

Progress in ATRP-derived materials for biomedical applications

Abbreviations: 3D, Three-dimensional; β -CD, β -cyclodextrin; ϵ -CL, ϵ -caprolactone; μ L-scale, Microliter-scale; ADM, Adamantane; AEMA, Acetyl ethyl methacrylate; AGET, Activators generated by electron transfer; APTES, (3-aminopropyl)triethoxysilane; ARGET, Activators regenerated by electron transfer; ATRA, Atom transfer radical addition; ATRP, Atom transfer radical polymerization; AuNR, Gold nanorod; BiB, Bromoisobutryl; BiBADA, 12-(2-bromoisobutyramido)dodecanoic acid; BiBB, Bromoisobutryl bromide; bioATRP, Biocatalytic ATRP; BPC, Biomolecule–polymer conjugate; bPEA, PEA brushes; CA, Contrast agent; CAP, Cell-affinity peptide; Chol, Cholesterol; CNT, Carbon nanotube; CPT, Camptothecin; CRP, Controlled radical polymerization; CSTR, Continuous stirred-tank reactor; CuAAC, Copper-catalyzed azide/alkyne cycloaddition; CYFRA 21–1, Cytokeratin fragment antigen 21–1; DDS, Drug delivery systems; DLC, Diamond-like carbon; DNH, Double-network hydrogel; DOX, Doxorubicin; DyDS, Dynamic delivery system; eATRP, Electrochemically mediated ATRP; ECM, Extracellular matrix; EPR, Enhanced permeability and retention; FA, Focal adhesion; FITC, Fluorescein-5-isothiocyanate; FMMA, Ferrocenylmethyl methacrylate; FN, Fibronectin; FR/NIR, Far-red/near-infrared; FRP, Free radical polymerization; GCE, Glassy carbon electrode; Gd, Gadolinium; GQD, Graphene quantum dot; HAP, Hydroxyapatite; HAP NC, HAP nanocrystal; HEMA, Hydroxyethyl methacrylate; hMSC, Human mesenchymal stem cell; HUVEC, Human umbilical vein endothelial cell; I_2 , Free radical initiator; ICAR, Initiators for continuous activator regeneration; QD, Quantum dot; LCST, Lower critical solution temperature; LOD, Limit of detection; M°/L , Activator or catalyst; Me₆TREN, tris[2-(dimethylamino)ethyl]amine; mechanoATRP, Mechanically induced ATRP; miRNA, MicroRNA; MMSN, Magnetic mesoporous silica nanosphere; MnB, Magnetic nanobeads; Mo, Monomer; MOF, Metal-organic framework; MSN, Mesoporous silica nanoparticle; MWCNT, Multi-walled carbon nanotubes; MWCNT-COOH, Carboxyl-functionalized MWCNTs; NHC, N-heterocyclic carbene; NMP, Nitroxide-mediated polymerization; NP, Nanoparticle; N-ATRP, Normal ATRP; OCL, Hydrophobic oligo(caprolactone); OEG, Oligo(ethylene glycol); OEGMA, Oligo(ethylene glycol) monomethacrylate; OSA, Oligosaccharides; O-ATRP, Organocatalyzed ATRP; O-NB, O-nitrobenzyl; PAA, Poly(acrylic acid); PAM, Polyacrylamide; PBHP, Polymer Brush Hypersurface Photolithography; PBIB, Propargyl 2-bromoisobutyrate; PBIBEM, Poly(2-(2-bromoisobutyryloxy)ethyl methacrylate); PBP, Polymer brush pattern; PBS, Phosphate buffered saline; PbS, Lead sulfide; PC, Photoredox catalyst; PCLdiUPy, UPy-modified polycaprolactone; PDA, Polydopamine; PDC, Polymer–drug conjugate; P(DMAEMA-co-PHEMA), Poly([2-(dimethylamino) ethyl methacrylate]-co-[2-hydroxyethyl methacrylate]); PDMS, Poly(dimethylsiloxane); Pdot, Semiconducting polymer dot; PDT, Photodynamic therapy; PEA, Poly(ethyl acrylate); PEEK, Poly(ether ether ketone); PEG, Poly(ethylene glycol); PEGMA, Poly(ethylene glycol) methacrylate; PEI, Polyethylenimine; PET, Positron emission tomography; PF, Polyfluorene; PFTB, Poly(fluorene-alt-(4,7-bis(hexylthien)-2,1,3-benzothiadiazole)); PgA, Propargyl acrylate; PHEMA, Poly(2-hydroxyethyl methacrylate); photoATRP, Photoinduced ATRP; PISA, Polymerization-induced self-assembly; PLGA, Poly(lactic-co-glycolic acid); PLLA, Poly-L-lactic acid; PMCP, Poly[2-(methacryloyloxy)ethyl choline phosphate]; PMETA, Poly([2-methacryloyloxyethyl] trimethyl ammonium chloride); PMA, Poly(methyl acrylate); PMMA, Poly(methyl methacrylate); PMPC, Poly(2-methacryloyloxyethyl phosphorylcholine); PnBA, Poly(n-butyl acrylate); PNBA, Poly(2-nitrobenzyl acrylate); PNIPAAm, Poly(N-isopropylacrylamide); PNMEP, Poly[N-(2-methacryloyloxyethyl)pyrrolidone]; PNuA, Peptide nucleic acid; POEOMA, Poly[oligo(ethylene oxide) monomethyl ether methacrylate]; POEGMA-b-PHEMA, Poly(oligo(ethylene glycol) monomethacrylate)-b- Poly(2-hydroxyethyl methacrylate); P(EGMA-co-GMA), Poly([ethylene glycol methyl ether methacrylate]-co-[glycidyl methacrylate]); PPC, Protein–polymer conjugate; PPE, Poly(p-phenyleneethynylene); PPV, Poly(p-phenylenevinylene); PSA, Polysaccharides; PSBMA, Poly(2-(methacryloyloxy) ethyl) dimethyl-(3-sulfopropyl) ammonium hydroxide; PSEMA, Poly(sulfoethyl methacrylate); PSPMA, Poly(3-sulfopropyl methacrylate potassium salt); PTA, Poly[2-(*tert*-butylaminoethyl) methacrylate]; PtBA, Poly(*tert*-butyl acrylate); PTH, Phenylphenothiazine; PTh-g-PAU, Poly(thiophene)-g-poly(acrylate urethane); PTT, Photothermal therapy; PVA, Polyvinyl alcohol; PVDF, Poly(vinylidene fluoride); RAFT, Addition–fragmentation chain transfer; RBC, Red blood cell; RDPD, Reactive dissipative particle dynamics; RDRP, Reversible deactivation radical polymerizations; ROMP, Ring-opening metathesis polymerization; ROS, Reactive oxygen species; R-ATRP, Reverse ATRP; R-X, Alkyl halide or initiator; SARA, Supplemental activator and reducing agent; SAv, Streptavidin; SbMA, Sulfobetaine methacrylate; SBMA, 2-(methacryloyloxy) ethyl) dimethyl-(3-sulfopropyl) ammonium hydroxide; SCP, Sequence-controlled polymer; SDA, Strand-displacement amplification; SDP, Sequence-defined polymers; seATRP, Sacrificial-anode ATRP; siRNA, Small interfering RNA; SMC, Smooth muscle cell; SPECT, Single photon emission computed tomography; SPMA, 3-sulfopropyl methacrylate potassium; SR&NI, Simultaneous reverse and normal initiation; SI-ATRP, Surface-initiated ATRP; SI-CRP, Surface-initiated CRP; SS-ATRP, Subsurface-initiated ATRP; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl; TERM, Tissue engineering and regenerative medicine; t-DNA, Target DNA; Ti-P15, Ti plates with randomly adsorbed P15; UCNP, Lanthanide-doped upconverting nanoparticle; UPy, Ureidopyrimidinone; X-M^{t+1}/L, Transition metal complex in its higher oxidation state.

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ABSTRACT

The continuing wave of technological breakthroughs and advances is critical for engineering well-defined materials, particularly biomaterials, with tailored microstructure and properties. Over the last few decades, controlled radical polymerization (CRP) has become a very promising option for the synthesis of precise polymeric materials with an unprecedented degree of control over molecular architecture. Atom transfer radical polymerization (ATRP), one of the most robust and efficient CRPs, has been at the forefront of the synthesis of well-defined polymers with controlled/predetermined molecular weights, polydispersity, topology, composition, and site-specific functionality. ATRP has been leveraged to prepare a wide range of polymers with properties tailored for a number of biomedical applications. Furthermore, ATRP can also be utilized to introduce stimuli-responsive properties into the chemical structure of polymers. Moreover, the degradation behavior of ATRP-derived polymers can be tailored by incorporating chemical bonds susceptible to hydrolysis or proteolysis. This strategy allows the design of degradable polymers for *in vivo* applications. This review summarizes the recent advances in ATRP for the design of functional materials and techniques implemented to advance the biomedical field, such as surface modification and functionalization. Additionally, the latest applications and progress of ATRP-derived materials in various biomedical arenas such as drug delivery, tissue engineering, bioimaging, and biosensing are reported. Lastly, the current limitations and future perspectives of ATRP-derived biomaterials are carefully discussed to support further improvement of their properties and performance for translatability into the clinic. Moving forward, there is a need for further development of ATRP to align with green chemistry principles. This entails exploring the use of renewable monomers, environmentally friendly and nontoxic solvents, as well as metal-free and biocompatible catalysts. Additionally, researchers should thoroughly investigate the bioactivity, biodegradation behavior, and *in vivo* fate of ATRP-derived polymers and polymer conjugates before considering their translation into clinical applications.

1. Introduction

The controlled formation of robust carbon–carbon bonds capable of withstanding the thermomechanical vibrations of macromolecules is a fundamental concept essential for creating diverse complex molecular architectures in both natural and modern organic chemistry [1,2]. The versatility of carbon chemistry has facilitated the fabrication of a wide range of organic materials, encompassing small molecules to substantial macromolecular structures, including emerging synthetic polymers [3,4]. Over the past three decades, chemical and materials science advancements have transformed health science, leading to significant progress in using biomaterials for medical applications [5–10]. However, for *in vivo* applications, candidate materials must meet strict criteria concerning biocompatibility, biodegradation, and bioactivity. In some instances, they may also require specific self-assembly properties, immune system modulation or evasion, and the ability to change their size or morphology [11–17]. These requirements drive the development of novel biomaterials, known as mission-oriented biomaterials, through molecular design and engineering of functional polymers [18–23]. Correspondingly, emerging biomaterials design is experiencing a paradigm shift from designing simple structures to tailor-making complex structures for specific applications [24]. Such properties originate not only from the chemical composition, but also from the architecture and sequence of monomers in polymer chains [25].

Accordingly, the controlled formation of carbon–carbon bonds is crucial for novel (bio)molecular design, which cannot be achieved primarily through conventional synthetic approaches like free radical polymerization (FRP) [26]. Consequently, there has been an urgent need to develop controlled polymerization techniques to synthesize polymers with well-defined molecular structures and predefined functions [27]. In classical FRP, a high concentration of reactive radicals leads to rapid propagation of growing chains, resulting in random formation of carbon–carbon bonds. Moreover, the abundant reactive radicals are prone to collision, leading to rapid termination reactions. These phenomena hinder FRP from achieving macromolecules with highly controlled architecture and low polydispersity. Despite the development of anionic living polymerization, which allows control of structure and composition of

polymers, achieving pure living polymerization through radical polymerization is not possible [28].

Fortunately, controlled radical polymerization (CRP) techniques, also known as reversible-deactivation radical polymerization (RDRP), emerged in the mid-1990 s, providing polymer chemists with an invaluable toolbox to create polymers with controlled and predictable features [27,29]. In contrast to living polymerization, RDRP may involve termination and/or chain transfer reactions. As schematically illustrated in Fig. 1A, the key concept of RDRP techniques is to establish a dynamic equilibrium between active (growing radicals) and dormant states, overcoming the limitations of achieving well-defined polymers through radical polymerization [30]. In RDRP, the rate constant of the backward reaction (k_d) is significantly higher than that of the forward reaction (k_a). As a result, the concentration of free radicals (P_n^*) is very low compared to FRP, leading to a reduced number of termination reactions. This radical-starving mechanism in RDRP causes a linear increase in molecular weight and an exponential decrease in polydispersity as the reaction progresses (Fig. 1B). Additionally, while the lifetime of radicals is short, lasting only about 1 s in FRP, the reversible deactivation of propagating radicals significantly extends the lifespan of radicals in the RDRP mechanism, as shown in Fig. 1C. Nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP), and reversible addition–fragmentation chain transfer (RAFT) are the most popular CRP methods. Reversible deactivation of propagating radicals in ATRP and NMP, and degenerative transfer in RAFT, offer control of the chemical makeup of the synthesized polymers and, thus, the structure and properties of the resulting material [27,31]. These more-desirable strategies for material design are classified as living polymerizations due to a lower concentration of radicals during chain propagation and a decrease in termination rates through the introduction of a dynamic equilibrium with a dormant state of the polymer chains.

Because of its robust and versatile nature, tolerance to the impurities and trace amounts of oxygen, commercial availability of reagents, and facile reaction setup, ATRP has become the most-widely utilized CRP technique [33]. ATRP, developed by Matyjaszewski and Sawamoto in the mid-1990 s, stands as one of the most effective and widely used methods for conducting CRP [34,35]. Since its inception, modified versions of ATRP have been developed to design new polymers or polymer conjugates [36,37]. ATRP and its modified versions have been widely used to make well-defined polymers with unique architectural features (e.g., star polymers) and tailored properties (e.g., programmed degradation) [38–40]. For instance, electrochemically mediated ATRP (eATRP) was utilized to

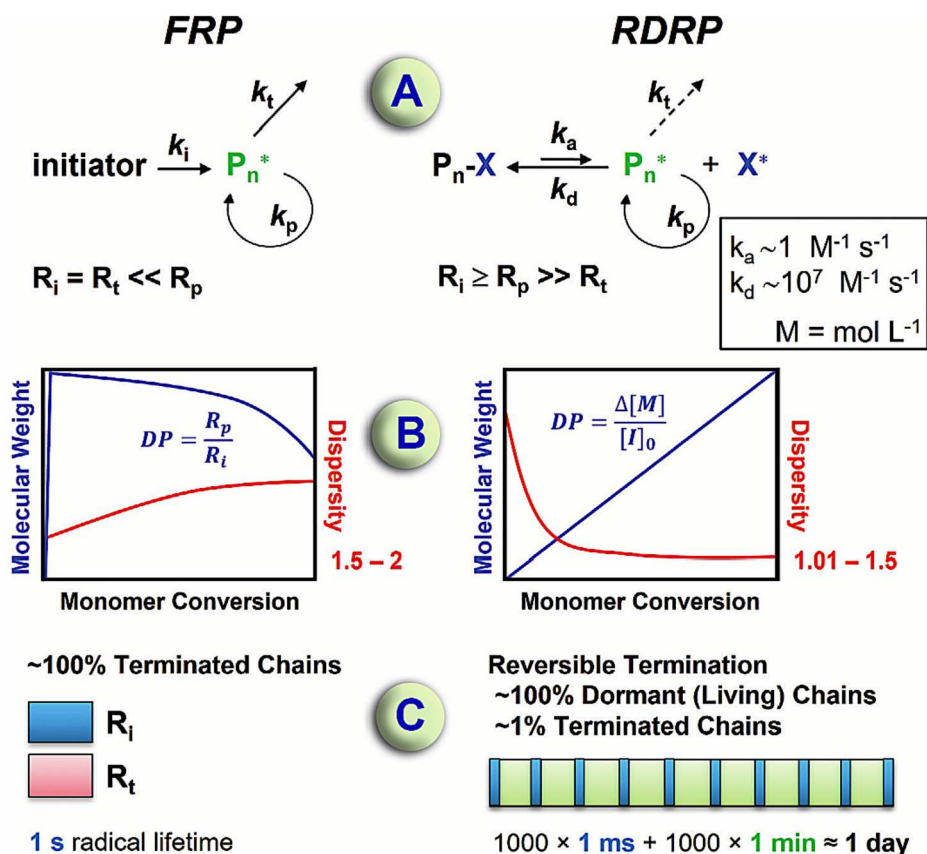


Fig. 1. Comparison of radical polymerization processes. (A) Free radical polymerization (FRP) and reversible-deactivation radical polymerization (RDRP). In FRP, the rate of initiation, termination, and propagation reactions is denoted by R_i , R_t , and R_p , respectively, with corresponding rate constants k_i , k_t , and k_p . In contrast, RDRP involves radical activation and deactivation, with rate constants denoted as k_a and k_d , respectively. The termination rate for RDRP strategies is significantly lower than for FRP, owing to the low concentration of radicals (P_n^*), which prolongs the lifetime of dormant chains. Additionally, k_d is significantly greater than k_a , promoting the backward reaction and leading to the accumulation of dormant radicals. Reproduced with permission from [32]. Copyright 2021 Elsevier.

Table 1

Applications of ATRP in the development of advanced and complex materials: Possible ATRP reaction pathways, benefits, and shortcomings.

Classification	Reagents	Catalyst level (ppm)	Oxygen tolerance	Comments, advantages, and disadvantages	Ref
N-ATRP	R-X, M^I/L , Mo	~5000	Weak	- Approaching equilibrium from left side - Extremely low concentration of radicals - High catalyst content - Irreversible formation and accumulation of $X-M^{I+1}/L$ species - Not cytocompatible	[34]
R-ATRP	R-X, $X-M^{I+1}/L$, Mo, I_2	~5000	Weak	- Approaching equilibrium from right side - Higher stability of M^{I+1}/L <i>In situ</i> formation of activator - Unfavorable creation of auxiliary radical species (I^*) - High catalyst content - Not cytocompatible	[147]
Modified R-ATRP	R-X, $X-M^{I+1}/L$, Mo, Cu(0)/Fe(0)		Weak	- Approaching equilibrium from right side - Cytocompatibility (especially when using Fe(0)) - Higher stability of M^{I+1}/L <i>In situ</i> formation of activator	[148]
SR&NI ATRP	R-X, M^I/L , $X-M^{I+1}/L$, Mo, I_2	~2000	Weak	- Approaching equilibrium from both left and right sides - Higher stability of M^{I+1}/L <i>In situ</i> formation of activator plus addition of activator - Unfavorable creation of auxiliary radical species (I^*) - High catalyst content	[69]
AGET ATRP	R-X, $X-M^{I+1}/L$, Mo, reducing agent (e.g., ascorbic acid)	<500	Moderate	- Approaching equilibrium from right side - Higher stability of M^{I+1}/L <i>In situ</i> formation of activator - Moderate oxygen tolerance because reducing agents remove dissolved oxygen - Highly active catalyst complexes can be used, in contrast to N-ATRP - Unfavorable addition of auxiliary chemicals	[149]
ARGET ATRP	R-X, $X-M^{I+1}/L$, Mo, FDA-approved reducing agent (e.g., ascorbic acid)	<50	Moderate	- Relatively high catalyst content - Continuous regeneration of highly active catalyst complexes - Polymerization rate depends on activator regeneration rate - Highly active catalyst complexes can be used - Low catalyst content (10-fold lower than AGET ATRP) which inhibits irreversible formation and accumulation of $X-M^{I+1}/L$ species - Green synthesis procedure (low catalyst level, inexpensive ligands, FDA-approved reducing agents) - Oxidized byproduct impurities in the obtained polymer resulting from auxiliary reducing agents	[72,150]
ICAR ATRP	R-X, $X-M^{I+1}/L$, Mo, I_2	<100	Moderate	- Mechanism similar to ARGET ATRP process but uses free radical initiator to regenerate activator - Polymerization rate depends on I_2 decomposition rate - Very low catalyst content which does not require removal after polymerization (in most applications) I_2 decomposition and I^* release is carried out at lower temperature compared to ARGET ATRP - Byproduct impurities in the obtained polymer resulting from free radical initiators	[151]

(continued on next page)

Table 1 (continued)

Classification	Reagents	Catalyst level (ppm)	Oxygen tolerance	Comments, advantages, and disadvantages	Ref
SARA ATRP	R-X, X-M ^{t+1} /L, Mo, SARA agent (zerovalent metals, reducing agents)	<50	Moderate	• Cu(0) plays roles both in activation and reactivation processes • Other zerovalent metals (silver, zinc, magnesium) can be used instead of copper • Possible to use (in)organic reducing agents • Biocompatible • Low catalyst content • Oxidized byproduct impurities in the obtained polymer resulting from auxiliary reducing agents	[148]
eATRP	R-X, X-M ^{t+1} /L, Mo	<50	Moderate	• Direct electron transfer from electrodes to reduce deactivator, i.e., deactivator regeneration via an electrochemical stimulant (external stimulant) • Creation of fewer byproducts and a more benign process • Low catalyst content • Biocompatible • Polymerization conditions can be fine-tuned through external parameters (applied potential, current) • Problems when used in bulk insulating systems, where electron transfer from electrode surface to inner layers of insulating materials can be problematic	[84,88]
photoATRP	R-X, X-M ^{t+1} /L, Mo, (photosensitizers)	<10	Moderate to high (for PICAR ATRP)PICAR: photoinduced initiators for continuous activator regeneration	• Uses exogeneous stimuli, similar to eATRP • Harnesses photons' energy by ligands/dyes and releases electrons to regenerate catalyst • Benign process • Low catalyst content • Biocompatible • High spatiotemporal control over polymerization • Polymerization conditions can be fine-tuned through external parameters (applied potential, current) • Problems when used in opaque or translucent systems • Impurities from PC residue (byproducts)	[152,153]
O-ATRP (metal-free ATRP)	R-X, X-M ^{t+1} /L, Mo, PC	<50	Moderate	• O-ATRP is a type of photoATRP in which catalyst regeneration is mediated by photoredox catalyst (PC) • Metal-free and biocompatible • Shallow penetration of UV/visible light into body tissue • Impurities from PC residue (byproducts)	[154,155]
mechanoATRP	R-X, X-M ^{t+1} /L, Mo, mechanophores	0	Moderate	• Uses exogeneous stimuli, similar to photoATRP • Harnesses mechanical energy by mechanophores and releases electrons to regenerate catalyst • Metal-free and biocompatible • Remote activation using mechanophores with high spatiotemporal control over polymerization • Deep tissue penetration • Impurities from mechanophore residue (byproducts)	[156,157]
bioATRP	R-X, X-M ^{t+1} /L, Mo, auxiliary enzyme system	0	High	• Utilizes enzymes or cell/organism processes to catalyze an ATRP reaction • Bio-benign reaction conditions, biodegradation, and non-toxicity • High oxygen tolerance and even oxygen-fueled ATRP is possible	[158,159]

precisely graft polymer brushes onto a polyether sulfone (PEU) membrane. These polymer brushes were based on thermosensitive poly (N-isopropylacrylamide) (PNIPAAm) and PNIPAAm-based random copolymers [41]. Additionally, owing to their advantages, metal-free versions of other CRP techniques such as metal-free RAFT, have also been developed for synthesizing well-defined polymers [42,43]. In an article published in 2012 by Matyjaszewski and colleagues, biorelated materials obtained by ATRP were carefully reviewed [44]. They discussed how these advanced polymers could be used to make drug delivery systems (DDS), molecular imaging probes, and scaffolds for tissue engineering. This review article not only provides an update to the Matyjaszewski article but widens the scope of research and application of ATRP-derived materials for biomedical applications.

