarsky, J. C. Bartholomew and A. B. Ames, *FASEB J.*, 1999, **13**, 411–418.
- C. H. Park, K.-U. Lee, J.-Y. Park, E.-H. Koh, H.-S. Kim and J. Lee, *Pharmazie*, 2010, **65**, 580–584.
- R. Martinent, D. Du, J. López-Andarias, N. Sakai and S. Matile, *ChemBioChem*, 2020, **22**, 253–259.
- C. Carbone, E. Arena, V. Pepe, O. Prezzavento, I. Cacciatore, H. Turkez, A. Marrazzo, A. Di Stefano and G. Puglisi, *Colloids Surf., B*, 2017, **155**, 238–247.

- 41 R. C. Thomas and L. J. Reed, *J. Am. Chem. Soc.*, 1956, **78**, 6148–6149.
- 42 K. Endo, T. Shiroy and N. Murata, *Polym. J.*, 2005, **37**, 512–516.
- 43 X. Zhang and R. M. Waymouth, *J. Am. Chem. Soc.*, 2017, **139**, 3822–3833.
- 44 Y. Wang, S. Sun and P. Wu, *Adv. Funct. Mater.*, 2021, **31**, 2101494.
- 45 H. Chen, T. Liu, P. Zhou, S. Li, J. Ren, H. He, J. Wang, N. Wang and S. Guo, *Adv. Mater.*, 2020, **32**, 1905661.
- 46 Z. Chen, Q. Cheng, H. Chen, Y. Wu, J. Ding, X. Wu, H. Yang, H. Liu, W. Chen, X. Tang, X. Lu, Y. Li and Y. Li, *Adv. Mater.*, 2023, **35**, 2300513.
- 47 M. M. Kristensen, K. B. Løvschall and A. N. Zelikin, *ACS Macro Lett.*, 2023, **12**, 955–960.
- 48 H. Yang, W. Shen, W. Liu, L. Chen, P. Zhang, C. Xiao and X. Chen, *Biomacromolecules*, 2018, **19**, 4492–4503.
- 49 T. Du, B. Shen, J. Dai, M. Zhang, X. Chen, P. Yu and Y. Liu, *J. Am. Chem. Soc.*, 2023, **145**, 27788–27799.
- 50 B.-S. Wang, Q. Zhang, Z.-Q. Wang, C.-Y. Shi, X.-Q. Gong, H. Tian and D.-H. Qu, *Angew. Chem., Int. Ed.*, 2023, **62**, e202215329.
- 51 M. Haktaniyan and M. Bradley, *Chem. Soc. Rev.*, 2022, **51**, 8584–8611.
- 52 Z. Zhao-Qi, T. Pei-Duo, W. Li, Q. Zheng-Hui, L. Jing-An, L. Hang, G. Shao-Kang, L. Cun-Guo and W. Hong-Yan, *Chem. Eng. J.*, 2022, **451**, 139096.
- 53 S. Mu, Y. Zhu, Y. Wang, S. Qu, Y. Huang, L. Zheng, S. Duan, B. Yu, M. Qin and F.-J. Xu, *Adv. Mater.*, 2022, **34**, 2204065.
- 54 R. Salvayre, A. Negre-Salvayre and C. Camaré, *Biochimie*, 2016, **125**, 281–296.
- 55 H. Xu, P. She, B. Ma, Z. Zhao, G. Li and Y. Wang, *Biomaterials*, 2022, **288**, 121734.
- 56 W. Fang, D. Jiaqiang, Q. Hao, L. Dongfan, G. Dong, C. Jinjin, Z. Yanfeng, C. Yilong and H. Xijing, *Chem. Eng. J.*, 2023, **466**, 143071.
- 57 C. Cui, L. Mei, D. Wang, P. Jia, Q. Zhou and W. Liu, *Nat. Commun.*, 2023, **14**, 7707.
- 58 G. Gasparini, E. K. Bang, G. Molinard, D. V. Tulumello, S. Ward, S. O. Kelley, A. Roux, N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2014, **136**, 6069–6074.
- 59 C. Wang, B. Wang, S. Zou, B. Wang, G. Liu, F. Zhang, Q. Wang, Q. He and L. Zhang, *Biomater. Sci.*, 2021, 5977–5987.