The ATRP strategy allows controlled growth of propagating radicals during polymerization, preventing termination reactions by reducing the concentration of active radicals. This has led to the design of well-defined polymers with tailored architectural features and chemical properties. Using ATRP, it becomes possible to incorporate desirable physicochemical properties (e.g., softness, mechanical strength, firmness, damping properties, self-healing capability, tissue adhesiveness, processability, *in vivo* stability and/or biodegradation, and bioactivity) into a single polymeric structure. This is achieved by precisely controlling molecular weight and architecture and carefully selecting appropriate (co)monomers [45,46]. ATRP has facilitated the synthesis of structurally unique polymers, such as star polymers, dendrimers, and bottlebrush polymers, exhibiting unprecedented properties, such as the unexpected rheological behavior of bottlebrush polymers. These structures are invaluable for creating smart DDS, bioimaging probes, tissue engineering scaffolds, and wound dressings. Moreover, ATRP can produce hybrid nanostructures, like hairy nanoparticles (NPs), that not only display interesting properties but also prevent aggregation of nanostructures and enhance their dispersibility in solvents or polymer matrices [47–49]. Furthermore, surface-initiated ATRP (SI-ATRP) is a versatile and robust strategy for grafting high-density polymer brushes onto surfaces with various chemistries. This approach leads to significant changes in the surface chemistry of tissue engineering scaffolds, medical implants, or nanocarriers, allowing for precise adjustments in their biocompatibility, antifouling properties, immunogenicity, loading capacity, or stimuli-responsiveness [50–52]. Additionally, an ATRP-based signal amplification strategy has enabled the creation of ultrasensitive biosensors capable of detecting very low concentrations of biomacromolecules, such as nucleic acids. As a result, these biosensors facilitate early detection of disease biomarkers, enhancing diagnostic capabilities [53,54].

Various reagents have been used in the different types of ATRP strategies. As depicted in Table 1, these reagents include (co) monomers, alkyl halides (i.e., ATRP initiator), transition metal complexes (i.e., activator and/or deactivator), free radical initiators, zerovalent metals, and reducing agents. Additionally, externally controlled versions of ATRP, such as photoATRP, eATRP, and mechanoATRP, utilize various types of photosensitizers and mechanophores for catalyst regeneration, enabling spatiotemporal control over polymerization [55]. In photoATRP, naturally derived photosensitizers like riboflavin can be used instead of synthetic ones, avoiding complex synthesis and expensive raw chemicals [36,37]. Furthermore, using renewable monomers and environmentally friendly solvents such as water will lead to the synthesis of well-defined and sustainable polymers through greener ATRP processes [32,56,57].

ATRP has the potential to progress by incorporating chemically versatile and renewable monomers, along with environmentally friendly solvents, thus aligning with the principles of green chemistry [32]. Additionally, an important research area in ATRP involves investigating novel catalyst-free systems or oxygen-tolerant biocompatible catalytic systems. The development of ATRP strategies that are orthogonal and/or operate under mild reaction conditions is also a key focus in the field [58].

In this review, the concepts that resulted in the development of ATRP and its derivatives are discussed thoroughly in Section 2. Leveraging ATRP for designing advanced polymer structures, such as star polymers and dendrimers, is discussed in Section 3. This section also describes various strategies for conducting ATRP in special environments, such as confined systems. ATRP for conjugating polymers to other (macro)molecules, such as proteins (e.g., enzymes, antibodies), peptides, and drugs, are discussed in Section 4. Section 5 highlights the utilization of ATRP for surface modification, with an emphasis on the “grafting-from” method using the SI-ATRP strategy, allowing the synthesis of polymer brushes with high density [59]. In this section, ATRP-induced surface modification is classified based on the material type (i.e., surface chemistry), such as metals, ceramics, semiconductors, and polymers. Section 6 describes the biomedical applications of (bio)(macro)molecules that are modified or synthesized using ATRP. Special attention is given to drug and gene delivery systems, tissue engineering, bioimaging, theranostic platforms, and biosensors. The last section discusses the current limitations and future perspective of ATRP for biomedical applications.

2. ATRP: Fundamentals and evolution

The formation of carbon–carbon bonds is a fundamental requirement in the synthesis of most polymers. Additionally, the formation of carbon–carbon and carbon–heteroatom bonds plays an essential role in modern organic synthesis [60,61]. However, in such bond-formation strategies, the formation of byproducts or unfavorable species is unavoidable, especially as the molecular weight of the polymer increases during synthesis. As a result, a wide variety of molecular structures, spanning different molecular weights and branching patterns, is observed for polymers synthesized by traditional methods like FRP. This phenomenon restricts the application of FRP in designing polymers with well-defined molecular structures.

ATRP originated from atom transfer radical addition (ATRA), a technique first reported by Kharasch *et al.*, and later modified through the accidental discovery of its catalytic system [62,63]. Further development made ATRA technique a robust tool for carbon – carbon bond formation. In ATRA, the abstraction of halogen atoms from alkyl halides, in the presence of transition metal catalysts, leads to the formation of radical intermediates that react with olefin molecules. The removed halides reversibly attach to the catalysts, and re-abstraction of halogen results in recombination of the halogen atom with the alkyl species [64,65]. This process leads to the formation of a new carbon–carbon bond while retaining the alkyl halide species, providing a valuable capability for repetitive carbon–carbon bond formation, which is highly valuable for polymer synthesis.

Inspired by this repetitive bond formation, Sawamoto and Matyjaszewski independently published seminal papers on polymer synthesis using ATRP [34,35]. Matyjaszewski and Wang utilized a catalytic system that included 2,2'-bipyridine (bpy) ligands for the controlled polymerization of styrene and methyl acrylate. The main reagents in such a strategy include alkyl halides (i.e., ATRP initiators) and transition metal complexes containing a few ligands, known as activators.

In traditional or normal ATRP (N-ATRP), the halide atom attaches to the metal complex (M^I/L), leaving a radical and a deactivator, i.e., the complex with the metal ion in its higher oxidation state or $X-M^{II+1}/L$ (forward reaction). The created radicals are reactive species that undergo polymerization with neighboring monomers before deactivation by $X-M^{II+1}/L$ to yield dormant species and M^I/L (the backward reaction). This frequent accelerate-and-brake strategy in ATRP prevents local heat accumulation and autoacceleration, i.e., it prohibits the fast but haphazard attachment of monomers to propagating radicals that is common in conventional FRP. Additionally, as the backward reaction is much faster, the concentration of propagating radicals remains extremely low, resulting in rare termination reactions. Although k_t is comparable to that of FRP, the very low concentration of propagating radicals results in the rate of the termination reaction approaching zero. The ATRP catalyst includes transition metal complexes (e.g., Cu, Fe) containing few ligands. These ligands not only solubilize transition metals, but also adjust their redox potential for halogen exchange during repetitive carbon-carbon bond formation. Accordingly, the ligand type affects the reaction medium selection (solvent type) and the activation rate constant in ATRP [66]. Fig. 2A schematically illustrates the mechanism of ATRP, where active species are represented by awakened snakes and dormant species by sleeping snakes. Monomers are depicted as mice, which serve as food for the snakes to grow. The snakes falling from the table represent the backward reaction, which is much faster than their climb up the ladder, representing the forward reaction. This results in many more sleeping snakes on the ground (dormant species) than on the table (active species). Additionally, Fig. 2B shows various modified versions of ATRP.

The ATRP mechanism shows that equilibrium can be approached not only from the left side (as in N-ATRP) but also from the right side, leading to the development of reverse ATRP (R-ATRP) [67]. In R-ATRP, the reagents include an alkyl halide (R-X), a transition metal complex in its higher oxidation state ($X-M^{II+1}/L$), and a free radical initiator (I_2) such as an azo compound. The radical initiator decomposes to produce radical species, as in FRP, which then react with monomers to produce propagating radicals (R^*). R-ATRP benefits from higher oxidative stability of $X-M^{II+1}/L$ species compared to less-stable M^I/L activators. In a modified version of R-ATRP, Cu^0 (in the form of wires, foils, etc.) is utilized instead, transforming Cu^{II} into Cu^I by donating an electron through comproportionation [68]. On the other hand, it is possible to approach the ATRP equilibrium from both left and right sides simultaneously using a reverse and normal initiation (SR&NI) ATRP approach [69]. Activators generated by electron transfer (AGET) originated from the SR&NI method, where the ATRP initiator and $X-M^{II+1}/L$ are utilized as starting reagents. A chemical reducing agent (e.g., ascorbic acid) is added to the reaction medium to reduce $X-M^{II+1}/L$ into activator, which eliminates the unfavorable creation of auxiliary radical species [70]. Similarly, in AGET ATRP, activators are fabricated *in situ*, allowing the use of a lower concentration of copper catalyst. Furthermore, reducing agents can remove dissolved oxygen, indicating some degree of oxygen tolerance [71]. Despite its similarity to N-ATRP, AGET ATRP stands out due to its ability to utilize highly active catalyst complexes, setting it apart from N-ATRP.

Development of AGET led to the design of activators regenerated by electron transfer (ARGET) ATRP, where highly active catalyst complexes are continuously regenerated after addition at the ppm level [72]. In other words, this strategy addresses the challenges associated with radical termination processes that result in irreversible formation and accumulation of $X-M^{II+1}/L$ species, which necessitates the use of high catalyst amounts [72]. Continuous catalyst regeneration in ARGET ATRP reduces the required catalyst quantity by approximately 10-fold compared to AGET, as shown in Table 1. ARGET represents a green synthesis method that employs very low levels of catalyst, along with inexpensive ligands and non-toxic reducing agents such as ascorbic acid and stannous octoate

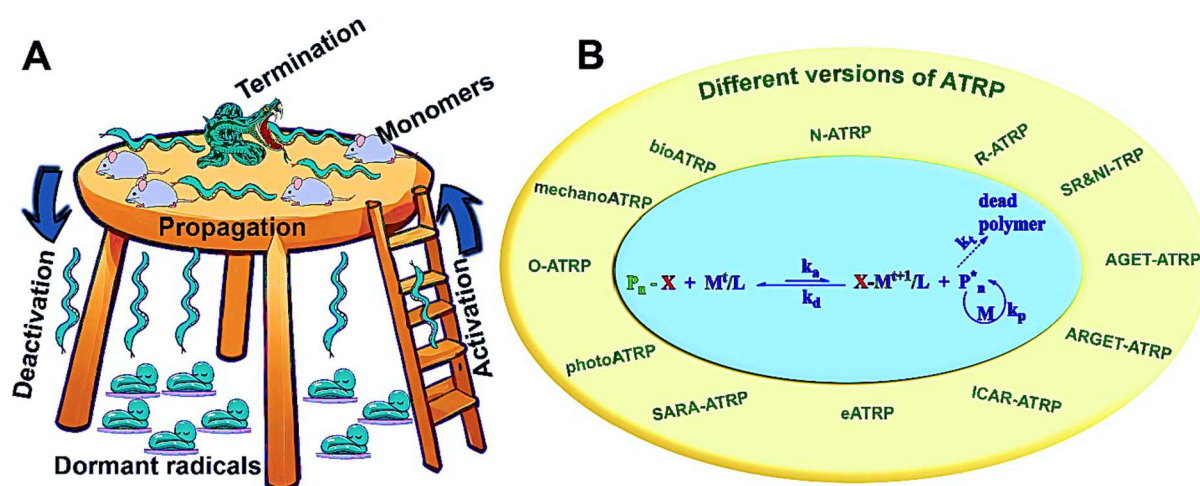


Fig. 2. Mechanism and various types of ATRP reactions. (A) Dormant species are represented by sleeping snakes. Some of the snakes are awakened, feel hungry, and try to climb up the ladder to reach the table full of mice (monomers). However, they fall down again after consuming a few mice and return to sleep. If a hungry snake on the table tries to accidentally eat another snake, it results in a larger snake that dies (termination). (B) General mechanism and various types of ATRP reactions. Schematic A was partially designed using Microsoft Bing Image Creator.

[73,74].

Initiators for continuous activator regeneration (ICAR) mechanistically resembles a reverse ARGET ATRP process, in which activator regeneration is carried out using organic radicals created by the decomposition of a free radical initiator. As a result, the polymerization rate is significantly influenced by the decomposition rate of free radicals rather than the regeneration rate of activators. Similar to ARGET, ICAR utilizes low concentration of copper catalysts, which means that, for most applications, there is no need for catalyst removal after complete polymerization. However, unlike reverse ARGET, ICAR should be carried out at lower temperatures to decelerate the reaction kinetics and ensure a long-lasting decomposition of radical initiators [55]. For a detailed and comprehensive discussion on the impact of various parameters (e.g., monomer, solvent, and catalyst) on ATRP kinetics, interested readers can refer to a review article authored by Krys and Matyjaszewski [75].

As previously stated, zerovalent copper (Cu^0) can participate in a comproportionation reaction with divalent copper to generate monovalent copper activator, effectively acting as a reducing agent in ARGET [76]. In simpler terms, Cu^0 gradually transfers electrons to $\text{X-M}^{+1}/\text{L}$ species, leading to the regeneration of activators. Moreover, although less common, zerovalent copper can directly react with initiators (R-X), thereby serving as an additional activator for ATRP. This evolution led to the development of the supplemental activator and reducing agent (SARA) ATRP, where Cu^0 fulfills both activation and reactivation roles [77,78]. To improve the biocompatibility of SARA, metals other than copper, such as elemental silver, zinc, magnesium, or iron, have been explored [79,80]. Furthermore, other (in)organic reducing agents, such as sulfites, can be used as SARA agents [81]. For example, sodium dithionite has been employed as a non-toxic SARA agent to synthesize polymers with narrow molecular weight distributions for biomedical applications [82]. However, the use of reducing agents can result in oxidized byproducts, which may remain as impurities in the final product. Therefore, chemists have been actively investigating alternative approaches that eliminate the need for reducing agents, making it an attractive field of study.

Electrochemical processes, where electron transfer is directly mediated by an applied potential, hold significant value. They not only aid in understanding reaction mechanisms but also provide a robust strategy for organic synthesis, such as electropolymerization [83]. Through direct electron transfer, reagents can be reduced, leading to fewer byproducts and a more environmentally friendly process. In electrochemically mediated ATRP (eATRP), deactivator regeneration is achieved using an electrochemical stimulus. Precise control over the applied potential, current, and time allows for fine-tuning the rate and conversion of eATRP [84,85]. Furthermore, the eATRP setup offers the advantage of creating gradient polymer brushes [86]. For a simplified eATRP setup, a sacrificial counter electrode, known as seATRP, could be used [87]. For more in-depth information about the fundamental aspects of eATRP and its applications in creating architecturally defined polymers, interested readers can refer to a review article by Matyjaszewski's group [88].

Similar to eATRP, photoinduced ATRP (photoATRP) is light-induced ATRP, also known as photocatalytic ATRP. PhotoATRP utilizes exogenous stimuli in the form of light or electromagnetic waves for spatiotemporal control over polymerization [89,90]. By combining the advantages of ATRP with the robustness of photochemistry techniques, photoATRP can produce well-defined polymers [91–94]. Various photoinitiating systems, such as semiconducting NPs, transition metal complexes, and naturally derived dyes, can be used in photoATRP [93,94]. For example, organocatalyzed ATRP (O-ATRP) or metal-free ATRP is a type of photoATRP where the activation or deactivation of polymer chains is mediated by an organic chromophore, known as a photoredox catalyst (PC) [95–97].

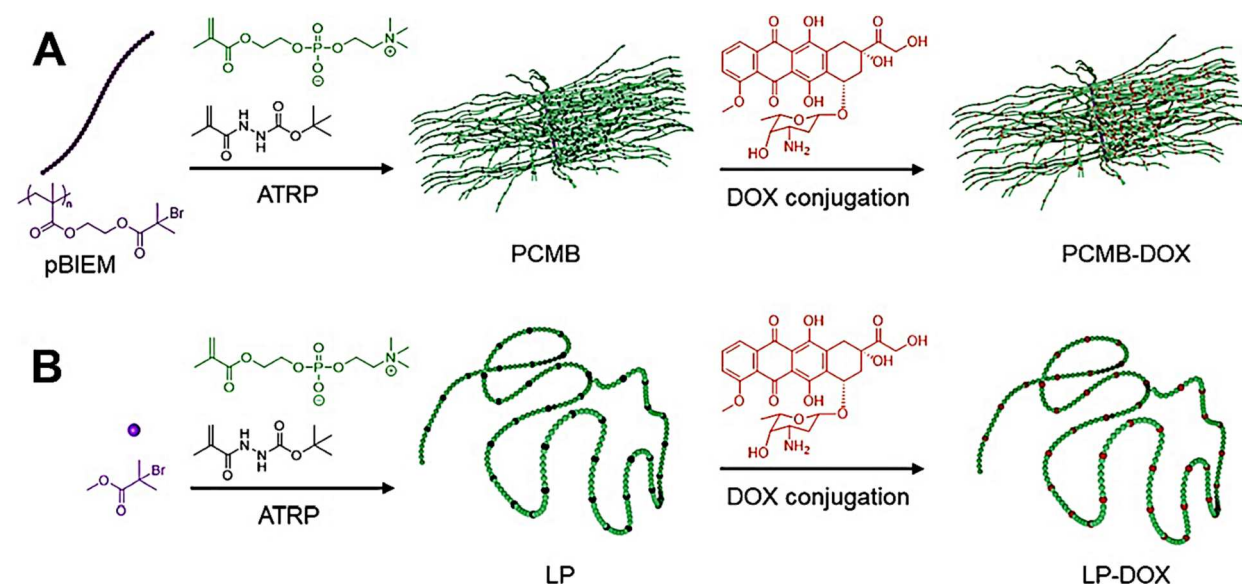


Fig. 3. Schematic depicting DOX conjugation with linear and bottlebrush polymers. (A) Synthetic pathway for bottlebrush polymer–DOX conjugates: chains were grafted from pBIEM chains via ATRP followed by DOX conjugation. (B) Synthetic pathway for linear polymer–DOX conjugates. Reprinted with permission from [186]. Copyright 2021 American Chemical Society.

The first PC based on iridium was used for controlled polymerization of methyl methacrylate (MMA) in 2012 [93]. Subsequently, the iridium-based PC was replaced by more sustainable organic PCs based on perylene and 10-phenylphenothiazine (PTH) [95,98]. In O-ATRP, the PC can activate the polymerization process through oxidative quenching of its excited state (PC*) to directly reduce the alkyl halide (activation). Alternatively, PC* may not directly reduce the alkyl halide; instead, it can obtain an electron from an electron donor (D) to generate a radical anion (PC^{•-}) and subsequently activate dormant species [99]. This latter strategy, known as reductive quenching, involves the use of dual catalytic systems and UV light to produce activators (Cu^I/L species). In photoATRP, activator regeneration is achieved through photoexcitation, which can occur directly (via ligands) or indirectly (via photosensitizers or photoinitiators) [100,101]. Ligand to metal charge transfer (LMCT) plays a critical role in reducing the deactivator and facilitating the activation of polymerization.

UV and visible light are useful for photoATRP, but they have limitations due to their inability to penetrate nontransparent materials like living tissues, which restricts their applications in bioimaging or chemical reactions in biological systems [102]. However, they can still be utilized to make polymers or polymer-modified systems, followed by *in vivo* use after the required purification processes [103]. In contrast, NIR light, with longer wavelengths and lower-energy photons, can penetrate deeper into living tissue, allowing for energy transfer and bioimaging in living systems [104]. Although low-energy NIR photons lack sufficient energy to directly trigger chemical reactions like photoATRP, they can initiate controlled polymerization by producing photosensitizer-induced reactive species [105–107]. Moreover, NIR can be converted into UV/visible light with higher energy using upconverting NPs (UCNPs), which have promising applications in bioimaging, nanomedicine, and theranostic fields [108,109]. UCNPs, with anti-Stokes emission, enable the construction of materials that respond to NIR radiation, making them valuable tools for biological applications [110]. UCNPs have been used to remotely activate gene expression in living cells and for deep brain stimulation [111,112]. PhotoATRP can be initiated by NIR using UCNPs, which is highly beneficial for nontransparent systems, such as biological systems [113]. Alternatively, homogenous photocatalysts can harness UV or visible light to produce active species, but they suffer from limitations such as side reactions and the requirement for costly purification processes. Therefore, the development of heterogeneous photocatalytic systems that can utilize energy from visible or NIR photons, excite and transfer electrons to activate an ATRP catalyst, and initiate ATRP with easy removal and reusability, holds great promise [114]. In such systems, photocatalysts (e.g., eosin Y or tetraphenylporphyrin [PTH]) may be immobilized on solid substrates (e.g., NPs or fibers) or embedded in a hydrogel [114–116]. Another innovative approach involves incorporating photocatalysts into polymers containing an extended conjugated network. In this strategy, photocatalyst molecules can be copolymerized or crosslinked with other aromatic molecules to tune their light-absorption properties. In this context, Dadashi-Silab *et al.* developed a heterogeneous catalyst based on conjugated microporous polymers (CMPs) for green/red light-induced ATRP [117]. CMPs have unique features due to their extended π -electron conjugation and porous structure, making them interesting for various applications [118]. This group used phenothiazine (PTZ) as a photocatalyst and dimethoxybenzene as a crosslinker to create a PTZ-CMP double catalytic system. While PTZ itself efficiently absorbs UV light, the PTZ-CMP network exhibited photocatalytic activity in

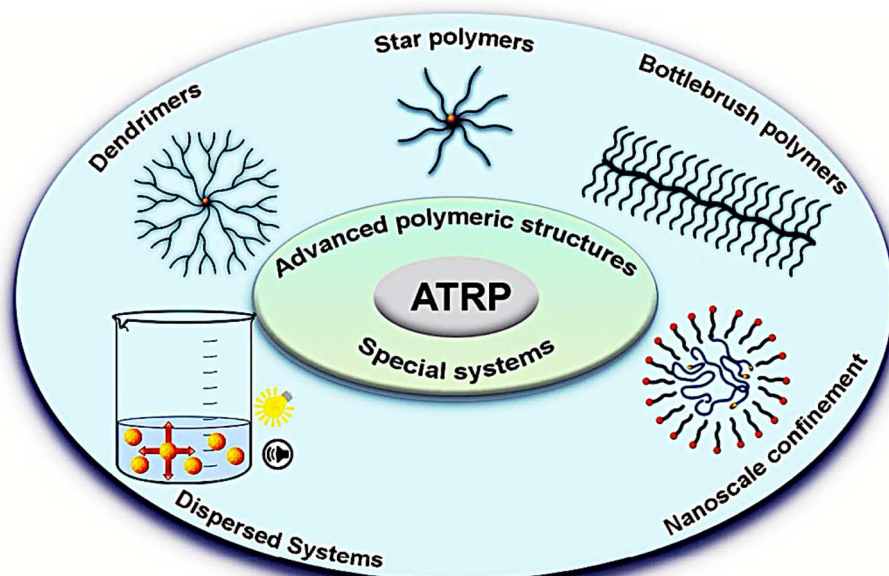


Fig. 4. ATRP in special systems for designing architecturally complex polymeric structures. ATRP has been widely investigated to create novel polymers with unique architectural features, including star polymers, dendrimers, and bottlebrush polymers. When subjected to nanoscale confinement of polymeric macromolecules and/or radicals, these macromolecules exhibit distinct relaxation behavior, influencing their movement and the kinetics of the ATRP reaction. ATRP conducted in dispersed media offers the advantage of facilitating heat transfer, as the solvent acts as a heat sink. Additionally, externally controlled variants of ATRP, such as photoATRP and mechanoATRP, benefit from improved transmission of electromagnetic and ultrasonic waves through the continuous phase of dispersed systems.

the visible light region, shifting towards higher wavelengths [117]. Furthermore, the extended π -electron delocalization in conjugated oligo-/polymers (e.g., oligo-/polyaniline, oligo-/polypyrrole, and oligo-/polythiophenes) can possibly be exploited to make heterogeneous photocatalysts for photoATRP [119,120]. Additionally, carbon dots (CDs) have also been used as a green PC and photosensitizer in the photoATRP of MMA [121]. Doped CDs serve as a biocompatible photocatalyst for photoATRP when exposed to blue LED light. More recently, Ma *et al.* discovered a new class of high-efficiency organic photocatalysts [97]. They found that oxygen-doped anthanthrene (ODA) strongly absorbs visible light, and its molar extinction coefficient is sufficient for use in O-ATRP under sunlight. For a comprehensive understanding of photomediated CRP strategies and their applications in creating well-defined polymers, interested readers can refer to a review article by Matyjaszewski's group [91]. However, it should be noted that this review paper does not specifically focus on the applications of the derived polymers.

Other external stimuli have also been investigated, such as electric current, mechanical vibrations, and ultrasound [55]. For example, sono- or mechanoATRP utilizes the energy of ultrasonic waves for controlled polymer synthesis [122]. While photoATRP harnesses the energy of photons through ligands or dyes to generate electrons, in sono- or mechanoATRP, electron generation is mediated by the piezoelectric effect, which converts mechanical vibrations into electrical charge [122,123]. In sonoATRP, mechanophores such as piezoelectric NPs (e.g., barium titanate or zinc oxide) use mechanical power and convert it to electrical charge [124]. The most critical step in such applications is the interfacial electron transfer from piezoelectric nanostructures to ATRP catalysts. Mechanochemistry, in which an applied force directly results in chemical transformation, also shows great potential [125–127]. Mechanochemical covalent bond scission can be employed for the release of small molecules or reactive species (e.g., singlet oxygen) [128,129]. Some mechanophores can respond to mechanical loads by releasing chemicals, which are known as chemical responses [125,130,131]. These chemical responses can be used to directly or indirectly initiate or suppress ATRP. For example, in a recent study, researchers incorporated an azo-based mechanophore into a hydrogel, which produced free radicals in response to mechanical perturbations [132]. When combined with water and oxygen, these free radicals were converted into ROS, which were used for mechanochemical dynamic therapy of various types of cancer. Similarly, these free radicals or ROS species can be used to initiate or suppress controlled polymerization. Additionally, polymer mechanochemistry can be utilized to release an ATRP trigger (e.g., activator) via bond cleavage or disassembly, similar to the release mechanism of drug molecules [133–135]. This mechanophore-based molecular release can be combined with ATRP techniques to develop more robust ATRP methods. Remote activation of mechanophores using ultrasound, which can penetrate deep into body tissues, offers a potential solution to the limitations associated with photoATRP [132]. High-intensity focused ultrasound (HIFU) can be used for spatiotemporal control of ATRP, similar to the approach used in photoATRP. However, no published reports using this strategy in the field have been found yet. For a comprehensive discussion on various ATRP methodologies, their mechanisms, and catalytic systems, readers can refer to a review article by Matyjaszewski's group [136].

Biocatalytic ATRP (bioATRP) involves the use of enzymes (e.g., metalloproteins) or whole cells/organisms to catalyze ATRP reactions [137–140]. For instance, hemoglobin-catalyzed ATRP was used to synthesize PNIPAAm [141]. In this work, the catalytic activity of hemoglobin initiated the ATRP, utilizing its ATRPase activity. "ATRPase" refers to the use of a biomacromolecule as a catalyst for conducting ATRP. This approach offers several benefits such as bio-friendly reaction conditions, biodegradability, and non-toxicity. Another method called SI-bioATRP was used to grow copolymer brushes in a controlled manner, allowing adjustment of the surface's bioaffinity [138]. Various enzymes, including metalloproteins like lactase, can also be used as ATRPases to synthesize well-defined synthetic polymers [142]. Furthermore, cellular processes involving redox reactions can be harnessed to induce CRP under biologically relevant conditions [137]. As iron ions are commonly found in nature, especially in bacterial processes, they can be used to mediate ATRP reactions instead of copper ions, which are less abundant in biological systems. In this context, three types of bacteria were used to catalyze biocompatible AGET ATRP for the synthesis of poly(ethylene glycol methyl ether methacrylate) (PEGMA). These bacterial redox systems initiate a redox transformation of the Fe/L activator, allowing the ATRP reactions to occur [137]. Mild ATRP conditions, including ambient temperature, pressure, aqueous media, and neutral pH, are highly desirable due to the delicate nature of

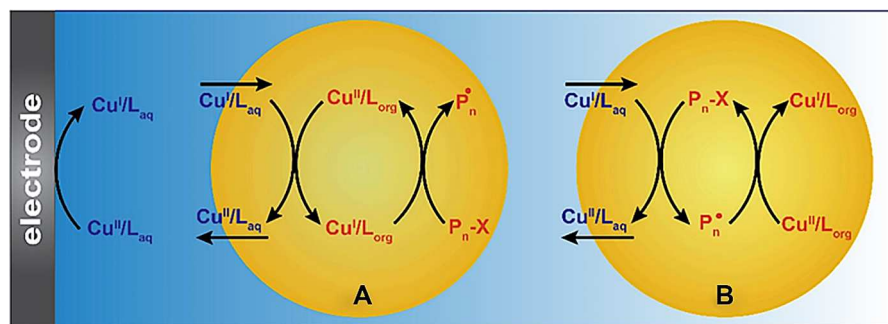


Fig. 5. Schematic describing the mechanism of eATRP in miniemulsion using a dual catalytic system. (A) The continuous phase contains a hydrophilic catalyst in its higher oxidation state. This catalyst grabs an electron at the electrode surface, is converted to its lower oxidation state, and diffuses in the dispersed phase. When it touches a droplet's surface, it delivers an electron to reduce a hydrophobic catalyst (at higher oxidation state) inside the droplet. The reduced hydrophobic catalyst serves as activator for ATRP. The oxidized hydrophilic catalyst diffuses back to the electrode's surface to grab another electron and repeat the process. (B) At the droplet surface, the hydrophilic catalyst may also deliver an electron to dormant species, converting them to radicals. Reprinted with permission from [201]. Copyright 2016 American Chemical Society.

many materials, which cannot sustain harsh chemical conditions. As result, there is a significant interest in ATRP techniques that provide reasonable kinetics under these mild conditions at room temperature. For example, ARGET ATRP in phosphate buffered saline (PBS) at 20 °C was employed to form lysozyme-poly(glycerol monomethacrylate) conjugates [143]. Ligand design highly affects the activity of ATRP activators and the reaction kinetics of polymerization [144]. While N-ATRP utilizes a high amount of CuBr and is performed at relatively high temperatures (~60 °C), Whitfield *et al.* showed that a ppm level concentration of CuBr can trigger the ATRP reaction even at room temperature [145]. In this study, the researchers intentionally used a higher concentration of tris[2-(dimethylamino)ethyl]amine (Me6TREN), which is an ATRP activator known for its high activity, in comparison to CuBr. In a similar recent study, it was reported that using a CuBr/Me6TREN catalyst, ATRP of methyl acrylate can be performed at room temperature due to a new mechanism [146]. In this work, the term “oxygen-enhanced ATRP mechanism” was introduced, which is characterized by improved reaction kinetics attributed to the formation of a Cu superoxido complex. Surprisingly, they discovered that when the catalyst is formed under ambient conditions with the presence of oxygen, the conventional ATRP strategy can be enhanced, allowing ATRP to be performed at room temperature. This oxygen-enhanced ATRP mechanism holds great promise for conducting ATRP in biological systems. Additionally, it offers the potential for chemical modification of delicate nanostructures, such as laser-induced graphene on polyimide film, which are sensitive to high temperatures and prolonged solvent exposure. Fast kinetics of the ATRP process under ambient conditions are especially important for (nano)(bio)materials. For a comprehensive overview, Table 1 summarizes various types of ATRP techniques and their respective advantages and disadvantages.

3. ATRP in the design of complex polymers

3.1. Advanced polymer structures

ATRP has been used to make various types of (co)polymers with well-defined molecular structures and numerous biomedical applications [160,161]. However, this section focuses on the application of ATRP in synthesizing architecturally unique polymers, including star polymers, dendrimers, and bottlebrush polymers (Fig. 4). We recommend reading a comprehensive article authored by Matyjaszewski *et al.*, providing an in-depth review of various macromolecular structures synthesized by ATRP [33]. However, they mainly focus on the design of macromolecular structures without diving too deep into their biomedical applications.

Star polymers are branched macromolecules in which linear chains, known as arms, are connected to a central core [162]. These three-dimensional (3D) polymers significantly differ from linear polymers in topological features, resulting in different

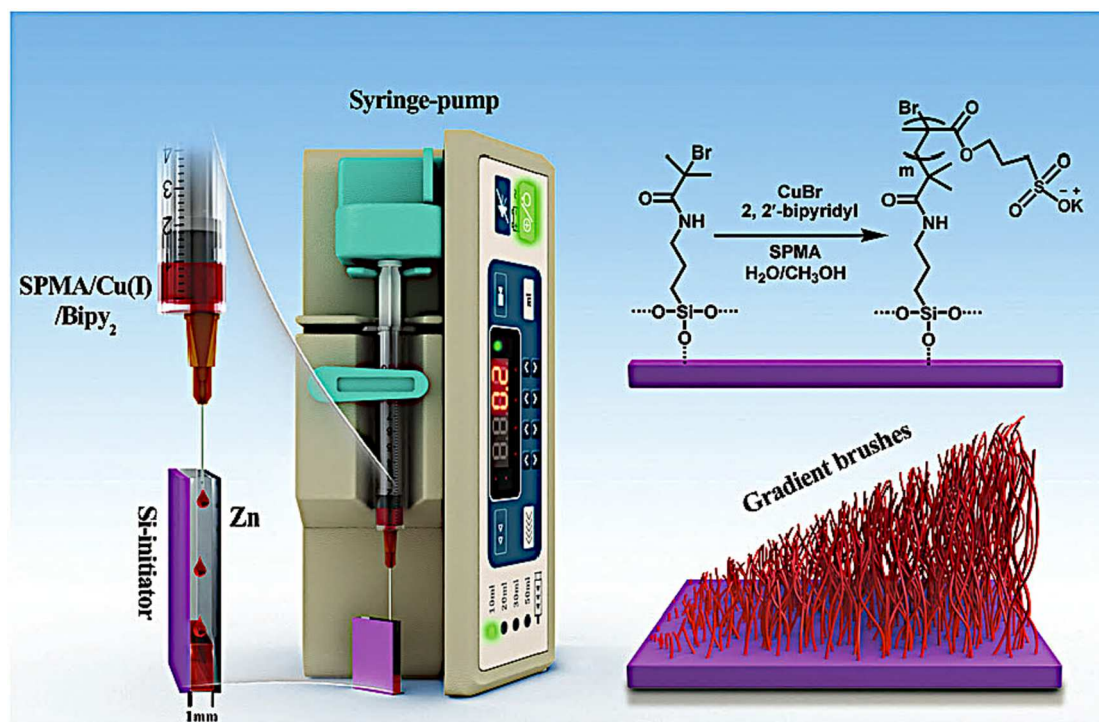


Fig. 6. Schematic depicting the fabrication process of gradient polymer-coated surfaces using a microfluidic system. One side of the microfluidic channel was surface-modified by an ATRP initiator. Injecting tiny volumes of a solution containing activator and monomer (3-sulfopropyl methacrylate potassium salt, SPMA) using a syringe pump fills the microchannel over time. Deeper regions fill sooner and have higher contact time with the solution (higher polymerization time) which results in growing longer polymer chains (PSPMA) at the bottom. Reprinted by permission [210]. Copyright 2019 Royal Society of Chemistry.

physicochemical properties and rheological performance [163,164]. Due to their unique structure and properties, star polymers have been widely utilized in biomedical fields such as drug delivery and bioimaging [165,166].

To make star polymers using ATRP, the “grafting-to”, “grafting-from”, or “grafting-through” methodologies can be used. In the grafting-from method (i.e., core-first methodology) the core (e.g., a (macro)molecule or NP) is functionalized with multiple ATRP initiation sites, enabling control over the number of arms, their dispersity, and length. Natural macromolecules are usually used as the core; for example, β -cyclodextrin (β -CD) was utilized as a multifunctional core to grow a diblock copolymer using the photoATRP strategy [167]. The β -CD was reacted with 2-bromoisobutryl bromide (BiBB) to produce a β -CD-based core containing seven ATRP initiating sites [167]. The hydrophobic β -CD core enables the encapsulation of hydrophobic species, such as drugs and photosensitizers, while the hydrophilic arms improve its biocompatibility and enable embedding of hydrophilic drugs.

In another study, tannic acid was used as a multifunctional core to grow poly (methyl methacrylate) (PMMA) and poly[oligo (ethylene oxide) monomethyl ether methacrylate] (POEOMA) arms [168]. Tannic acid is a plant-derived polyphenol which benefits from biodegradation, antimicrobial, and antiviral properties [169]. It has 25 hydroxyl groups that allow for chemical functionalization, such as tethering ATRP initiators. In this work, BiBB was used to create 25 ATRP initiation sites linked to the tannic acid core via cleavable phenolic ester linkages, allowing controlled degradation under alkaline conditions [168,170].

Dendrimers are highly branched and nearly monodisperse macromolecules with 3D structure [171]. They include a core, an internal part rich in branches, and a shell that enables further modifications. Contrary to star polymers, in which branch density decreases significantly toward the surface, dendrimers' branch density increases at higher generations, i.e., from the core toward the surface. Composition of dendrimers and generation number greatly impact the properties and applications of the dendrimers. Because of their special topological features, dendrimers have been used in various biomedical applications such as drug delivery, gene transfection, theranostic platforms, and bioimaging [172]. Moreover, some dendrimers show antimicrobial (e.g., cationic amphiphilic dendrimers) or antiviral properties which indicates their potential utilization as therapeutics in biomedicine [173]. In addition, specific architectural features and the nanoscale size of dendrimers allow high loading of both hydrophilic and hydrophobic drugs in interior parts [174].

Regardless of their inherent properties, they can be densely grafted by other (co)polymers using SI-ATRP, in which the dendrimers can serve as macroinitiators for ATRP. In fact, numerous surface functional groups on dendrimers are interesting sites for chemical modifications such as installing ATRP initiators or clickable functionalities for further conjugation with polymers or small molecules. In addition, drug molecules or imaging probes can be covalently attached to the dendrimers through stimuli-labile linkages. For example, stimuli-cleavable linkages that are susceptible to pH, redox moieties, enzymes, or ROS can be utilized to release drug molecules from the dendrimer surface [175,176].

Bottlebrush polymers have a linear polymeric backbone densely grafted with long polymeric side chains, and a backbone length greater than the side chain length. The unique architectural features of these grafted polymers result in unusual properties such as wormlike morphology and lower tendency toward entanglement [177]. CRP strategies and click chemistry are the most important

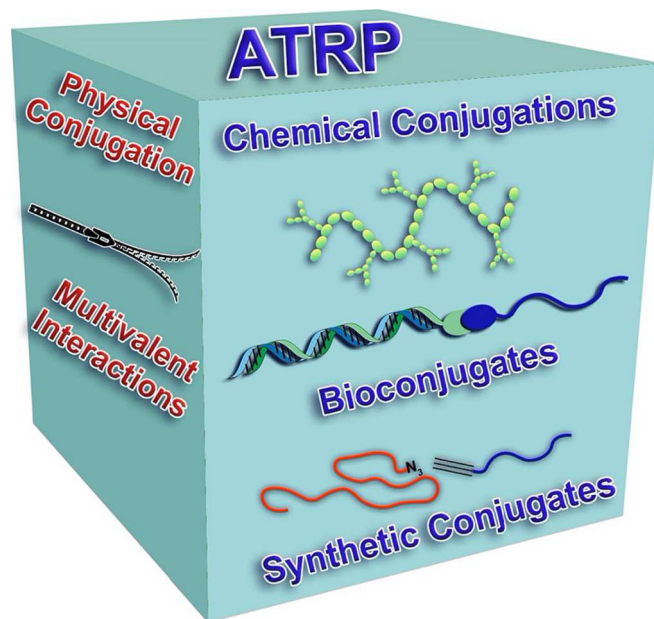


Fig. 7. ATRP in molecular conjugation. Physical conjugation of bulky molecules is dependent on multi/polyvalent interactions, resembling the mechanism of a zipper. On the other hand, chemical interactions are categorized into two groups: bioconjugates and synthetic conjugates. In the case of a bioconjugate, a synthetic polymer derived from ATRP is connected to a biomolecule through robust covalent bonding. Meanwhile, a synthetic conjugate involves the firm attachment of two or more ATRP-derived polymers through covalent bonding. For chemical conjugation, orthogonal chemistry, particularly an orthogonal click reaction, is commonly utilized.

tools for making bottlebrush (co)polymers. In the grafting-from strategy, the bottlebrush polymer backbone can be made via RAFT, ATRP, or ring-opening metathesis polymerization (ROMP) followed by installation of ATRP initiation sites and controlled polymerization [178]. Furthermore, in the grafting-onto method, the polymeric backbone and side chains can be prepared separately by CRP strategies followed by chemical conjugation of side chains to the backbone using click reactions with high yield. Steric repulsions between side chains, which usually have random coil morphology, can inhibit high density grafting, especially in the grafting-onto methodology. With the grafting-through methodology it is possible to make side chains as macromonomers (typically norbornene derivatives) before reacting them to make bottlebrush polymers [178,179].

The grafting-onto strategy was used for the synthesis of a bottlebrush polymer containing V-shaped side chains, in which ATRP was used in combination with RAFT and ring-opening polymerization (ROP) [180]. The RAFT strategy was used to make poly(glycidyl methacrylate) (PGMA), which constitutes the backbone, followed by installation of clickable azido groups on the side chains. V-shaped side chains were synthesized using an ATRP initiator containing two bromoisobutyryl (BiB) functional groups at the two ends and one clickable alkyne group in the middle. The synthesized polymer, with a pendant clickable group in the middle, was grafted onto a clickable PGMA backbone via click chemistry, resulting in V-shaped side chains [180,181]. A similar grafting-onto methodology was utilized for a bottlebrush polymer with poly(2-hydroxyethyl methacrylate) (PHEMA) as backbone and three polymers (polyethylene glycol or PEG, polystyrene or PS, and poly(butyl acrylate) or PBA) as side chains, using a combination of ATRP and click chemistry [182].

A grafting-from methodology was used for synthesis of a bottlebrush copolymer with poly(2-(2-bromoisobutyryloxy)ethyl methacrylate) (PBiBEM) as backbone grafted with PBA [183]. Two ATRP strategies, ICAR ATRP and SARA ATRP, were used to graft PBA chains from PBiBEM. It should be noted that bottlebrush copolymers can be part of more complex macromolecular structures, for example, the arms of a star-like polymer [178]. Furthermore, it is worth mentioning that special types of degradable bottlebrush copolymers can be made when stimuli-cleavable linkages are used to attach side chains to the backbone [184,185].

Special architectural features of bottlebrush polymers enable the embedding of large quantities of drug molecules. In addition, the high density of their grafted side chains, which can be end functionalized and conjugated with various drug molecules, biomolecules, and imaging probes, further confirms their vast potential usefulness in the biomedical field. For example, in one study, doxorubicin (DOX) molecules were conjugated to a bottlebrush copolymer via acid-labile hydrazone linkages, as shown in Fig. 3 [186]. Compared to their linear counterparts, bottlebrush copolymers showed high structural stability under physiological conditions, longer blood circulation half-life, and enhanced antitumor activity [186].

Natural systems produce proteins with not only monodisperse chain length but also monodisperse sequence. Mimicking such a high level of control over monodispersity in synthetic polymers is a great challenge in chemistry and polymer science. Sequence-defined polymers (SDP) or sequence-controlled polymers (SCPs) can bring about a paradigm shift in polymer technology. In SDP, the properties of polymers can be adjusted based merely on an alternation in sequencing while preserving the chemical composition [187,188]. In addition, it is believed that SDP can be used in data storage, similar to RNA/DNA, which could revolutionize modern storage devices [189]. RDRP strategies, especially ATRP, can be further developed to enable precision polymerization and creation of SDPs [190]. Achieving control over propagation of intermediate radical species in RDRP strategies enables full control over polymerization, i.e., precision polymerization. We think that precise control over reagent feeding (e.g., in diffusion-controlled systems such as microfluidics or mini-emulsion) in a monomer-starved CRP can be a fruitful strategy to control radical propagation in RDRP. Controlled feeding of monomers, based on half-life of propagating radicals, can result in one monomer addition at each step. For example, suppose that the ATRP is occurring in a microfluidic system with semipermeable microchannel walls separating monomer solutions. If an external (e.g., electric field or light) or internal (radical species) stimulant can adjust the permeability of the microchannel, sequencing is achievable. Every time the stimulant is on, the first monomer is allowed to pass, while turning off the stimulant results in passage of the second monomer. Additionally, continuous flow chemistry and automated synthesis can further our control over polymerization [191,192]. In an elegant paper, automated flow chemistry, which combines benefits of flow chemistry and automated synthesis, was used to make proteins artificially [193]. Another elegant, recent paper, used a semiautomated flow chemistry method for making SDPs based on PLGA for data storage [194]. Precision polymers will open a new horizon to synthesize complex chemical systems with reversible interactions, necessary for developing new forms of synthetic life from scratch [24]. In this work, Matyjaszewski *et al.* discussed how precision polymers can be synthesized using modern chemistry tools such as CRP [24]. While they carefully discussed the dynamic interactions and self-assembly of precision polymers to produce complex and organized structures that mimic the complexity of living biological matter, they did not focus on their applications in medicine.