- 60 Y. Zhu, M. Lin, W. Hu, J. Wang, Z.-G. Zhang, K. Zhang, B. Yu and F.-J. Xu, *Angew. Chem., Int. Ed.*, 2022, **61**, e202200535.
- 61 C. Chai, Y. Guo, Z. Huang, Z. Zhang, S. Yang, W. Li, Y. Zhao and J. Hao, *Langmuir*, 2020, **36**, 10448–10459.
- 62 L. Caikun, L. Ruilin, J. Mengqi, X. Xiao, C. Yun, L. Pengfei and Z. Shiyong, *Chem. Mater.*, 2023, **35**, 2588–2599.
- 63 X. Ke, S. Tang, Z. Dong, K. Ren, P. Yu, X. Xu, J. Yang, J. Luo and J. Li, *Chem. Eng. J.*, 2022, **443**, 136206.
- 64 C.-Y. Shi, D.-D. He, Q. Zhang, F. Tong, Z.-T. Shi, H. Tian and D.-H. Qu, *Natl. Sci. Rev.*, 2023, **10**, nwac139.
- 65 C. Cui, Y. Sun, X. Nie, X. Yang, F. Wang and W. Liu, *Adv. Funct. Mater.*, 2023, **33**, 2307543.
- 66 L. Hu, P. L. Chee, S. Sugiarto, Y. Yu, C. Shi, R. Yan, Z. Yao, X. Shi, J. Zhi, D. Kai, H.-D. Yu and W. Huang, *Adv. Mater.*, 2023, **35**, 2205326.
- 67 Y. Ren, Z. Liu, G. Jin, M. Yang, Y. Shao, W. Li, Y. Wu, L. Liu and F. Yan, *Adv. Mater.*, 2021, **33**, 2008486.
- 68 G. Sun, P. Wang, Y. Jiang, H. Sun, T. Liu, G. Li, W. Yu, C. Meng and S. Guo, *Nano Energy*, 2023, **110**, 108367.
- 69 D. Gao, G. Thangavel, J. Lee, J. Lv, Y. Li, J.-H. Ciou, J. Xiong, T. Park and P. S. Lee, *Nat. Commun.*, 2023, **14**, 1990.
- 70 J. Wang, Y. Zheng, T. Cui, T. Huang, H. Liu, J. Zhu, L. Song and Y. Hu, *Adv. Funct. Mater.*, 2024, **34**, 2312383.
- 71 D. Pei, S. Yu, X. Zhang, Y. Chen, M. Li and C. Li, *Chem. Eng. J.*, 2022, **445**, 136741.
- 72 C. Dang, M. Wang, J. Yu, Y. Chen, S. Zhou, X. Feng, D. Liu and H. Qi, *Adv. Funct. Mater.*, 2019, **29**, 1902467.
- 73 K. Amir, G. Sreekanth, W. Chia-Hua, L. Hong-Wei, P. Yi-Fang, I. W. Judy, G. Dipti, L. Ying-Chih and L. Hong-Cheu, *Nano Energy*, 2021, **90**, 106525.
- 74 C.-Y. Shi, Q. Zhang, B.-S. Wang, D.-D. He, H. Tian and D.-H. Qu, *CCS Chem.*, 2022, **5**, 1422–1432.
- 75 C. Wang, W. Huang, Y. Zhou, L. He, Z. He, Z. Chen, X. He, S. Tian, J. Liao, B. Lu, Y. Wei and M. Wang, *Bioact. Mater.*, 2020, **5**, 82–91.
- 76 C. Cai, S. Wu, Y. Zhang, F. Li, Z. Tan and S. Dong, *Adv. Sci.*, 2022, **9**, 2203630.
- 77 H. N. Tran, I. G. Kim, J. H. Kim, E.-J. Chung and I. Noh, *Biomater. Res.*, 2022, **26**, 75.
- 78 Q. Zhang, Y.-X. Deng, H.-X. Luo, C.-Y. Shi, G. M. Geise, B. L. Feringa, H. Tian and D.-H. Qu, *J. Am. Chem. Soc.*, 2019, **141**, 12804–12814.
- 79 W. Fang, Z. Mu, Y. He, K. Kong, K. Jiang, R. Tang and Z. Liu, *Nature*, 2023, **619**, 293–299.