3.2. Special systems

While ATRP is a well-established technology in terms of chemistry, exploring and engineering reaction conditions, such as performing ATRP in dispersed media, can further enhance this technique. Performing ATRP in such special systems expands its applications to design architecturally interesting polymers that cannot be achieved through conventional bulk polymerization. In particular, ATRP in dispersed media, as depicted in Fig. 4, offers additional advantages, such as improved heat transfer, facilitated by the solvent acting as a heat sink. Additionally, ATRP in dispersed emulsions benefits from lower viscosity and the use of environmentally friendly solvents, especially water, which effectively addresses challenges associated with volatile organic compounds (VOC) [195]. Moreover, the application of microfluidic systems allows for controlled fluid flow in a laminar regime, introducing an additional dimension of control over the polymerization process. In addition, these dispersed systems and microfluidic devices are better suited to facilitate the diffusion of external stimulants, such as sound and electromagnetic waves, providing more precise control and versatility to the ATRP process.

Nanoscale confinement, such as nanopores and nanoemulsions, may affect the conformation and relaxation behavior of single macromolecules as well as polymerization kinetics by affecting propagating radicals' movement and direction. However, at higher length scales (e.g., microcapsules, (mini)emulsions, and microfluidics) confinement mainly affects fluid flow behavior and mass transfer operations, rather than affecting single-molecule movement. In other words, effects of walls or interface on macromolecules cannot be ignored for macromolecules, as their size is comparable to that of nanocavities. In addition, in a thin layer of fluids, fluid behavior adjacent to the cavity wall is fundamentally different from fluid behavior in regions sufficiently far from walls. This indicates the solvent's effect on macromolecules and polymerization reagents may differ in nanocavities compared to cavities at higher length scales. In addition, nano- or micro-confined dispersed systems such as emulsions benefit from superior heat and mass transfer, compared to bulk systems.

Miniemulsion polymerization enables construction of hydrophobic polymer particles from monomer droplets in a confined environment. Compared to bulk polymerization, miniemulsion polymerization benefits from lower reaction rates because of lower concentration of reagents, better heat transfer, low viscosity product, and use of benign aqueous solutions [196,197]. These unique features can be combined with the advantages of CRP strategies to make well-defined hydrophobic polymers [198–200]. Such dispersed and confined systems allow more control over polymerization reactions. Performing ATRP in miniemulsion requires a hydrophobic catalyst in the dispersed phase (e.g., oil droplets) while in the continuous aqueous phase a hydrophilic catalyst can be used to serve as electron carrier. For example, in an eATRP setup, a dual catalytic system was used to carry electrons from the working electrode (WE) through the aqueous phase to the dispersed phase [201]. This system resembles the functions of a redox couple in dye-sensitized solar cells that carries an electric charge from cathode to photosensitizers [202]. The water-soluble ATRP catalyst grabs an electron at the WE surface and donates it to the organosoluble ATRP catalyst at the water–droplet interface (Fig. 5). It is obvious that, regardless of electrochemical parameters like applied potential, the mobility of catalyst molecules in the aqueous phase is a determining factor in polymerization rate. It was revealed that reactivity is independent of the redox potential of catalysts, in contrast to conventional ATRP [201]. This dual catalytic system was further utilized and modified by others [203].

CRP in miniemulsion systems can also exploit remote stimulants such as light and sound to trigger electron generation inside droplets, as previously discussed for photoATRP and mechanoATRP. PhotoATRP in dispersed systems is not only superior for heat transfer and preventing hot spots in the system, but light can also pass-through water more easily than bulk systems, which may be opaque or translucent, or may increase light absorption as the polymerization proceeds. Moreover, mechanoATRP can be conducted easily, with a simple experimental setup using a popular ultrasonic bath or ultrasonic probe.

On the other hand, CRP in microenvironments enables us to make polymers with special architectural features, like gradient polymers. In such systems, polymer chain length is affected by cavity length scales instead of parameters that affect polymerization, such as temperature, light intensity, concentration of radicals, and so forth. Gradient polymer-modified surfaces, in which the length or composition of polymer brushes changes continually along a specific axis, have special applications in biomedical fields such as cell separation.

The SI-ATRP strategy can be utilized to make gradient polymer brushes in microfluidic channels where the merits of laminar fluid flow are exploited in combination with controlled polymerization reactions. In a laminar fluid flow regime, the mass transfer operation is dominated mainly by molecular diffusion rather than convection, in contrast to the turbulent flow regime common in normal experimental setups. A gradient in the concentration of involved species (i.e., monomers, catalysts, or reducing agents), and/or their diffusion coefficients, or a change in external stimulant parameters (e.g., light intensity for photoATRP and applied potential for eATRP) can induce gradients in polymer brushes. For example, PNIPAAm brushes were fabricated through an eATRP method in which a smooth change in the applied potential resulted in an alternation in the concentration of *in situ*-generated catalyst species ($\text{Cu}^{\text{I}}/\text{L}$), followed by gradient growth of polymer chains [204]. In addition, this strategy can be easily used to make patterned surfaces, by applying an electrical potential to specific regions on the surface or masking a conductive surface with insulating materials. An alternate strategy for making gradient polymer brushes or patterned surfaces is to use eATRP with a gradient change in electrode distance, which affects diffusion time of activator molecules [205]. This patterning and/or making complex gradient brushes can also be achieved via other external triggers, for example, light irradiation which allows control over wavelength (i.e., energy of individual photons) and intensity [206,207]. Furthermore, gradient polymer brushes have recently been made in 3D spaces [208].

Film coating strategies, such as dip coating or spin coating, can also be used for making gradient polymer brushes, and parameters like withdrawal speed and spin coating speed can be finely adjusted. For example, in the dip coating process, the brush thickness can be modulated by withdrawal speed [209]. A dip coating process can be reversed with a system that gradually fills a microchannel whose internal surface is modified by anchoring an ATRP initiator.

To this end, and with inspiration from seATRP, a vertical microfluidic system with ATRP initiator-modified surfaces was designed in which tiny volumes of activator or monomer solutions were gradually pump into a microchannel to create polymer brushes with gradient length as illustrated in Fig. 6 [210]. Since the exposure time increases with depth, longer brushes are observed at the bottom. A solution containing catalyst and monomer (3-sulfopropyl methacrylate potassium salt, SPMA) was injected into a microchannel where SI-ATRP results in gradient PSPMA brushes. It was observed that brush thickness can be adjusted through flow rate (i.e., polymerization time) and concentration of monomer and activators [210]. This strategy could be used repetitively to make di- or triblock copolymers for which the injected solution composition alters between intermittent steps.

However, it should be noted that the potential of microfluidics in CRP is much higher than just controlling polymerization time. In other words, microfluidic systems can be designed to control the way the reactant or catalyst comes into contact, and to adjust the mixing profile of the involved species. Another great advantage of microfluidics is its ability to control reaction conditions via external stimulants. For example, while homogeneous heating and/or cooling of the reaction medium at higher length scales (e.g., in a beaker) is a challenge, micro-scale devices can be used for effective, on-demand heating and cooling. Moreover, because of the low depth,

microfluidic systems can be used more easily and efficiently in systems with remote stimulants, such as photoATRP. In addition, microfluidic systems usually utilize small volumes of reagents which makes them appealing from both economic and environmental perspectives, and microfluidic-assisted ATRP can be considered a sustainable and green procedure. These ideas have resulted in the concept of microliter-scale (μL -scale) ATRP, which focuses on applications of controlled polymerization at μL -scale [211]. Microfluidic devices facilitate the use of zerovalent metal-based ATRP because a metal plate can constitute a wall in the microfluidic system. Moreover, mounting microelectrodes in microchannels allows the use of eATRP or seATRP methodologies. Microfluidics and μL -scale systems may take us a step forward in democratizing polymer synthesis (polymer synthesis for non-experts) and facilitating on-demand synthesis of polymers. Of note, Matyjaszewski *et al.* have recently described the relationship between green chemistry and ATRP and how combining these strategies can produce sustainable, well-defined polymers [32].

Polymer brush patterns (PBPs) are important in biomedical fields such as biosensing devices and tissue engineering. Lithography is a robust strategy for patternable immobilization of ATRP initiators, where subsequent SI-ATRP results in a patterned brush-modified surface. Carbonell *et al.* coined a method named “Polymer Brush Hypersurface Photolithography” (PBHP) for fabrication of PBPs with high spatial resolution (known as polymeric pixels) via SI-ATRP on a silicon wafer surface [212]. This method not only allows creation of brush-modified surfaces with micrometer-scale resolution but also enables control over brush length and composition. The aforementioned polymer brushes that possess different length, composition, or patterns are considered to be hierarchical polymer brushes, and their synthesis and biomedical applications were recently reviewed [213].

O-ATRP procedure can occur in continuous flow reactors to continually produce well-defined polymers [214]. This strategy combines the merits of ATRP with the advantages of flow chemistry. Continuous flow of a small volume of reagents enables better control over reaction conditions; in addition, more effective irradiation for this O-ATRP methodology resulted in higher initiator efficiency (even at very low photocatalyst loadings), improved polymerization, and low-dispersity polymers [215].

Although these strategies are invaluable in research and development of the CRP field, translation of ATRP to industry requires special requirements for large-scale production of well-defined polymers. Specifically, economic and safety issues should be considered when engineering reactors for CRP strategies [215]. When scaling up from experimental setups, such as beakers and microfluidics, to industrial-scale reactors like continuous stirred-tank reactors (CSTRs) or tubular reactors, the engineer must consider various factors. The reaction mechanism and conditions (e.g., temperature and pressure) and the reagent and (side) product conditions should be considered in the design of storage vessels, feeding systems, heating/cooling systems, condensers, mixers, reactor lining, purification unit operations (e.g., distillation column, filter bags, and crystallizer), and so forth. As polymerization reactions at industrial scales are usually carried out in continuous flow reactors, the design criteria for continuous flow reactors should be noted when translating CRPs into industry [216,217]. Doing CRP in continuous flow reactors is an important step toward scaling up to industrial applications [215,218].

In this section, we have briefly reviewed some of the special systems that can be exploited for ATRP; however, additional systems may be available.

4. ATRP in molecular conjugation

Conjugation of two (macro)molecules can be carried out via physical or chemical interactions, as depicted in Fig. 7. While individual physical interactions are relatively weak, they benefit from reversibility and fast formation kinetics. On the other hand, covalent interactions are much more robust but typically suffer from irreversibility and slower kinetics. Various chemistries, such as click reactions and carbodiimide chemistry, can be utilized for conjugation of a (bio)(macro)molecule and a preformed polymer via the grafting-to methodology [181]. In such conditions, an ATRP strategy creates well-defined polymers, and click reactions enable efficient conjugation under mild reaction conditions [219,220]. Moreover, using the grafting-from methodology, polymers can grow from initiation site(s) on (bio)(macro)molecules such as oligonucleotides and nucleic acids, peptides, proteins, and carbohydrates to make bioconjugates [221,222].

On the other hand, strong physical interactions between bulky polymers and (bio)(macro)molecules require the use of multivalent/polyvalent physical interactions to compensate for challenges associated with the relative weakness of physical bonds [223]. This phenomenon has prompted nature to use the four letters of the DNA sequence, in which every two complementary base pairs interact via two or three hydrogen bonds, to hold the two bulky strands of DNA together firmly [224]. The strength of multiple hydrogen bonding interactions in oligonucleotides can be exploited to design strong physical anchors between two macromolecules. Thus, two macromolecules containing two complementary oligonucleotides in their structure can conjugate through hybridization, with the benefits of appropriate strength and reversibility [225]. Similarly, a class of synthetic polymers, known as supramolecular polymeric materials, utilize physical interactions in the molecular structure of supramonomers or supramolecular polymers [226]. This section discusses the conjugation of two or more macromolecules through physical and chemical interactions and the role of ATRP methodologies in such conjugations.

4.1. Chemical conjugation

Both grafting-to and grafting-from strategies require accessible, reactive functional groups for chemical conjugation. In fact, since complex (bio)molecules like proteins form hierarchical structures, such as supramolecular assemblies, the accessibility of reactive functionalities is important when making bioconjugates. In this section, we give an overview of the chemical conjugation strategies for making bioconjugates and synthetic conjugates [227,228].

4.1.1. Bioconjugates

Biomolecule–polymer conjugates (BPCs) combine the bioactivity and functions of biological entities with appealing features of synthetic polymers such as stability, stimuli-responsiveness, mechanical properties, and electrical conductivity [229,230]. In addition, they are capable of functioning as bioimaging probes for understanding inter- or intracellular events and designing theranostic platforms [231,232]. CRP strategies such as ATRP play a crucial role in making BPCs [221,233]. Chemical, enzymatic, and photo-induced reactions are also vital to the process [234].

Proteins with complex but delicate hierarchical structures can be modified with polymers for various purposes. For example, the circulation time of protein therapeutics can be enhanced using a PEG coating [235,236]. Protein–polymer conjugates (PPCs) constitute the most well-known class of BPCs and there are several FDA-approved PEGylated protein therapeutics on the market today [237]. Reactive chemical functional groups on the outer surface of proteins serve as handles permitting attachment of monomers (in the grafting-from methodology) or polymers (in the grafting-to methodology). However, steric hinderance, low concentration of reactive species, the fragile nature of many biomolecules, and challenges associated with chemical interference require the use of special types of chemistry, especially click reactions.

For click ligation, non-natural clickable groups should be installed on the protein sequence before conjugation with polymers or immobilization of ATRP initiators. Cysteine, lysine, and tyrosine residues on the primary structure of proteins have been widely used for chemical modifications. Cysteine residues are relatively rare in protein sequences, allowing for site-specific or chemoselective conjugation [238]. Thiol moieties can react with maleimide through Michael addition, resulting in formation of carbon–sulfur (i.e., thioether) bonds. Moreover, iodoacetamide and 3-arylpropionitriles can also react with thiol moieties to create carbon–sulfur bonds [229,239]. On the other hand, redox-labile disulfide bonds can be created via pyridyl disulfide exchange [240].

Contrary to cysteine, lysine residues are relatively abundant in protein sequences and can covalently attach to other polymers through N-hydroxysuccinimidyl (NHS) esters, acid halides, isocyanates, isothiocyanates, squaric acid, benzoyl fluorides, and nitro-phenyl carbonates [229,238,241]. Because of their less-reactive nature, tyrosine residues have attracted much less attention for protein conjugation [229].

Carbodiimide chemistry and acid halide reactivity have been exploited for modification of peptides [242,243]. In addition, reactive side chains on polymers (e.g., strained rings and clickable groups) can be utilized to conjugate short peptides. For example, in a recent work, short peptides were conjugated by using a ring-opening reaction of pendant epoxy moieties of poly([ethylene glycol methyl ether methacrylate]–co-[glycidyl methacrylate]) (P(EGMA-co-GMA)) [244].

The conjugation of oligo- or polysaccharides (OSA or PSA) with synthetic polymers is another important class of bioconjugation [245]. An ATRP initiator can be grafted on hydroxyl, amine, and carboxylic acid functionalities on pyranose rings of OSA/PSA or aldehyde groups of oxidized OSAs/PSAs to initiate a grafting-from strategy [246]. For example, BiBB was grafted to hydroxyl groups of dextran followed by polymerization of oligo(ethylene glycol) monomethacrylate (OEGMA) to produce bottlebrush-like macromolecules [247]. In another work, α -bromoisobutyric acid was used to convert a biomolecular initiator for ATRP via an esterification reaction [248]. More complete reviews on PSA–polymer conjugates using ATRP can be found elsewhere [249,250].

As an invaluable building block of life on our planet, carbohydrate polymers play important roles in many biological processes, indicating that conjugation with synthetic polymers should profoundly enhance their physicochemical properties and functionalities. In a review article, the Matyjaszewski's group has discussed the applications of reactive dissipative particle dynamics (RDPD) strategies for preparation of polymer–carbohydrate conjugates known as glycopolymers [251]. The prepared, tailor-made glycopolymer brushes can interact with lectin proteins on plasma membranes, thereby affecting cellular functions such as differentiation, resulting in numerous biomedical applications such as biosensing. Another review by the Matyjaszewski's group discusses the conjugation of polymers to various biomacromolecules, such as proteins and vesicles, using grafting-to and grafting-from methodologies, along with their applications [252].

4.1.2. Synthetic conjugates

For synthetic conjugates, the general concepts and chemistries are similar to bioconjugates, and covalent attachment is done through reactive handles (e.g., clickable groups, strained rings, amines, halide acids, and carboxylic acids) as end groups or side chains. This section gives an overview of some special kinds of polymer–polymer conjugates. Polymer–drug conjugates (PDCs) are an important class of synthetic conjugates, and they play a vital role in increasing the solubility and stability of drug molecules [253].

Making diblock copolymers containing hydrophilic and hydrophobic species is a popular type of conjugation. For example, such a strategy can be utilized in polymerization-induced self-assembly (PISA), for drug delivery or similar applications [254,255]. Other conjugated polymers that benefit from unique optoelectronic properties are appealing macromolecules for a range of biomedical applications including bioimaging, theranostics, and photothermal therapy [256]. They can be conjugated with other types of polymers to enhance properties like biocompatibility, water solubility, and processability. For example, poly(p-phenylenevinylene) (PPV) end-functionalized by α -bromoester (an acid halide initiator) was used as an ATRP macroinitiator for methyl methacrylate (MMA) polymerization to make PPV-*b*-PMMA copolymers [257]. In another research article, ATRP was used to make poly(thiophene)-graft-poly(acrylate urethane) (PTh-g-PAU) [258]. Such macromolecular conjugates can be used in applications such as bioimaging and photothermal therapy of cancer.

Other conjugates exist that combine the fascinating properties of various polymers. For example, ATRP can be used to make conjugated polymers with two or more segments, where each segment has unique properties or architecture. Conjugation can be used to induce biodegradability, electroactivity (e.g., poly(vinylidene fluoride) or PVDF), or single/multiple stimuli-responsiveness, combining the desired functions into a single molecule, a multifunctional polymer. Conjugation also enables the creation of architecturally complex structures from simple, well-studied architectures. For example, linear and bottlebrush polymers can be conjugated

to make special kinds of polymers, which will be discussed in following sections. Moreover, it is possible to conjugate bottlebrush copolymers to star polymers or dendrimers to make more complex architectural structures. Such fascinating molecular structures usually result in unprecedented properties for the obtained polymers. Furthermore, formation of hierarchical self-assemblies may be expected for such complex architectures, and this will open new doors to further research and development.

4.2. Physical conjugation

Physical bioconjugates can be classified into two subclasses, including: 1) nature-inspired biorecognition motifs based on specific multivalent/polyvalent physical interactions and 2) less-specific electrostatic interactions. The most important members of the first class include complementary nucleobases introduced as end or middle segments in biomolecules and polymers. For example, ATRP initiators containing an oligonucleotide segment can undergo oligonucleotide hybridization and immobilize onto a biomolecule before polymerization [259]. In addition, DNA can be used as macroinitiator for ATRP to make copolymers of DNA with synthetic polymers [260]. DNA-polymer conjugates enable the construction of spectacular functional nanostructures with unprecedented structural complexity and unique physicochemical properties for applications throughout the biomedical field [261].

Oligonucleotide hybridization is, in fact, just one subclass of a broader class, based on non-covalent but specific interactions between complementary molecular-recognition motifs, known as non-covalent click chemistry [262]. Other important members include streptavidin (SAv)-biotin, cucurbituril-adamantane, cyclodextrin-adamantane, and cucurbituril-ferrocene interactions that can be exploited to make physical bioconjugates. For instance, biotinylated ATRP initiators (i.e., a biotin-containing BiB end group, or biotin-BiB) were conjugated to streptavidin to make a macroinitiator for ATRP [263]. This work used the specific physical interactions between biotin and streptavidin to immobilize ATRP initiators on streptavidin. Such physical and specific interactions are fast and occur under biologically relevant conditions, making them of utmost importance in biomedicine for protein or cell manipulation. In another work, physical interactions between β -CD trimer and adamantane (ADM) was used to conjugate β -CD-g-polyacrylamide (PAM) (β -CD-g-PAM) (a star polymer) to ADM-g-PMMA [264]. Trivalent physical interactions, between β -CD trimer and three ADM-g-PMMA molecules, were used to make miktoarm star polymers. β -CD trimer was synthesized using a copper-catalyzed azide/alkyne cycloaddition (CuAAC) click reaction [265]. Moreover, ADM was modified by an ATRP initiator and serves as the ATRP initiation site for controlled polymerization of methyl methacrylate from ADM, to yield ADM-g-PMMA. Each arm of the resulted star polymers is a polymer-polymer conjugate with specific physical interactions in the middle [264].

5. Surface modification and functionalization via ATRP

In the biomedical field, the surface properties of materials greatly impact their interactions with biological systems (e.g., biocompatibility, immunogenicity, and protein adsorption) and their *in vivo* fates [266]. For example, surface properties mainly define the biocompatibility of medical implants and the circulation time of NPs. Surface modification strategies aim to alter the interface properties (biocompatibility, wettability, and friction) of implanted devices for special applications. ATRP can be used to make surface-modified materials via “grafting-from”, “grafting-to”, or “grafting-through” methodologies at different length scales. ATRP provides a robust tool to make polymer-decorated surfaces with different surface chemistries and shapes at various length scales.

Surfaces with a diverse range of materials, including inorganic materials (e.g., ceramic, metals (oxides), semiconductors), organic materials (e.g., polymers) and biological entities (e.g., proteins), can be modified by polymers via an ATRP strategy as represented in Table 2. Normally, the first step in the grafting-from methodology is tethering ATRP initiator (via physical interactions or chemical bonding) on the surface, followed by controlled polymerization. ATRP initiators normally include two or more end/middle functionalities: 1) BiB and 2) reactive (e.g., acid halide, alkoxysilane, and chlorosilane) or anchorable functional groups (catechol and disulfide). The latter enable tethering on the surface, while the former provide initiation sites for controlled polymerization.

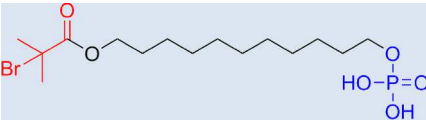
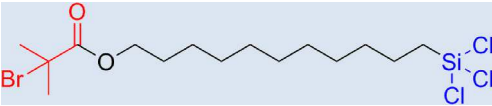
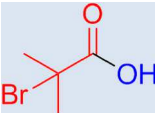
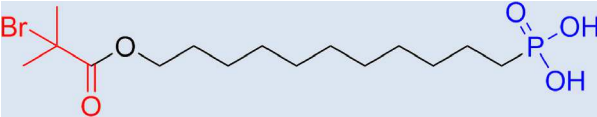
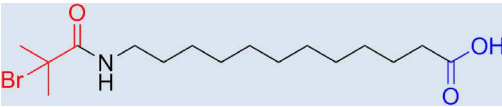
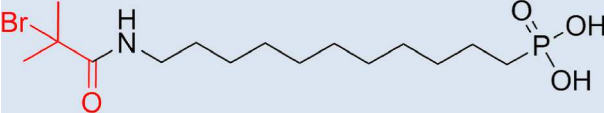
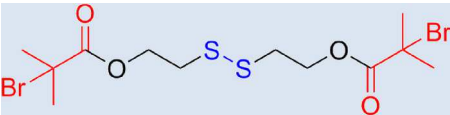
Moreover, the grafting-to strategy requires two complementary reactive handles—one on the surface and one on the polymer chain end—for efficient attachment. Click chemistry, which enables orthogonal and efficient chemical reactions under mild conditions, is one of the most widely applied solutions for this methodology [267]. Grafting-to via ATRP typically involves two steps: first, making polymer chains containing clickable end-functionalities (or other reactive handles), and then immobilizing the polymer chains on the surface through a click reaction. Orthogonality ensures the intactness of the immobilized clickable group on the surface in the reaction medium [268]. Another robust strategy for conjugating polymers and reactive handles on the surface is carbodiimide chemistry.

This section discusses the application of ATRP in creating polymer-modified surfaces with different surface chemistries, with special focus on the grafting-from methodology. In concave surfaces, complications related to mass transfer and steric hindrances can alter the efficient grafting of polymer chains to the nanomaterial surface, such as within the lumen of nanotubes/nanopores or on irregularly shaped NPs. Nevertheless, the ATRP-mediated grafting of growing polymer chains (referred to as the grafting-from methodology) presents a robust strategy to produce polymer-decorated surfaces with various shapes (concave, flat, or convex) and curvatures at multiple length scales, ranging from nano- to macroscale [269]. Compared to conventional polymer decoration strategies with limited grafting densities, the grafting-from methodology, using tethered ATRP initiators, can yield a dense forest of polymer brushes on the surface. In fact, SI-ATRP can be effectively used to grow dense and uniform polymer brushes from a wide range of nanomaterials, resulting in a polymer corona that significantly influences the nanomaterial's interactions with biological entities [270,271]. As the surface functionalities and their modification strategies depend on the inherent chemistry of materials, the classification in this section is arranged based on the type of material.

Materials that combine inorganic and organic segments often exhibit unique properties, and they are commonly found in natural structures [271,272]. Therefore, the combination of inorganic materials with organic polymers results in hybrid materials that exhibit

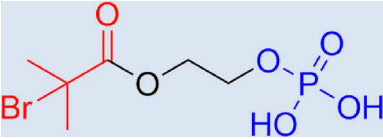
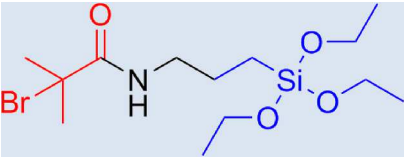
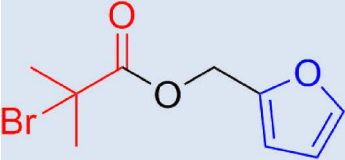
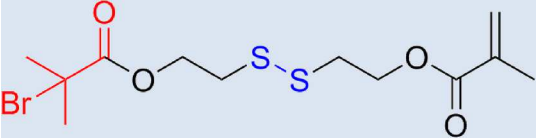
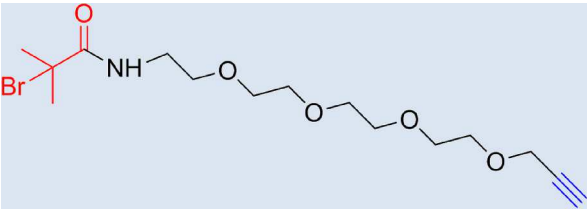
Table 2

Tethering ATRP initiators on materials with different surface functionalities.

Attachment type	Substrate material	Surface functional groups	Anchoring group	ATRP initiator example	Ref
Chemical bonding	Silica/mica	OH	Phosphonic acid		[276]
	Silica	OH	Trichlorosilane		[277]
	n-HAP NC	OH	Carboxylic acid		[278]
	Metal alloy	OH	Phosphonic acid		[279]
	Ti	OH	Carboxylic acid		[280]
	Ti/TiO ₂	OH	Phosphonic acid		[281]
	Gold	–	Thiol		[282]

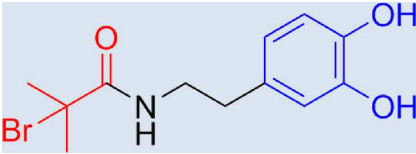
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Table 2 (continued)

Attachment type	Substrate material	Surface functional groups	Anchoring group	ATRP initiator example	Ref
	Fe ₃ O ₄ NPs	OH	Phosphonic acid		[283]
	Metal oxide	OH	Alkoxysilane		[284]
		–	Furan		[285]
	Polystyrene	Vinyl	Vinyl		[49]
	Virus-like NPs	Azide	Alkyne (click)		[286]

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Table 2 (continued)

Attachment type	Substrate material	Surface functional groups	Anchoring group	ATRP initiator example	Ref
Physical interactions	Gold	Au ³⁺	Catechol		[287]
	various	Local charges	Charged species	Polyelectrolyte macroinitiator	[288]

novel and advantageous properties. An illustrative example is hairy NPs that combine brush (co)polymers with nanoscale inorganic materials, resulting in multifunctional materials [273]. It is worth mentioning that, in a review article by the Matyjaszewski's group, the properties and applications of brush-modified materials or hairy NPs are thoroughly discussed [274]. These hybrid brush particle-based materials are mainly manufactured using a surface-initiated polymerization, especially surface-initiated RDRP, and exhibit properties different from those of conventional composite materials. Moreover, another review article by the Matyjaszewski's group presented the progress that has been made in utilization of SI-ATRP to make multifunctional hybrid materials [275]. They discussed the properties of these advanced materials and summarized their potential applications in making nanocomposites. However, their applications in the biomedical field were not discussed. In this section, we focus on grafting polymer chains onto materials at different scales, from nano to micro to macroscales.

5.1. Glass, silicon, and silica

Glass and silicon are commonly used as substrates in the biomedical field, such as microfluidics and organ-on-a-chip (OOC) devices [289]. The inertness of these materials' surfaces highlights the significance of modifying them with hydrophilic and bioactive polymers. Polymer brush-modified silica finds application in bioseparation processes like size exclusion chromatography (SEC) [290]. These materials have hydroxyl groups on their surface, or hydroxyl groups can be introduced through etching using strong oxidants like piranha solution. Subsequently, reactive chemical functionalities, such as alkoxy silane and chlorosilane, based on silane compounds, are widely used to modify such substrates [291]. For example, (3-aminopropyl)triethoxysilane (APTES) is a commonly used aminosilane for surface modification of silica because it reacts easily with hydroxyl groups on these materials, resulting in a more reactive amine group. This amine functionality can be conjugated with an ATRP initiator containing a reactive functional group (e.g., acid halide), like 2-bromoisobutyryl bromide (BiBB) [292].

On the other hand, alkoxy silane and chlorosilane functional groups can be added to ATRP initiators, such as 3-(triethoxysilyl) propyl 2-bromo-2-methylpropanoate [293,294]. These ATRP initiators have two reactive handles: one for attaching to the substrate and the other for enabling controlled polymerization. This modification strategy has been applied to silicon wafers [295], glass [296], silica NPs [297], and silica-coated NPs [294]. For example, the SI-seATRP method was used to attach (co)polymer brushes to silica NPs with a high number density and relatively low dispersity [298]. The ATRP initiator containing both chlorosilane and bromoisobutyrate (BiB) functionalities was grafted onto silica NPs (average diameter 15.8 nm) with density of 1.5 nm^{-2} , as shown in Fig. 8. Subsequently, various (co)polymers were grafted from silica NPs using ATRP, resulting in a high graft density of 0.93 nm^{-2} .

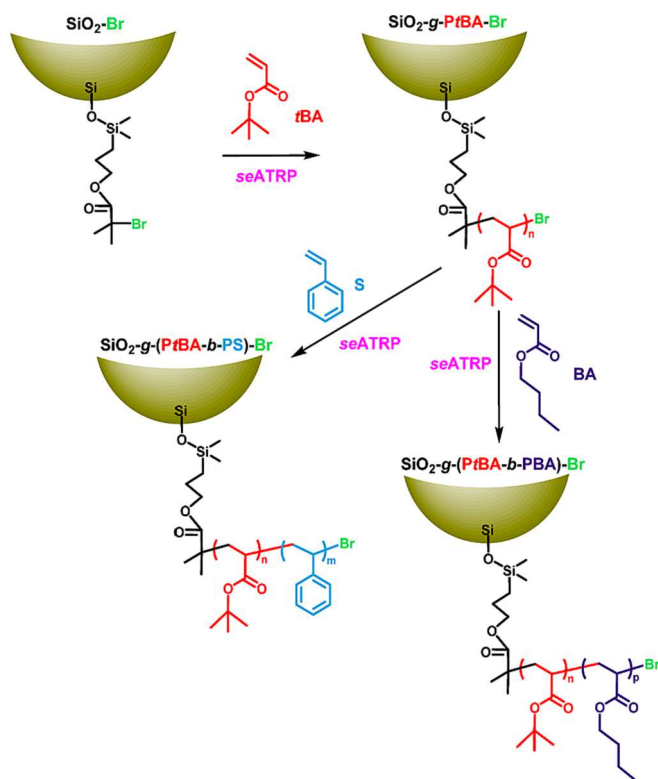


Fig. 8. Schematic depicting the synthesis of polymer brush coating on silicon nanoparticles via seATRP. The process involves several synthetic steps: (i) grafting of ATRP initiator on silicon (SI) NPs, (ii) controlled polymerization of *tert*-butyl acrylate via seATRP, and (iii) copolymerization with butyl acrylate or styrene via seATRP [298].

In some cases, surface treatment (e.g., plasma treatment) may be necessary to create, or increase the number of, hydroxyl groups on the substrate before attaching the ATRP initiator. For instance, poly(2-hydroxyethyl methacrylate)-*co*-poly(2-aminoethyl methacrylate hydrochloride)(PHEMA-*co*-P(2-AEMA·HCl)) brushes were grafted from silicon wafers via ATRP [299]. Oxygen plasma treatment was carried out to activate the silicon surface (i.e., to create hydroxyl functional groups) before immobilizing alkoxy silane-functionalized ATRP initiators. Alternatively, alkoxy silanes containing clickable functional groups, such as azide, can be easily conjugated with ATRP initiators that have complementary functional groups, such as alkyne, with high yield and under mild reaction conditions [300].

Additionally, silica surfaces contain abundant silanol groups (Si-O-H), which can be used for silanization with organosiloxanes or organophosphonic acids to attach an ATRP initiator through Si-O-Si or Si-O-P linkages [301,302]. However, the susceptibility of siloxane linkages to hydrolysis emphasizes the importance and stability of phosphonate-based linkages, which can form monodendate, didendate, or tridendate linkages on substrates [303,304]. Organophosphonates, in particular, serve as valuable anchoring agents for grafting polymer brushes onto the surfaces of silica and mica [276]. In a specific study, two ATRP initiators were used for the surface modification of silica. The first initiator featured a bromosilane reactive handle, while the second had organophosphonate moieties as anchoring groups. These initiators differed in the separation lengths (aliphatic chains) between the surface anchoring groups and the polymerization initiation sites. Under the same reaction conditions, the measured thickness of the polymer coating was significantly greater for surfaces modified with Si-Br (i.e., 120 nm) compared to 2 nm for P-Br. This difference was attributed to a lower number density of grafted initiators for P-Br-modified silica, resulting in a coiled conformation of polymer chains, in contrast to a stretched chain conformation in densely grafted Si-Br initiators (Fig. 9).

5.2. Metals

Most metals are unstable under physiological conditions and require surface modification before being used *in vivo*. Typically, the surfaces of most metals are coated with a thin layer of oxide due to partial oxidation under environmental conditions. This oxide layer may or may not protect the underlying metal from further oxidation, depending on the specific metal (e.g., aluminum and iron exhibit different behaviors). Surface modification strategies for metal oxides are similar to those used for metals, which will be discussed in the next section. Moreover, metal NPs can be coated with a silica shell before SI-ATRP to prevent oxidation or improve biocompatibility. The Stöber process is commonly used for *in situ* coating with a silica shell. For example, silver NPs (AgNPs) were coated with a silica shell before the immobilization of an initiator and SI-ATRP for growing PMMA brushes [305]. Surface modification for these “metal

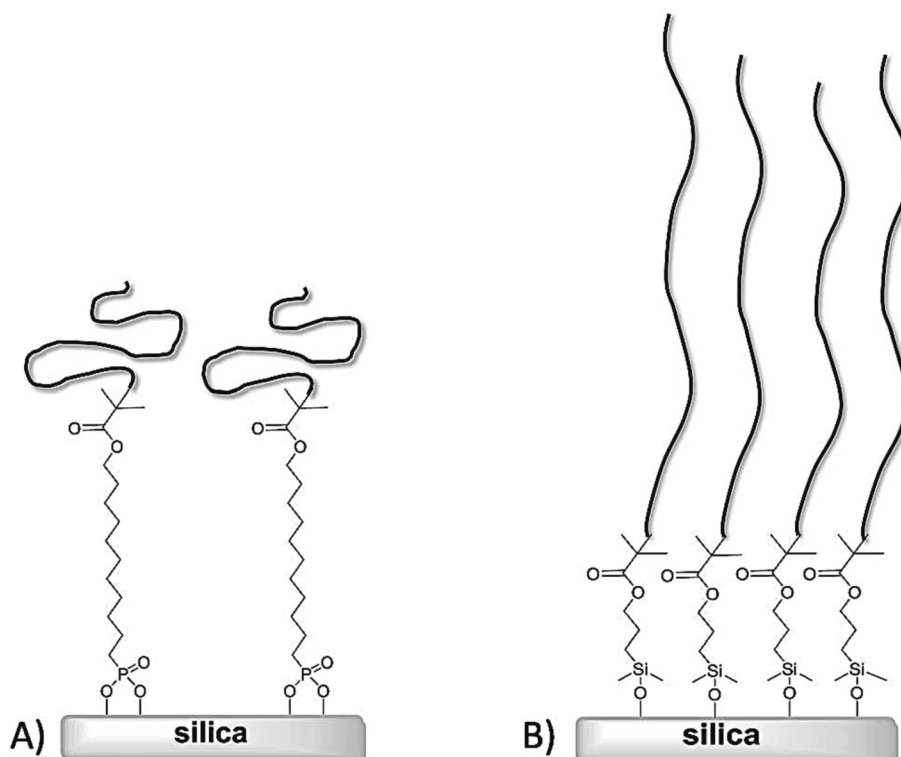


Fig. 9. Schematic displaying polymer brush grafting from a silica surface. (A) The use of an ATRP initiator containing organophosphonate moieties results in lower thickness of the polymer coating, likely due to a lower density of grafted brushes, leading to coiled polymer chains. (B) The use of an ATRP initiator containing bromosilane moieties results in a significantly thicker polymer coating on the silica substrate, likely due to a higher grafting density of the ATRP initiator and the presence of extended polymer chains [276].

core / silica shell” structures is similar to the methodologies used for silica.

However, some metals, such as gold, can withstand harsh environmental conditions allowing direct use of gold NPs (AuNPs) in applications like photothermal therapy (PTT) of cancers and bioimaging [306]. Thiol and disulfide functional groups have a strong affinity for the relatively inert surface of AuNPs. In most cases, ATRP initiators containing thiol, disulfide, or sulfur end-functional groups are used to chemically tether ATRP initiators to the gold surface for SI-ATRP, utilizing relatively strong metal–ligand interactions [307]. This chemical immobilization technique results in a uniform and dense polymer corona around the nanomaterials. Unlike physical adsorption methods, these polymer brushes firmly attach to the core, ensuring high stability even under harsh *in vivo* conditions. In a recent study, gold films were functionalized with a thiol-based initiator for grafting thermosensitive brush polymers via SI-ARGET ATRP [308]. The initiator contained thiol and BiB moieties on two ends of an aliphatic chain. In another study, an ATRP initiator containing disulfide was used to grow an anionic corona around AuNPs using SI-ATRP [309]. Tethering disulfide on AuNPs typically forms a V-shaped molecule, with both ends capable of initiating controlled polymerization.

The use of N-heterocyclic carbenes (NHCs) ligands, which form strong and stable bonds with metals, has become a popular strategy in gold functionalization. Furthermore, the stability of NHCs in gold nanostructures can be tailored through chemical design of NHCs ligands [310]. As thiols are susceptible to oxidation degradation, NHCs ligands offer an attractive alternative for designing functionalized gold nanostructures for *in vivo* applications, thanks to their significantly higher stability [311]. In some studies, ATRP has been used to synthesize polymers with NHC-functionalization, followed by ligand exchange to produce polymer-modified Au nanostructures [312]. This approach allows the formation of polymer-modified Au nanostructures with improved long-term stability in biological environments. However, to date, there is still limited research on the utilization of ATRP and NHC ligands for Au modifications.

Another strategy involves using materials with inherent complexation properties. For instance, dopamine and phosphonic acid are chelating agents that firmly attach to metal NPs. Polydopamine (PDA) chemistry is a robust method to modify relatively inert surfaces such as AuNPs [313]. The unique chemical structure of dopamine and PDA, which includes catechol and amine functional groups, allows PDA to attach to various surface chemistries [313]. Additionally, materials based on phosphonic acid possess excellent metal complexation capability and high biocompatibility, making them attractive for surface modification of metals [314].

5.3. Metal oxides

Metal oxides, such as superparamagnetic iron oxide NPs (SPIONs), hold great promise for various biomedical applications, especially in drug delivery, bioimaging, theranostics, and image-guided therapy [315]. However, to enhance their biocompatibility and stability for improved efficacy, a common and effective approach is to coat them with hydrophilic polymers like PEG. By using hydrophilic brushes that firmly attach with high densities, the biocompatibility of SPIONs is improved, making them “stealth NPs” that can evade the immune system [316]. In previous sections, we have discussed various coupling agents, such as thiols, silanes, phosphonates, and carboxylic acids, which are typically used for the surface modification of metal oxides.

Silane-based initiators, such as alkoxy- and chlorosilane, as discussed earlier, are commonly used for the surface modification of metal oxides. For example, lauric acid-coated Fe_3O_4 NPs were immobilized with an ATRP initiator bearing alkoxy-silane (2-bromopropionamidepropyl trimethoxysilane), before conducting SI-ATRP of NIPAM [318 306]. Lauric acid is a fatty acid usually used to make colloidal dispersions of NPs. The resulting PNIPAAm brush-coated SPIONs served as a dual-sensitive (temperature and pH) theranostic platform, enabling controlled delivery of anticancer drugs and functioning as a bioimaging material for MRI contrast enhancement. In addition, the Matyjaszewski’s group developed a low-cost tetherable initiator that can be used for the fabrication of various metal oxide NPs, as depicted in Fig. 10 [317].

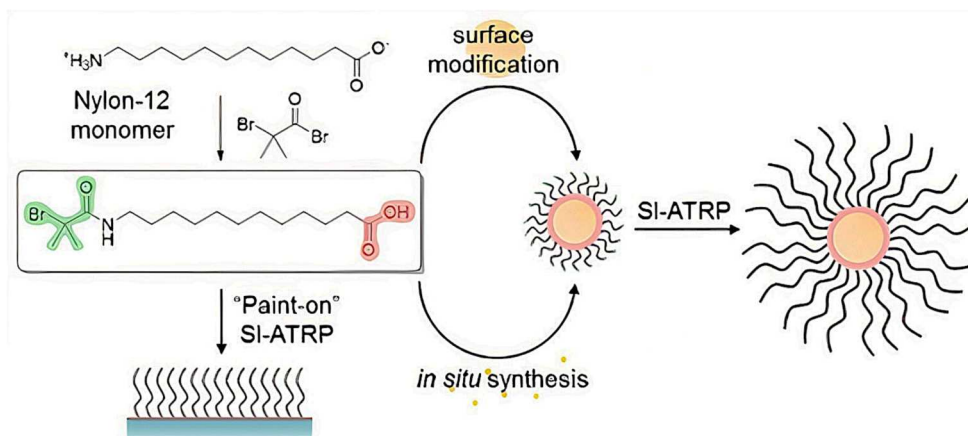


Fig. 10. Schematic depicting a universally tetherable ATRP initiator. This illustration describes the manufacturing process for an ATRP initiator that can be used for the modification of various materials. The initiator contains 2-bromoisobutyryl amide and carboxylic acid end-functional groups with an aliphatic chain (spacer) in the middle [317].

Oleic acid is commonly used to stabilize metal (oxide) NPs. Inspired by the chemical structure of this fatty acid, the Matyjaszewski's group developed a tetherable initiator that features carboxylate moieties on one end and 2-bromoisobutyryl amide on the other end. The carboxylate group facilitates anchoring to the metal surface, while 2-bromoisobutyryl enables SI-ATRP. This tethered initiator was also utilized to coat aluminum foil with polymer brushes. Chen *et al.* utilized an SI-photoATRP method with PTH as the photocatalyst to graft PAA onto the surface of UCNPs, creating UCNP@PAA for bioimaging applications [319].

5.4. Semiconductors

Conventional inorganic semiconductors (e.g., Si and Ge) and modern organic semiconductors (e.g., poly(3,4-ethylenedioxythiophene) [PEDOT]) are essential for designing (organic) electronics such as solar cells and display devices. In biomedical applications, doped (in)organic semiconducting materials, such as quantum dots (QDs) or NPs, exhibit advantageous and tunable optoelectronic properties for phototherapy, bioimaging, and biosensing [320]. To ensure their biocompatibility, coating these materials with polymers, especially densely grafted polymer brushes, using surface-initiated CRP (SI-CRP) methodologies can be an effective approach [321]. This section discusses the application of ATRP for surface modification of (in)organic semiconductors. QDs are nanoscale semiconductors with remarkable optical and electronic features, and they have been widely used in various applications from electronic devices to biotechnology [322]. Their superior optical properties, such as photobleaching stability and narrow emission spectra, make them invaluable for bioimaging [323]. However, challenges such as *in vivo* instability and poor dispersibility and solubility in aqueous solutions, limited biodistribution, and toxicity have restricted their applications [324]. To address the water insolubility of QDs, a promising approach is coating them with hydrophilic polymers like PEG brushes. For instance, in a study, poly (oligo(ethylene glycol) monomethacrylate) (POEGMA) was grafted onto silicon QDs [325]. These silicon-based QDs share similar chemistry with silicone, although the process details may vary slightly. The study involved a pretreatment to create hydroxy groups on Si QDs, followed by esterification using an ATRP initiator containing an acid halide end group [325].

In another study, the surface of ZnS QDs was modified by APTES and BiBB before SI-ATRP to grow PMMA from the QD surface, as illustrated in Fig. 11 [326–315]. The reactivity of amine and acid halide was leveraged to tether the initiator onto the QD surface. Alternatively, an esterification reaction between the hydroxyl groups on CdSe QDs and the acid halide end-group of the ATRP initiator was used to grow poly(glycidyl methacrylate) (PGMA) brushes from the QD surface [327].

Furthermore, amine-functionalized biotin was grafted to the epoxide pendant groups of PGMA via a ring-opening reaction, resulting in a multifunctional hybrid biotinylated-PGMA-g-CdSe. This approach significantly improved biocompatibility compared to bare CdSe QDs while preserving good optical properties [327]. It is important to note that while the surface chemistry of QDs is generally similar to their higher-length scale counterparts, edge effects are more pronounced in QDs, and edge functionalities may differ from other functional groups in the middle. For example, graphene oxide (GO) chemistry has shown that more carboxylic acid is

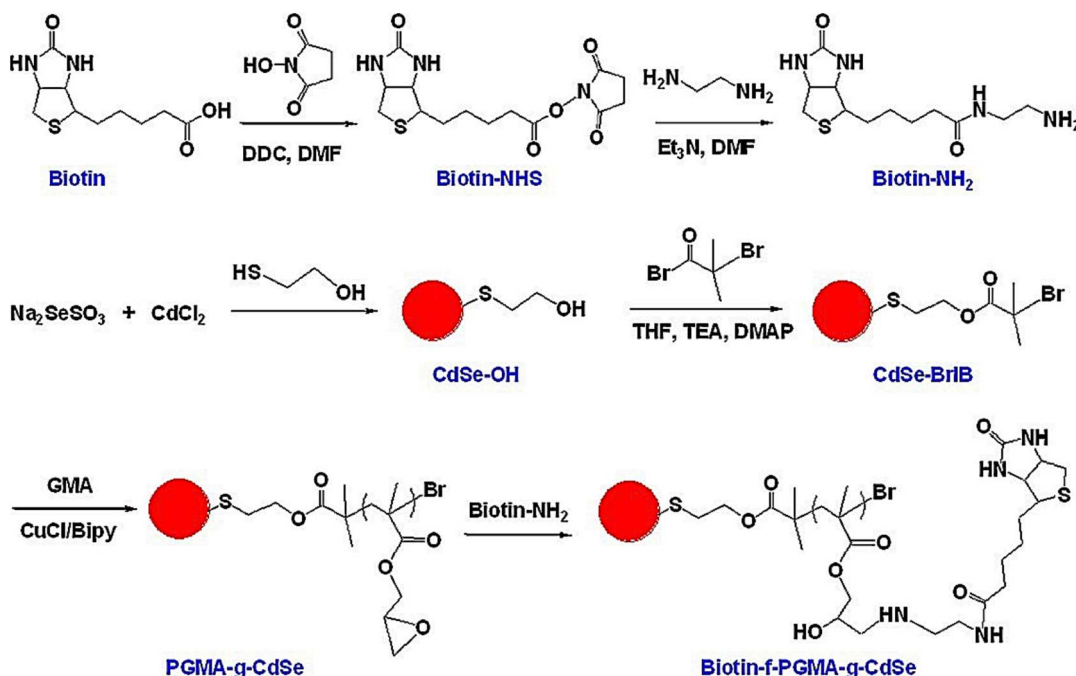


Fig. 11. Schematic describing a multistep approach for the synthesis of polymer brush-coated QD nanoparticles. Carboxylic acid functionalities on biotin were converted into more reactive amine functional groups using carbodiimide chemistry. Hydroxylated CdSe QDs were prepared and functionalized with an ATRP initiator. Poly(glycidyl methacrylate) (PGMA) brushes grow from the QD surface via ATRP, followed by the attachment of biotin moieties to the epoxide side groups of PGMA via a ring-opening reaction [327].

present on the edges, while more epoxy and hydroxyl are found in the basal plane [328].

Conjugated polymers such as polyaniline (PANI), polypyrrole (PPy), and polythiophenes exhibit semiconducting behavior and peculiar optical properties in doped states [329]. Their light-absorbing NPs find applications in photothermal therapy (PTT) of cancers [330]. However, most suffer from low water dispersibility and biocompatibility. To address this, poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) and poly(2-hydroxyethyl methacrylate) (PHEMA) (co)polymer brushes were grown from PPy NPs using SI-ATRP [331]. Resulting PPy NPs were water dispersible and showed dual responsive properties (pH and temperature). The surfaces of PPy NPs were modified by an alkoxysilane (N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, AAPS), and BiBB (the ATRP initiator) was tethered. Various chemical (e.g., via substitution on aromatic rings or side chains) or physical (e.g., via mussel-inspired chemistry) tethering methodologies can be used for surface modification of these conjugated polymer NPs. Care should be taken during molecular-scale modification (i.e., grafting an oligo-/polymer on a polymer backbone) to avoid disrupting the extended conjugated network of polymers [332]. For example, substitution on thiophene (e.g., in poly(3-hexylthiophene) [P3HT]) will not significantly affect the conjugated network of polythiophenes, while chemical modification of nitrogen atoms in quinoid or benzenoid rings of PANI can distort the conjugated network of the polymer backbone, resulting in diminished electrical conductivity.

5.5. Organic nanomaterials

Carbon-based nanomaterials, including carbon nanotubes (CNTs) and graphene, offer high electrical conductivity and thermal stability, making them promising for various biomedical applications such as PTT, imaging, and biosensing [333]. The chemical modification of pristine graphene and CNTs can be achieved using reactive chemicals like thionyl chloride [334]. Meanwhile, top-down strategies for producing graphene, such as Hummers' method, result in GO nanoplatelets decorated with various oxygenated functional groups [328,335], and commercially available CNTs already possess functional groups like hydroxyl and carboxyl, making them suitable for further chemical functionalization [336].

Various covalent and non-covalent strategies can be used to functionalize CNTs or graphene. One approach involves utilizing π - π stacking interactions between CNT or graphene sheets and pyrene moieties to adsorb molecules onto the surface. For instance, 1-pyrenebutanoic acid succinimidyl ester (PBASE) and 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBSE) were adsorbed onto CNTs via π - π stacking interactions [337], where pyrene moieties act as anchors to immobilize ATRP initiators on the surface. Although there are limited works on pyrene-containing molecules for SI-ATRP, using polymer-based initiators with multiple pyrene pendant groups can strengthen these interactions through a multivalency phenomenon [223]. Additionally, Diels-Alder cycloaddition provides another covalent immobilization chemistry for ATRP initiators on CNT surfaces, requiring the ATRP initiator to have a dienophile like furan in its chemical structure [285].

GO, with its abundance of oxygen functionalities, offers various chemistries for chemical modifications. Direct amidation of carboxylic acid, epoxide ring opening, esterification of carboxylic acids, etherification of hydroxyl groups, and reduction of carbonyl functionalities can all be used to modify GO or introduce other functionalities or molecules, such as ATRP initiators. For instance, GO platelets were modified with BiBB before grafting poly[2-(*tert*-butylaminoethyl) methacrylate] (PTA) brushes from their surfaces using ATRP and FRP [338]. ATRP showed a higher grafting density compared to FRP, with grafting percentages of 25.7 and 22.3 wt% for ATRP and FRP, respectively. Catechol and gallol moieties can also serve as anchors for immobilizing ATRP initiators on CNT and graphene surfaces. A copolymer of tannic acid and triethylenetetramine (TETA) was used to graft an ATRP initiator on CNT surfaces [339]. Tannic acid, containing gallol functionalities, allows attachment to various surfaces with different chemistries, similar to PDA, which contains catechol moieties.

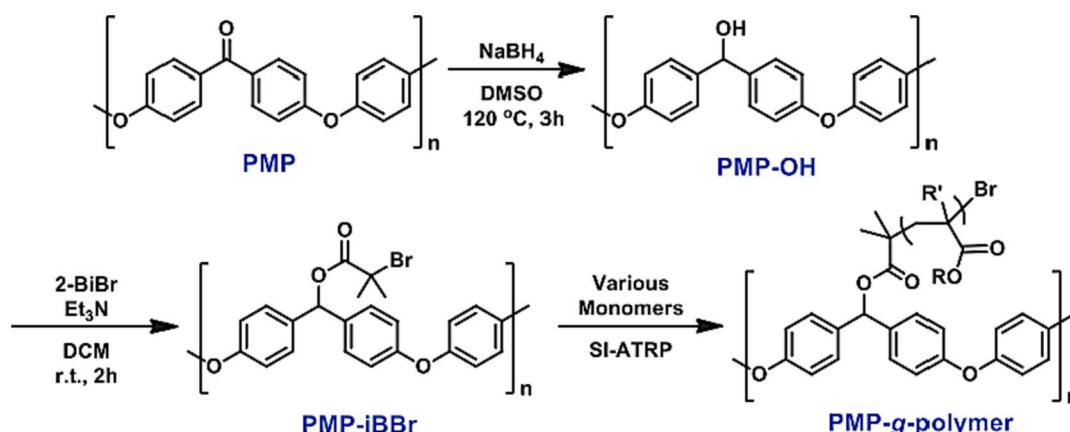


Fig. 12. Schematic highlighting the surface modification of PEEK microparticles using SI-ATRP. First, carbonyl groups on PEEK chains were chemically reduced to hydroxyl functional groups using sodium borohydride. Then 2-bromoisobutyryl bromide (BiBB), the ATRP initiator, was attached to PEEK chains via esterification of acid halide and hydroxyl functionalities on BiBB and PEEK, respectively. Various polymers were then grown from these ATRP initiation sites via SI-ATRP, resulting in polymer-grafted PEEK microparticles [340].

5.6. Polymers

ATRP can be used to functionalize the surfaces of various polymer-based materials, including NPs, nanofibers, nanogels, microgels, or hydrogels, similar to the materials discussed earlier. This approach is beneficial when specific surface chemical functionalities are required, and the choice of ATRP initiator becomes crucial. The conjugation of two polymers at the molecular scale will be covered in Section 5, which also delves into the process of polymerization-induced self-assembly (PISA) in NPs that self-assemble *in situ* after polymerization. For example, polyether ether ketone (PEEK) microparticles were modified with PDMAEMA brushes using SI-ATRP [340]. The process involved reducing carbonyl functional groups on the microparticle surface to hydroxyl groups using sodium borohydride, followed by esterification with BiBB to introduce initiation sites for ATRP (Fig. 12).

In a recent study, two SI-ATRP strategies (SARA ATRP and photoATRP) were used for the surface modification of PEEK, using two types of hydrophilic polymer brushes [341]. PDMAEMA and poly(2-hydroxyethyl acrylate) (PHEA) polymer brushes were grown from different PEEK sheets. The modified PEEK materials demonstrated enhanced hydrophilicity, improved mineralization, and enhanced osseointegration when tested for bone tissue engineering. PhotoATRP, known for its cytocompatibility and metal-free nature, was found to be a better choice for PEEK modification.

For some polymeric surfaces, prior treatment is necessary to graft the ATRP initiator and introduce reactive chemical functionalities. Plasma or chemical treatments are commonly used to introduce hydroxyl groups on inert surfaces of polymers. For instance, poly(dimethylsiloxane) (PDMS), a hydrophobic and inert polymer, can be treated with plasma to create surface functional groups. In the case of PDMS, SI-SARA ATRP was used to graft PDMAEMA, imparting antimicrobial properties [342]. ATRP initiator tethering on PDMS was achieved using 2-[4-(chloromethyl)phenyl]ethyl-trimethoxysilane (CPTS), where alkoxysilane was attached to the PDMS surface.

In some cases, ATRP initiators can have polymerizable functional groups that allow ATRP active sites to be embedded throughout the entire polymeric construct or in the outer part of the polymer. For instance, an acrylate monomer containing ATRP initiation sites (e.g., BiB moieties) can be copolymerized with other monomers to produce a hydrogel with embedded ATRP initiation sites [343]. In another study, crosslinked polystyrene (PS) NPs with a shell containing embedded ATRP initiation sites were synthesized to graft poly(methyl acrylate) (PMA) and obtain hairy NPs [49]. The incorporation of ATRP initiation sites in the outer shell was achieved through a two-step polymerization process, where crosslinked PS beads were first synthesized via miniemulsion polymerization of styrene and divinylbenzene (DVB), followed by grafting PMA from the initiator-anchored surface of PS beads to form an outer shell with PMA brushes [49].

Nanogels can play critical roles in drug delivery due to their nanoscale size and hydrated nature. Smart nanogels have been fabricated from stimuli-responsive polymers to release encapsulated drugs in a controlled manner and accumulate in tumor sites through the enhanced permeability and retention (EPR) effect. In this work, decorating the nanogel surface with targeting ligands, such as nanobodies, enabled active targeting of cancer cells [344–333]. ATRP is a versatile method for preparing and functionalizing hydrogel particles at the nano to microscale, where the ATRP initiator can be incorporated as a pendant group in the nanogel's monomer or crosslinker structure, allowing the initiating sites to be embedded throughout the nanogel rather than just on its surface [345,346].

The surface of one-dimensional (1D) polymeric constructs, such as nanofibers and nanotubes, can be modified with polymer brushes to enhance wettability and biocompatibility. These modified nanofibers can be utilized in the production of scaffolds for tissue engineering and wound dressings. For instance, SI-ATRP was employed to modify the bioinert surface of cellulose nanofiber (CNF) with densely grafted polymer brushes, resembling the bottlebrush structure of proteoglycans found in native articular cartilage tissue [347]. Hydroxyl functional groups were introduced onto CNF using a phosphorus pentoxide (P_2O_5) atmosphere as an oxidizing agent. Subsequently, hydrophilic poly[poly(ethylene glycol) methyl ether methacrylate] (PPEGMA) brushes were grown from CNF, with hydroxyl functionalities on CNF reacting with acid halide moieties on the ATRP initiator. Notably, the Matyjaszewski's group provided a review on the beneficial aspects of using ATRP to modify wood-based materials such as cellulose and lignin [348].

5.7. Biological entities

Hybrid structures that incorporate both biological entities (e.g., peptides, proteins, exosomes, viruses, bacteria, living cells) and polymers offer significant advantages. They bring together the strengths of both components within a single platform, enhancing the stability and therapeutic effectiveness of the biological entities [263,349]. The CRP is a technique that can modify natural polymers by attaching synthetic polymer chains. This modification can be applied to the molecular structure of peptides, either as side groups or end groups, or to the outer surface of self-assembled hierarchical structures like tertiary protein structures. In one instance, surface-initiated atom transfer radical polymerization (SI-ATRP) was employed to graft thermosensitive PNIPAAm onto a cyclic peptide. This modification enabled the cyclic peptide to self-assemble into nanotubes [350]. Moreover, an ATRP initiator can be strategically attached to multiple sites on a protein, offering versatile opportunities for tailored functionalization.

The chemical functionalities present on the outer surface of proteins' tertiary structures can serve as anchoring points for the immobilization of ATRP initiators, giving rise to novel protein-based macroinitiators. This method requires avoiding harsh reaction conditions to prevent undesirable protein denaturation [351]. Additionally, specific interactions, often referred to as non-covalent click chemistry, existing between proteins and other molecular species can be harnessed to effectively immobilize ATRP initiators. For instance, a biotin-functionalized ATRP initiator can be conjugated to the streptavidin protein through precise physical interactions, yielding an ATRP macroinitiator. Following controlled polymerization, this process results in the production of a streptavidin–polymer conjugate with minimal dispersity [263]. In the realm of PPCs, the uniformity of grafting and polymer chain length emerges as a

pivotal consideration, underscoring the significance of employing controlled radical polymerization (CRP) techniques to achieve well-defined PPCs [230,238]. Similarly, the synthesis of DNA–polymer conjugates is also feasible through ATRP [352]. These instances offer a glimpse into how polymers can be integrated into single-molecule biological entities. Yet, it is important to note that the modification of other biological entities composed of multiple molecules, such as vesicles and cells, is equally viable through various ATRP methodologies. ATRP initiators were anchored to virus-like NPs, followed by SI-ATRP of OEGMA macromers to create polymer brushes attached to the virus-like NPs [286]. Exosomes, small nanoscale extracellular vesicles secreted by eukaryotic cells, play a role in intercellular signaling in biological systems [353]. These exosomes have garnered attention as nanocarriers for designing DDS due to their biological origin, biocompatibility, tissue penetration, and small size [354]. However, the clinical application of exosomes faces challenges including poor stability, short circulation time, limited targeting ability, and low therapeutic loading capacity. Therefore, exosomes are promising candidates for modification through bioengineering or chemical methods [355]. For instance, click chemistry offers a versatile and robust approach for covalently attaching various biomolecules, such as targeting ligands, fluorescent probes, and oligonucleotides [356]. Recently, the surface of exosomes was modified by grafting well-defined polymers using a biocompatible photoATRP method [357]. This approach enhances the stability, pharmacokinetics, and circulation time of exosomes while maintaining their intrinsic properties, including bioactivity. Moreover, the interactions of exosomes with biological components and the plasma membrane can be tuned by adjusting grafting density and chain length. Lathwal *et al.* employed both grafting-to and grafting-from methods to attach diblock copolymers with a nucleic acid segment onto the exosome surface. Instead of using click chemistry or non-specific interactions, they used specific physical interactions between complementary DNA strands (DNA hybridization) for immobilizing the ATRP initiator (grafting-from strategy) or for linking a preformed block copolymer. Cholesterol (Chol), a component of the plasma membrane, can also serve as an anchor for molecules like oligonucleotides. Its hydrophobic core can integrate into the lipid bilayer, offering strong and reliable anchoring [358]. Both approaches share a common initial step involving the conjugation of an oligonucleotide with Chol, which is then immobilized onto the exosome surface, causing the negatively charged deoxyribonucleic acid (DNA) to extend outward from the lipid membrane. In the grafting-to strategy, a single-stranded DNA (ssDNA) linked to Chol (Chol-ssDNA) is used as the oligonucleotide. Subsequently, complementary DNA (DNA') is functionalized with BiB to create an oligonucleotide macroinitiator. An oligomeric monomer is then polymerized through ATRP to produce DNA'-POEGMA, forming a diblock copolymer. The DNA'-POEGMA is bonded to Chol-ssDNA-modified exosomes (Exo-ssDNA) through specific physical interactions between DNA and DNA'. In the grafting-from strategy, two complementary ssDNAs with Chol and ATRP-initiator end groups are hybridized to form a double-stranded DNA (dsDNA), which is then immobilized in the lipid membrane of exosomes (Fig. 13). The resultant Exo-dsDNA containing BiB units becomes a valuable resource for SI-ATRP of various (macro)monomers. This method of exosome surface modification with tailored polymers allows for the adjustment of stability and *in vivo* behavior of these biological entities [357].

Metabolic glycoengineering (MGE) approaches aim to incorporate non-natural building blocks into glycoconjugates, while CRP methods focus on embellishing or coating cell surfaces with synthetic polymers [359,360]. CRP employs more rigorous reaction conditions, such as high concentrations of free radicals, toxic catalysts, and non-aqueous solvents, which can be detrimental to living cells. However, recently developed cytocompatible ATRP techniques, like photoATRP, have significantly reduced toxic effects by minimizing or eliminating the use of transition-metal catalysts, working in aqueous media, and employing non-harmful reducing agents such as ascorbic acid. Cytocompatible grafting of synthetic polymers onto the surfaces of living cells and other organisms using ATRP can enhance their desired functions [361]. For instance, Kim *et al.* employed SI-ARGET ATRP in an aqueous environment with

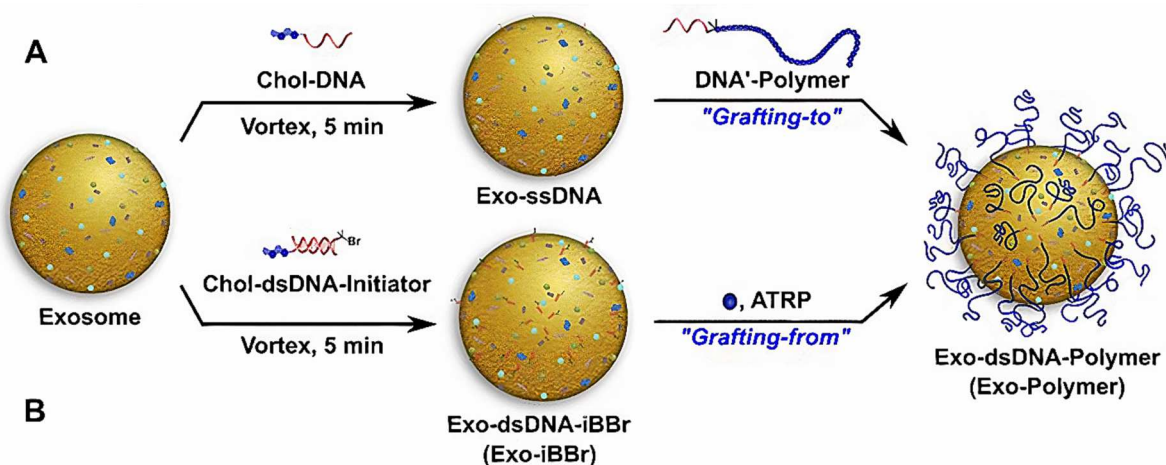


Fig. 13. Schematic depicting the synthetic pathways for generating polymer-modified exosomes. (A) Grafting-to approach: Single-stranded DNA (ssDNA) carrying a Chol moiety (Chol-ssDNA) was employed to immobilize ssDNA onto the exosome lipid membrane. Polymer chains linked to complementary DNA (DNA') were attached to the exosome surface through DNA hybridization. (B) Grafting-from method: Chol-ssDNA was immobilized onto exosome surfaces, followed by hybridization with DNA'-iBBR conjugates to form double-stranded DNA (dsDNA) initiation sites for ATRP. Polymer chains were then grown from these initiation sites on exosome surfaces, resulting in polymer brushes [357].

vitamin C as a reducing agent [349]. They used a PDA-based layer on cell surfaces, which not only immobilized the ATRP initiator (macroinitiator) onto the cell membrane but also shielded the cells from radical species due to PDA's radical-scavenging properties [362]. The entire process was cytocompatible and resulted in the creation of cell-polymer hybrids [360].

5.8. Material-independent methods

Immobilization via physical interactions provides a versatile approach for anchoring ATRP initiators onto surfaces with diverse chemical compositions. Electrostatic interactions, in particular, offer a versatile means of attachment that can be applied to various types of materials. Based on this concept, Li *et al.* proposed a universal method for growing polymer brush layers on different nanomaterials [288]. As shown in Fig. 14, they employed a polyelectrolyte macroinitiator (PMI) that adsorbs onto nanomaterials through electrostatic interactions. The PMI comprised a copolymer of BiBB-functionalized PHEMA and poly(trimethylammonium ethyl methacrylate) (PMAEM). In contrast to traditional small-molecule ATRP initiators that weakly adhere to substrates via a limited number of interaction sites, the PMI establishes robust physical interactions due to its multiple adhesion sites (polyvalent physical interactions). By layer-by-layer (LBL) assembly of the cationic macroinitiator and a polyanionic fluorescent probe, they generated a modified surface with abundant initiation sites for ATRP. The fluorescent probe featured a conjugated polymer doped with poly(styrene sulfonate) (PSS), a macromolecular dopant. The use of such macromolecular dopants mitigates the loss of electrical conductivity that can occur during the aging process of conjugated polymers, a concern when employing small molecule dopants like protonic acids [363].

Mussel-inspired chemistry can be considered a material-independent strategy since it can be applied to surfaces of various chemistries, such as ceramics, metal (oxides), and polymers [313]. Material-independent surface modification with dopamine and PDA relies on catechol and amine functional groups. PDA-modified surfaces provide numerous adhesion sites for physical interaction with virtually any surface [313,364]. Titania (TiO₂) was coated with a PDA layer through oxidative polymerization before tethering the BiBB initiator for SI-SARA ATRP [365]. Conversely, Zhu *et al.* reacted dopamine monomers with BiBB to create modified-dopamine monomers containing 2-bromoisobutyrate functional groups. This was followed by the polymerization of modified-dopamine and unmodified dopamine, resulting in the deposition of an adhesive PDA layer containing ATRP initiation sites on various surfaces [366]. SI-ARGET ATRP was utilized to grow PMMA brushes from PDA macroinitiator-modified surfaces, including glass, aluminum, steel, and polystyrene. Dopamine chemistry, which facilitates immobilization on various surfaces, and pyrene chemistry, which is well-suited for carbon-based nanomaterials, can be combined to create versatile oligo-/polymeric initiators capable of strongly adhering to surfaces of different chemistries. Employing this approach, Wei *et al.* synthesized a copolymer macroinitiator containing catechol, pyrene, and BiB functionalities in subsequent repeating units. This copolymer initiator can be effectively used on substrates of virtually any chemistry [367]. Furthermore, their study revealed a synergistic effect between these two functional groups in terms of anchoring to the surface, resulting in the grafting of a high density of polymer brushes from the GO surface.

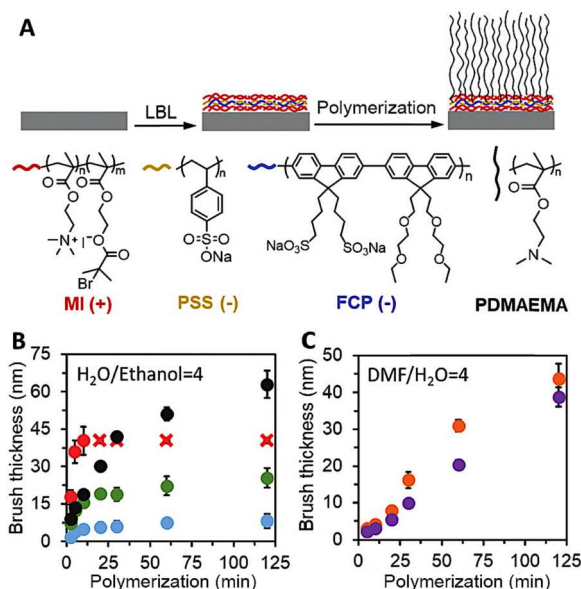


Fig. 14. Schematic illustrating the process of polymer-brush modification on nanomaterials through a material-independent approach. A polycationic macroinitiator derived from a random copolymer is firmly adsorbed onto the surface through polyvalent electrostatic interactions. This polyelectrolyte macroinitiator (PMI) provides numerous initiation sites for ATRP and serves as adsorption sites for anions. Subsequently, a layer-by-layer (LBL) assembly technique is employed to attach two polyanions onto the PMI-modified surface: (1) poly(styrene sulfonate) (PSS), acting as a dopant, and (2) FCP, the fluorescent probe. Eventually, PDMAEMA brushes are grown utilizing the surface-initiated ATRP (SI-ATRP) method [288].

6. Leveraging ATRP for biomedical applications

6.1. Drug delivery

During the last few decades, the delivery of therapeutics to target locations has significantly evolved. An appropriate delivery system should possess a high drug-loading capacity, a high circulation time, and the ability to circumvent biological barriers, evade the immune system, target specific cells, and release the drugs in a sustained and controlled manner [368,369]. Materials constitute the most crucial factor in designing novel DDS. These materials should be biocompatible and non-immunogenic, they should possess high stability under physiological conditions, and they should be smart, in that they can respond to special exo- and/or endogenous stimuli [370,371]. An overview of designed delivery systems shows that they are continually becoming more complex at multiple length scales; both their molecular architecture and their morphological features are becoming more intricate as well as more delicate [17,372]. On one hand are the advancements in modern organic chemistry such as the evolution of click chemistry, bioorthogonal and (photo)click chemistries, the dynamic chemistry toolbox, and emerging controlled polymerization techniques; on the other hand, are the great achievements in nanotechnology and biology. Leveraging both sets of tools allows materials scientists to design and fabricate novel delivery systems for precise targeting of specific cells [27,176,373,374].

Fabrication of well-defined macromolecular structures plays a pivotal role in designing complex chemical systems, such as novel DDS. In such complex systems, chemical composition, architecture, topology, and dynamic interactions of the constituting macromolecule(s) can be finely tuned through modern organic chemistry strategies, especially the CRP methods [24,375]. This section highlights the importance of the ATRP technique in making novel DDS, although fabrication of such complex chemical systems often requires various CRPs, the click and orthogonal chemistry toolbox, the dynamic chemistry toolbox, and other chemistries in sequence or in parallel.

Polymers with complex molecular structures that combine hydrophilic and hydrophobic segments are fruitful for making DDS. They can have different molecular architectures and various functionalities which empower them to self-assemble into unique morphologies and carry various payloads (e.g., therapeutics and NPs) to target locations [376]. In this context, complex polymeric structures such as bottlebrush copolymers have been investigated as potent DDS. The Wei's group made a series of cyclic brush (CB) copolymers containing heterogeneous polymer grafts for drug delivery applications, as schematically illustrated in Fig. 15 [377–379]. CB polymers are ring-like molecular structures with dense brushes protruding from the ring. The CB polymers outperform as DDS compared to their bottlebrush counterparts, because they can self-assemble to more stable unimolecular micelles. They made an amphiphilic CB copolymer with heterogeneous polymer brushes using a combination of ATRP, click chemistry, and ROP [379]. ATRP was utilized to make poly(oligo(ethylene glycol) monomethacrylate) (POEGMA)-*b*-PHEMA copolymer while click reactions were used to transform it into a closed ring, via a triazole linkage, and ROP was used to install polycaprolactone (PCL) brushes onto the 2-hydroxyethyl methacrylate (2-HEMA) side chains of PHEMA blocks. Hydrophilic oligo(ethylene glycol) (OEG) and hydrophobic oligo(caprolactone) (OCL) brushes were attached to the central ring, which is the diblock copolymer POEGMA-*b*-PHEMA. DOX molecules were encapsulated in the resulting self-assembled nanostructures. *In vitro* studies showed enhanced cytotoxicity of CB/DOX compared to bottlebrush/DOX systems [379].

The Wei's group further developed this strategy using stimuli-responsive linkages to make a smart DDS. They made a CB polymer based on HEMA, OEGMA, and PCL segments containing reduction-labile disulfide linkages [378]. The cyclic core includes PHEMA, which is grafted with PCL, and the PCL segments are further modified with POEGMA. In this structure, PCL and POEGMA are linked together via a reduction-sensitive disulfide linkage. ATRP was used to make the PHEMA in the ring structure and POEGMA chains in the polymer brushes. ROP and click reactions were used to make PCL and to close the ring, respectively. The obtained structure contains a hydrophilic internal layer, a hydrophobic inner shell, and a hydrophilic corona on the outer surface. This structure enables loading of both hydrophilic and hydrophobic therapeutics; moreover, the outer polymer corona enhances circulation time. After cellular uptake, the disulfide linkage is clipped off via redox species in the cytosol, like glutathione (GSH), resulting in detachment of POEGMA and disassembly of the NPs [378].

Some delivery systems are unimolecular, in which only one natural/synthetic polymer is used to make self-assembled nanocarriers,

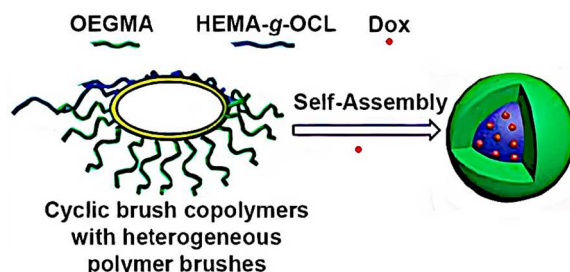


Fig. 15. Schematic highlighting the structure and process of self-assembly of an amphiphilic cyclic brush (CB) copolymer. The ring-like molecular structure of CB copolymer based on POEGMA-*b*-PHEMA was made through ATRP, containing heterogeneous hydrophilic OEG and hydrophobic OCL brushes. Self-assembly of CB makes unimolecular micelles embedded with DOX molecules. Reprinted with permission from [379]. Copyright 2018 American Chemical Society.

even though some auxiliary small molecules may be necessary (e.g., metal ions for complexation). Furthermore, there are delivery systems, such as vesicles and micelles, that are based on assemblies of amphiphilic (macro)molecules such as phospholipids [380,381]. In some of the unimolecular delivery systems, polymerization results in a special morphology (e.g., dendrimers) that can be directly utilized as a delivery platform [382]. However, most unimolecular systems rely on intra- or intermolecular interactions between the polymer chain(s) and a small molecule/ion (e.g., alginate/ Ca^{2+} system) to create self-assembled nanostructures. In addition, PISA, crystallization-driven self-assembly, and other kinds of self-assembly can be exploited to make nanoscale morphologies [383–385]. It is worth mentioning that morphological features of DDS are an important factor as they can affect the cellular uptake of nanocarriers and their fate *in vivo* [386]. Bia *et al.* managed to make unimolecular micelles based on cylindrical polymer brushes (CPBs) for improved cancer therapy [387]. Their investigations revealed that for anti-tumor efficacy, size reduction of DDS was a higher priority than anisotropy. Unimolecular micelles based on CPBs self-assemble in water to create DDS.

For creation of CPBs, they modified a cellulose backbone with ATRP initiation sites, followed by copolymerization of OEGMA

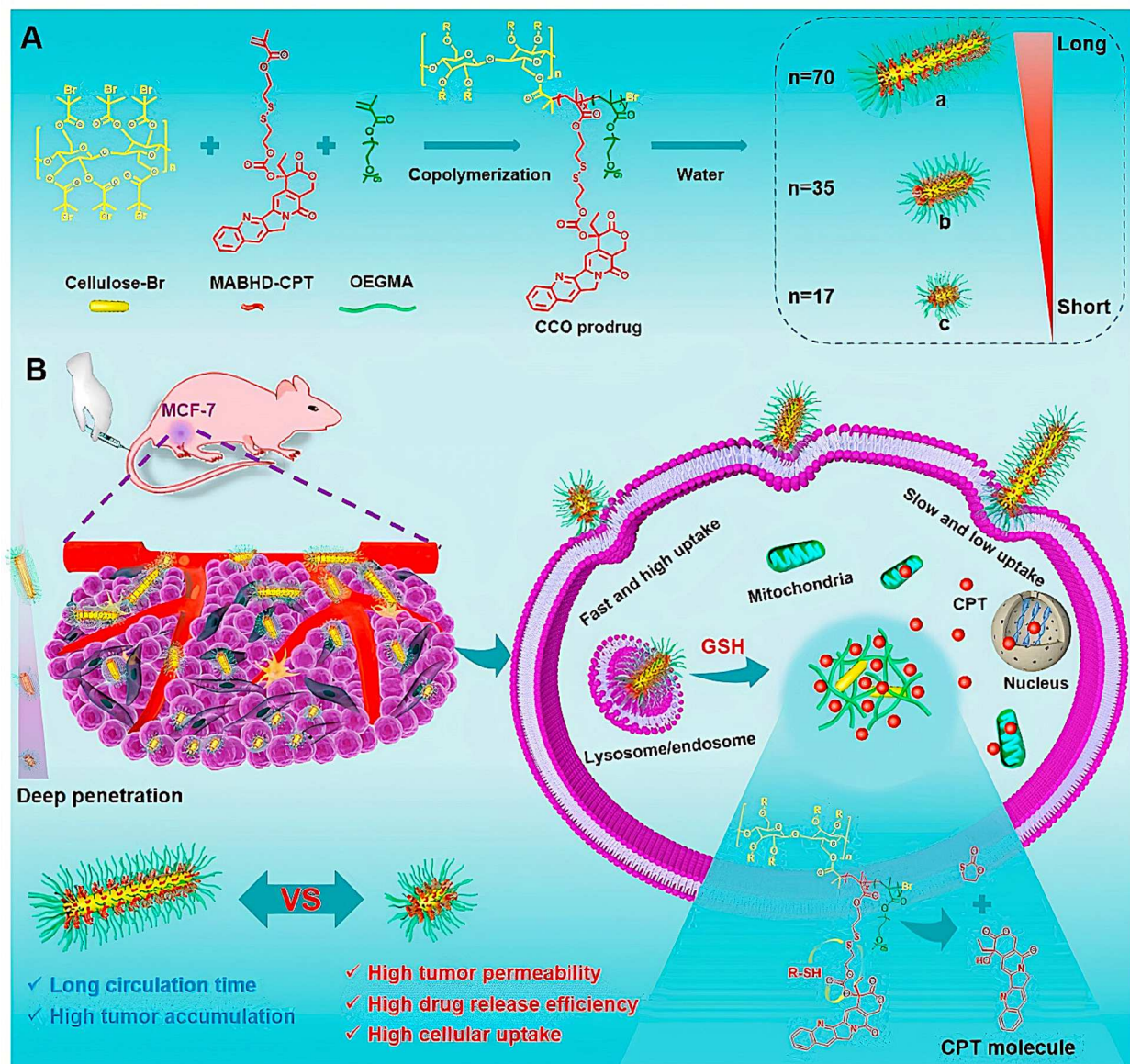


Fig. 16. Schematic depicting the synthetic route and anti-cancer mechanism of a unimolecular prodrug delivery platform. (A) ATRP to synthesize cylindrical polymer brushes (CPBs) based on cellulose, as backbone and two monomers: (1) prodrug monomer based on camptothecin (CPT)-modified 6-hydroxyhexyl methacrylate (MABHD-CPT) and (2) oligomeric ethylene glycol methacrylate (OEGMA); self-organization of copolymer to cylindrical morphology in water. (B) Tumor accumulation, tumor diffusion, and cancer cell internalization of prodrug micelles; disassembly of CPBs and disulfide bond cleavage in cytosol, resulting in release of CPT molecules; CPT molecules induce apoptosis while invading the mitochondria and nucleus of the cancer cell. Reproduced with permission from [387]. Copyright 2021 Elsevier.

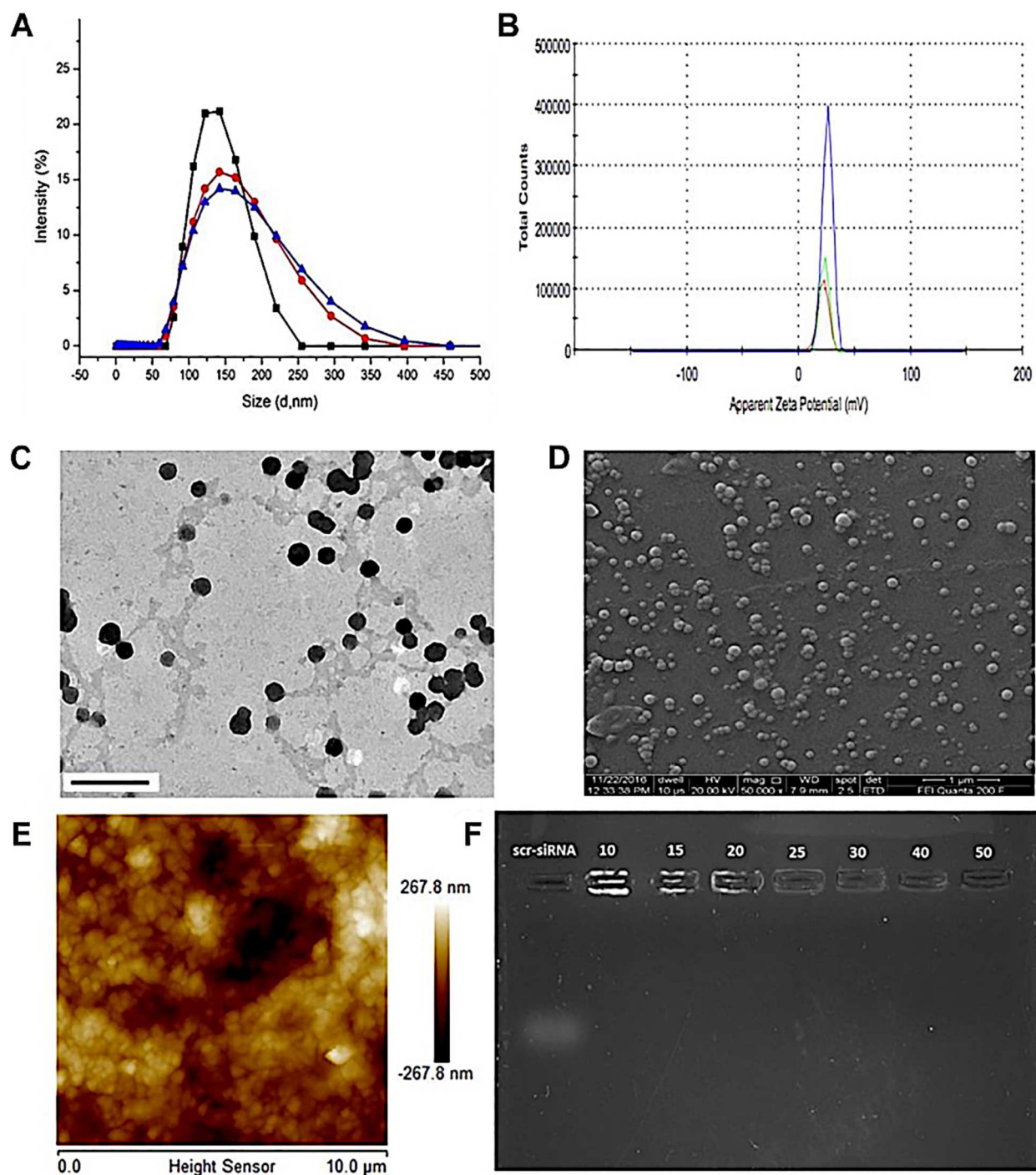


Fig. 17. Particle size analysis (dynamic laser scattering, DLS) and morphological observation for investigation of polyplexes containing DOX and scr-siRNA (DOX polyplexes scr-siRNA) at a fixed weight ratio of polymer/siRNA. (A) Analysis of size by DLS. (B) Apparent zeta potential, taking n equal to 3. (C) High-resolution transmission electron microscope (HRTEM), with scale bar fixed at 500 nm. (D) Field-emission scanning electron microscope (FESEM). (E) Atomic force microscope (AFM). (F) Agarose gel electrophoresis of DOX polyplexes scr-siRNA, having scr-siRNA as the negative control and varying the weight ratio of polymer to scr-siRNA between 10 and 50. Reproduced with permission from [391]. Copyright 2017 American Chemical Society.

macromonomer and a redox-sensitive prodrug monomer, as schematically illustrated in Fig. 16. In fact, in this one-step polymerization, cellulose with different lengths served as the macromolecular initiator for the ATRP. OEGMA formed the hydrophilic segment of the grafted copolymer while a GSH-sensitive prodrug monomer formed the hydrophobic segment. In the chemical structure of the prodrug monomer, camptothecin (CPT), a naturally occurring molecule with antitumor activity, is attached to methacrylate moieties via a redox-sensitive disulfide linkage. They used cellulose of different lengths (21, 40, 86 nm) to control micelle size. The obtained complex macromolecular structure self-assembled to unimolecular micelles with cylindrical morphology. It was observed that shorter micelles (less anisotropic and smaller) are internalized faster compared to bigger but more anisotropic counterparts (i.e., higher aspect

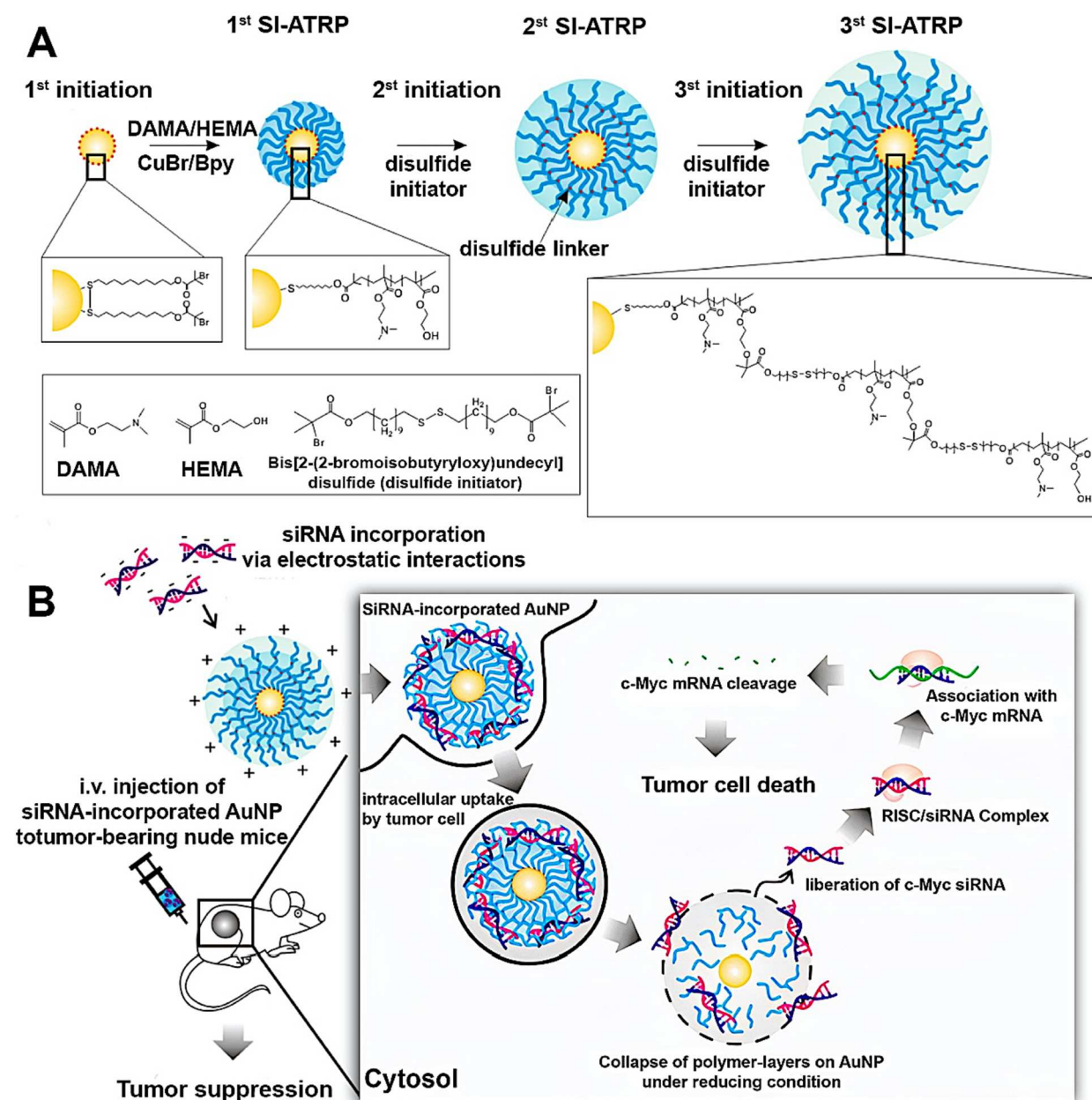


Fig. 18. Schematic depicting the preparation of polymer brush-coated gold nanoparticles. (A) Triple SI-ATRP to create three layers of polymer brushes on AuNP: in the first step an ATRP initiator containing a disulfide linkage and two BiB end groups was immobilized onto AuNP, followed by controlled polymerization of DAMA and HEMA to create the first layer; then an identical ATRP initiator containing a disulfide linkage and acid halide was conjugated to the copolymer brushes in the first layer via reaction between the hydroxyl group of PHEMA and the bromine of one BiB end group. The second layer of brush copolymer grows from the first layer via SI-ATRP. The third layer was created in a manner similar to the second layer. (B) siRNA was incorporated into polymer layers via electrostatic interactions between polycationic polymers and polyanionic siRNA. Reproduced with permission from [51]. Copyright 2018 American Chemical Society.

ratio). ATRP allowed for controlled polymerization of two bulky monomers to make well-defined polymer brushes from a cellulose backbone, resulting in relatively monodisperse micelles. Creating such complex molecular structures using FRP strategies is nearly impossible.

Disassembly of micelles or other nano-assemblies resulting from covalent-bond cleavage or charge alternation (e.g., charge reversal) have been widely used in making smart delivery systems. However, disassembly can be considered a subclass of morphological transformation in nano-assemblies. Morphological transformation can be triggered by physical (e.g., charge alternation in response to pH) or chemical (e.g., chemical bond cleavage) transformations. Morphology-transformable and/or size-tunable delivery platforms can facilitate the evasion of immune system, penetration into tumors, circumvention of physiological barriers (e.g., plasma membrane), and release of payload in a controlled manner [388,389]. We believe such dynamic delivery systems (DyDSs) that can respond to (e.g., through morphology transformation or size reduction) various exo- or endogenous stimuli will revolutionize the field in the near future. DyDSs may disassemble partially and reassemble on demand; they may also undergo morphology transformations. However, DyDSs would be based on complex chemical systems formed from one or several polymers with well-defined structures, low dispersity, predefined end-functionalities, specific side chains/brushes, and deliberately inserted linkages (e.g., stimuli responsive bridges). Such strict requirements highlight the utmost importance of CRP strategies, especially ATRP, as an indispensable part of making future delivery systems [390]. Complex chemical systems based on two or more different polymers have also been widely used to make delivery platforms. Usually, in these delivery systems, intermolecular interactions between two or more adjacent polymer chains result in chain collapse, forming nano-assemblies. Electrostatic interactions are the predominant interactions in making nano-assemblies based on multiple polymers.

Moreover, hydrogen bonding is also important in the creation of assemblies and hierarchical structures in natural or synthetic polymers. As genetic materials are negatively charged, cationic polymers synthesized using ATRP strategies have been widely utilized to make polymeric delivery vectors for genes [392,393]. For example, a polyplex (i.e., a combination of a cationic copolymer and anionic RNA) nanosystem with high biostability was developed using an ATRP strategy for simultaneous delivery of small interfering

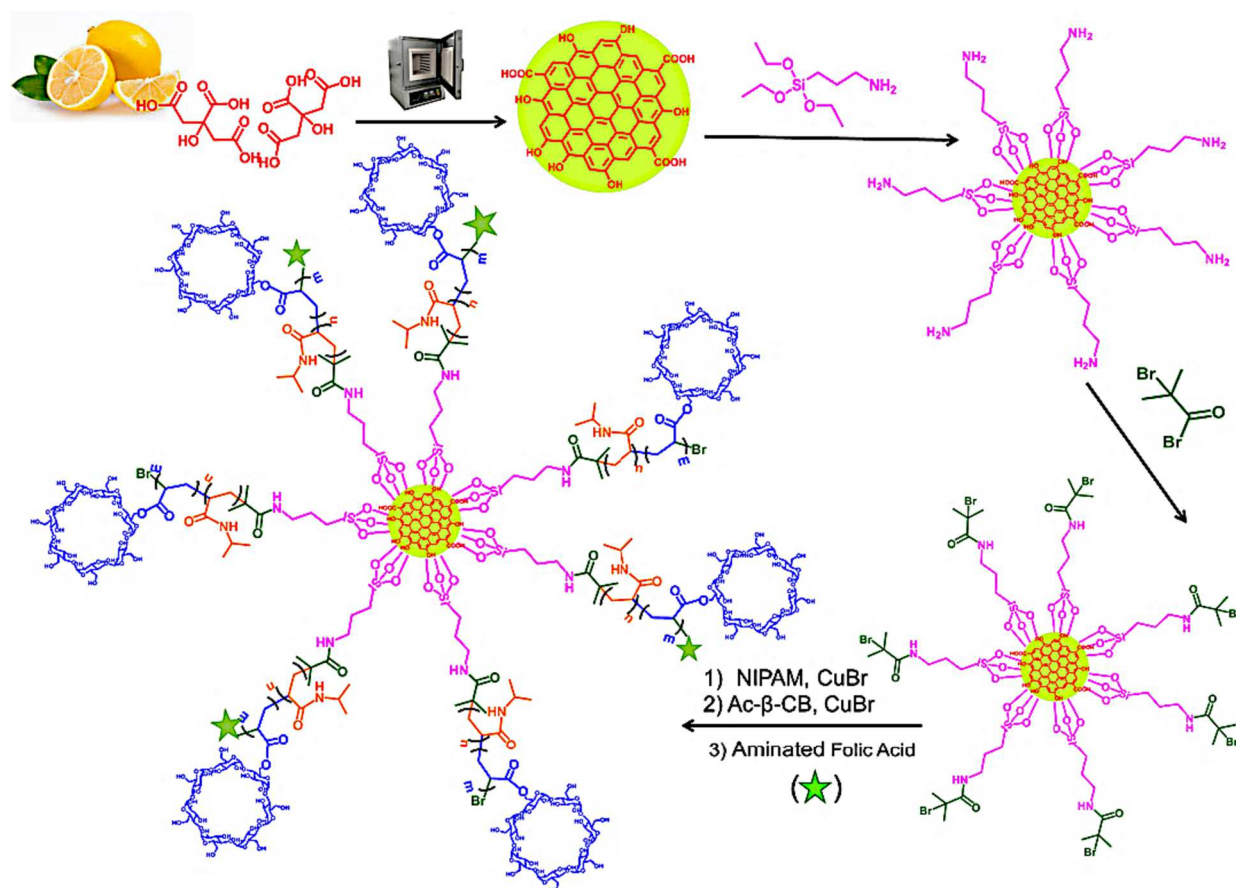


Fig. 19. Schematic describing the synthetic route of a stimuli-responsive theranostic system. Graphene quantum dots (GQDs) were synthesized using pyrolysis of citric acid followed by amination via alkoxylation chemistry. Initiation sites for ATRP were conjugated with aminated GQDs through amide linkages. Controlled polymerization of NIPAM and acrylated β -CD resulted in thermosensitive and pH-responsive copolymer brushes. Folic acid molecules were also grafted to the copolymer chain to enhance the targeting ability of the nanoplatform. Reprinted with permission from [50]. Copyright 2018 American Chemical Society.

RNA (siRNA) and DOX for synergistic cancer therapy [391]. An ATRP initiator containing redox-sensitive linkages was utilized for ROP of ϵ -caprolactone (ϵ -CL) to obtain a PCL-based macroinitiator.

Poly(ethylene glycol)methacrylate (PEGMA), as macromonomer, was grafted from a PCL-macroinitiator to produce a macromolecular structure containing a hydrophobic PCL, a hydrophilic poly(PEGMA), and an interior disulfide linkage. Succinic anhydride was utilized to convert less-reactive hydroxyl groups at end chains of PEGMA into more reactive carboxyl functional groups, which were conjugated with polyethylenimine (PEI) using carbodiimide chemistry. This stealth cationic nanosystem allows redox-sensitive release (e.g., GSH in cytosol) of both payloads and resulted in ~ 29 -fold shrinkage of tumor size in animal studies [391]. As shown in Fig. 17, the morphology and particle size of DOX polyplexes scr-siRNA in the polymer matrix was changed by varying the ratio of polymer to scr-siRNA.

Gold nanostructures are frequently used in formulation of DDS and theranostic platforms. However, their biocompatibility and functional properties can be enhanced via polymer incorporation, especially in the form of brushes. SI-ATRP was used to decorate gold nanorods (AuNRs) with thermosensitive PNIPAAm brushes [394]. Prior to controlled polymerization, an ATRP initiator containing disulfide was immobilized on AuNRs. In another work, Kim *et al.* managed to prepare AuNP (18 nm) with a cationic copolymer corona with a multishelled structure, using SI-ATRP as shown in Fig. 18 [51]. Multiple ATRP processes were used to enhance the loading capacity of siRNA into the multishelled structure of the copolymer corona. A disulfide initiator was anchored onto AuNPs via a ligand-exchange method, followed by the first SI-ATRP to build the first layer (or shell) based on poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA)-*co*-HEMA brushes. To initiate the second SI-ATRP from the obtained star-shaped AuNPs, the first layer needed to be functionalized with an ATRP initiator. The initiator they used had two BiB end-functional groups, in which one bromine leaving group can be utilized for conjugation to the first shell by reacting with hydroxyl groups on HEMA residues on the copolymer.

After conjugation of the initiator, SI-ATRP proceeded to make the second layer, i.e., P(DMAEMA-*co*-HEMA) copolymer. The third layer (or additional layers) can be fabricated sequentially, in a similar way to the second layer. The resulting brushes are based on P(DMAEMA-*co*-HEMA) copolymers interconnected via redox-sensitive disulfide linkages as shown in Fig. 8A (third stage). The loading capacity of anionic siRNA could be adjusted by the number of shells, since siRNA electrostatically interacts with cationic brushes on AuNPs (because of the cationic DMAEMA residue in the copolymer).

The cleavable disulfide linkages enable controlled release of siRNA in the reducing environment of the cytoplasm. These researchers conducted another test where they fabricated AuNPs coated with anionic corona, based on PSEMA, using SI-ATRP with the same initiator [309]. Then PEI and siRNA were incorporated via an LBL assembly method. In other words, anionic homopolymer brushes were used instead of P(DMAEMA-*co*-HEMA) copolymer for siRNA loading, using a cationic PEI interlayer. PEI is a cationic polymer that is widely used as the gold standard in non-viral gene transfection [395]. Carbon nanomaterials are another important group of materials commonly used in formulation of drug, gene delivery, and theranostic platforms [396]. The unique optoelectronic properties, special morphology, and high surface area of graphene and carbon nanotubes can be harnessed for medical imaging, photodynamic therapy (PDT), PTT, and loading of various drug molecules. However, biocompatibility, loading capacity, and stimuli-responsive features of these nanostructures can be improved using polymer chains that grow via an ATRP strategy. GQDs were modified by copolymer brushes and utilized for targeted delivery of anticancer drugs [50].

As schematically illustrated in Fig. 19, GQDs decorated with BiB via alkoxysilane chemistry behaved as nanostructured initiators for ATRP. The ATRP strategy was used to grow eblock copolymer brushes of NIPAAm and acrylated β -CD from the GQD surface. These thermosensitive copolymer brushes show pH sensitivity, making them responsive in the tumor microenvironment, which is slightly acidic [397]. Moreover, targeting capabilities of these DDSs were enhanced via conjugation with folic acid species that can target folate receptors on cancer cells [398]. Because of the photoluminescence phenomenon observed for GQDs, this system can also be used in theranostic platforms [399]. The dense polymer brushes grown from GQDs not only introduce stimuli-responsiveness into the system but also enhance their biocompatibility and dispersion in aqueous medium. *In vitro* experiments confirmed pH-responsive release of embedded DOX molecules [50].

Mesoporous silica NPs (MSNs) are another important group of inorganic biocompatible materials highly potent for making DDSs [400]. Like other inorganic materials, their physicochemical properties and functional features can be further enhanced using polymers [401]. Cationic PDMAEMA brushes were grafted from MSNs using ATRP [402]. MSNs were modified using an ATRP initiator containing a silane functionality. Surface erosion of biodegradable MSNs results in detachment of polymeric brushes and release of siRNA [402]. Dai *et al.* used a light-induced SI-ATRP methodology to graft nanodiamond with poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) brushes [103]. This photoinitiated ATRP utilizes FeBr_3 as catalyst and UV light to trigger polymerization. The obtained nanodiamond particles with enhanced water dispersibility and reduced toxicity were used as a DDS for intracellular DOX delivery. The results confirmed effective cell internalization of PMPC-grafted nanodiamonds and effective transportation of DOX molecules in HepG2 cells, while preserving their cell-killing properties.

6.2. Bioimaging and medical diagnosis

Medical diagnosis and imaging strategies are important steps toward disease detection, monitoring, and treatment. Some medical diagnosis tools (e.g., electroencephalography and electrocardiography) are designed to measure biosignals, outputting graphs that can only be interpreted by specialists [83]. On the other hand, bioimaging techniques offer visual representations of organs, tissues, and internal compartments of the human body. Some widely applied medical imaging techniques are magnetic resonance imaging (MRI), X-ray imaging techniques (e.g., computed tomography or CT, mammography, and angiography), nuclear medicine imaging (e.g., single photon emission computed tomography [SPECT] and positron emission tomography [PET]), fluorescence imaging, and ultrasound imaging [403]. While computational techniques and artificial intelligence (AI) strategies have recently been the center of

attention in biomedical imaging, materials continue to play a central role in medical diagnosis and bioimaging [404,405]. For example, in MRI, contrast agents (Cas) are materials used for enhancing the quality of obtained images [406]. Accordingly, designing bioimaging probes with appropriate optical properties, good biocompatibility and biodistribution, and effective clearance from the human body is an ongoing field of research. Metals, metal oxides, and semiconducting (in)organic materials are well-known imaging probes in medicine.

Conjugated polymers have progressively replaced conventional inorganic (semi)conductors in various applications such as organic electronics and, more recently, the biomedical field [407]. Conjugated polymers with extended π -electron delocalization possess interesting optoelectronic properties and can be used in bioimaging and cancer therapy [256,408]. Fluorescent imaging probes based on conjugated polymer NPs (CPNPs) perform better than traditional fluorescent probes because they absorb and emit at higher wavelengths (e.g., near-infrared or NIR), which penetrate deeper into biological tissue than visible light [409]. For example, CPNPs

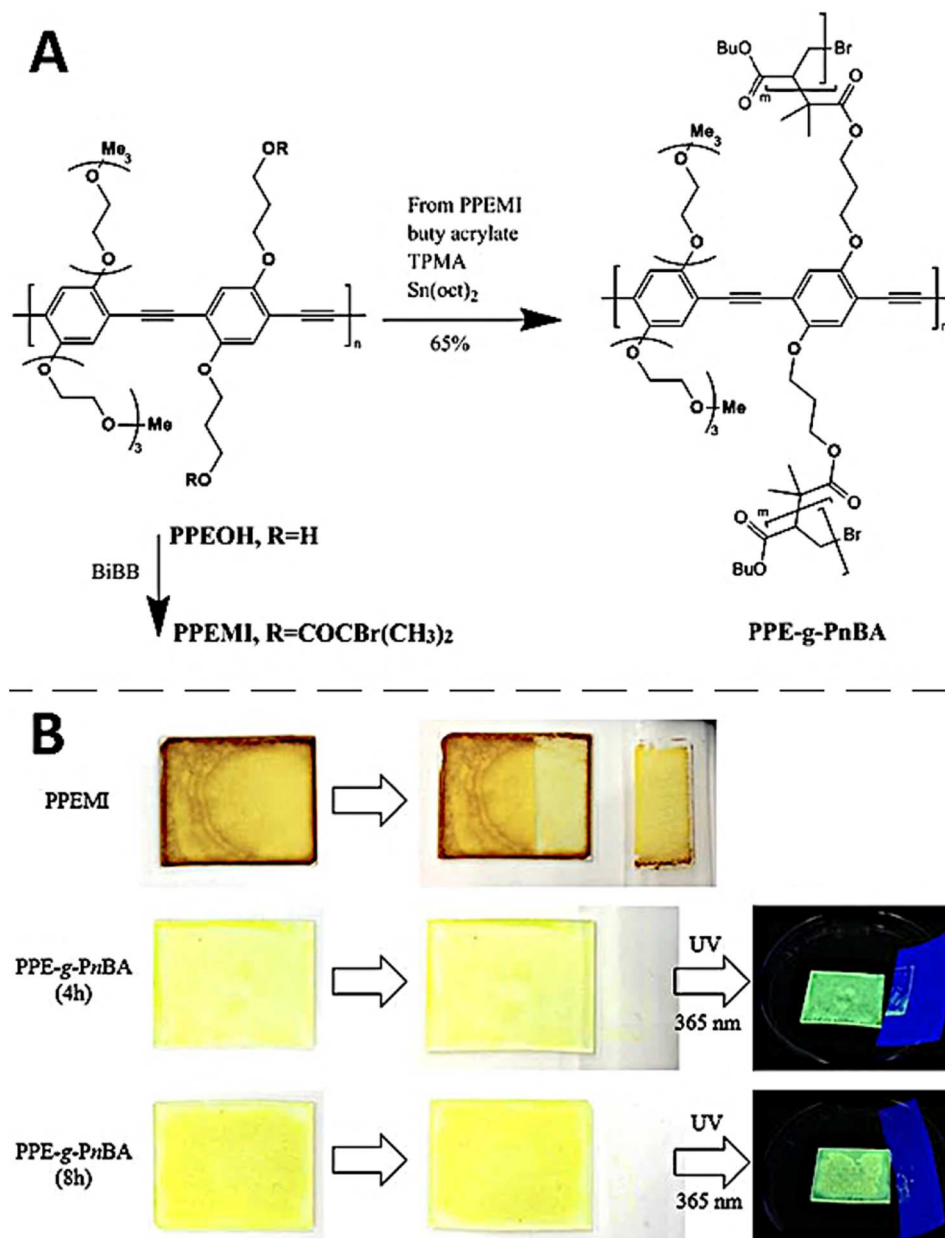


Fig. 20. Schematic illustrating the synthesis of a photoluminescent material via ARGET ATRP. (A) ATRP initiation sites were first introduced onto poly(p-phenyleneethynylene) (PPE) side chains, followed by polymerization of n-butyl acrylate via ARGET ATRP. Poly(n-butyl acrylate) (PnBA) chains grafted from the PPE backbone enhance the quantum yield of the polymer, which is valuable in medical imaging. (B) Dry adhesion tape test of drop-casted film of PPEMI and PPE-g-PnBA under UV at 365 nm. Reproduced by permission [411]. Copyright 2017 Elsevier.

were used in non-invasive fluorescence bioimaging of mouse brain to obtain a 3D map of the blood vessel network beneath the skull [410].

ATRP can be used to make conjugated polymers, or to modify conjugated polymers with other oligo-/polymers as side chains, or to make copolymers. In many cases, the main goal of such modifications is to enhance the water dispersibility of conjugated polymers. This modification may also affect the optoelectronic properties of the conjugated backbone. Moreover, such modifications can induce self-assembly when placed in an appropriate solvent, especially aqueous medium.

Conjugated (co)polymers can exhibit photoluminescent properties. However, their interaction with biological systems should be improved for effective utilization in the body. A photoluminescent copolymer based on a derivative of poly(p-phenyleneethynylene) (PPE) was modified by BiBB [411].

As illustrated in Fig. 20A, the modified PPE acted as a macroinitiator for ARGET ATRP in which poly(n-butyl acrylate) (PnBA) was grafted from the PPE backbone. The PnBA side chains enhance the quantum yield (via inhibition of interchain interactions), induce non-aggregating properties, and preserve interesting optical properties of the PPE backbone. Fig. 20B highlights the properties of the resulting adhesive, which is affected by the humidity, whereas a dry adhesion tape test revealed delamination for PPEMI compared to PPE-g-PnBA. These polymers can potentially be applied in bioimaging and biosensing applications.

Semiconducting polymer dots (Pdots) with interesting optoelectronic properties, tunable luminescence, and biocompatibility are promising for bioimaging and biosensing applications [412]. However, they suffer from disadvantages such as narrow emission bandwidth and relatively low quantum yield. Yang *et al.* found that fluorophores based on conjugated polymers that are encapsulated in a hydrophobic layer outperform neat Pdots in fluorescence quantum yield [413]. The conjugated backbone of poly(fluorene-alt-(4,7-bis(hexylthien)-2,1,3-benzothiadiazole)) (PFTB) includes fluorene, thiophene, and benzothiadiazole species that create a delocalized π -electron network along the main chain. PFTB was grafted to hydrophobic PCL to create a bottlebrush copolymer with conjugated backbone.

On the other hand, PCL-*b*-POEGMA copolymer was manufactured by ROP of ϵ -CL to create PCL and installing BiB end-chain functionalities that allow for controlled polymerization of OEGMA via ATRP. Amphiphilic PCL-*b*-POEGMA behaves like a surfactant, and its nanoprecipitation with conjugated bottlebrush polymer results in nano-assemblies in which bottlebrush is embedded in a PCL matrix wrapped with a hydrophilic corona based on POEGMA, as shown in Fig. 21. This system can be potentially used for far-red/near-infrared (FR/NIR) imaging [413].

In addition, fluorescent polymer can be created by polymerization of fluorescent monomers. For example, an amphiphilic fluorescent polymer was manufactured via growth of acrylated fluorescein monomer from a ϵ -polylysine backbone as ATRP macroinitiator [414]. The obtained macromolecules self-assemble to fluorescent NPs that can be used for live-cell imaging.

Lanthanides show interesting luminescent properties that can be exploited in bioimaging and fundamental biological applications [415]. Nanomaterials doped with lanthanides are invaluable tools for deep-tissue bioimaging, living-cell imaging, biophotonics, and nanothermometry, as they benefit from excellent NIR emission/absorption [416,417]. Luminescent lanthanide complexes may be encapsulated in polymer NPs [418].

However, in many cases, lanthanide ions are embedded in the microstructure of other nanomaterials such as MSNs, metal-organic frameworks (MOFs), and hydroxyapatite (HAp) [420]. Like other nanostructures, polymer modification is usually required to adjust their *in vivo* fate, and growing polymer brushes from the nanomaterial surface provides a robust strategy for such modifications. For

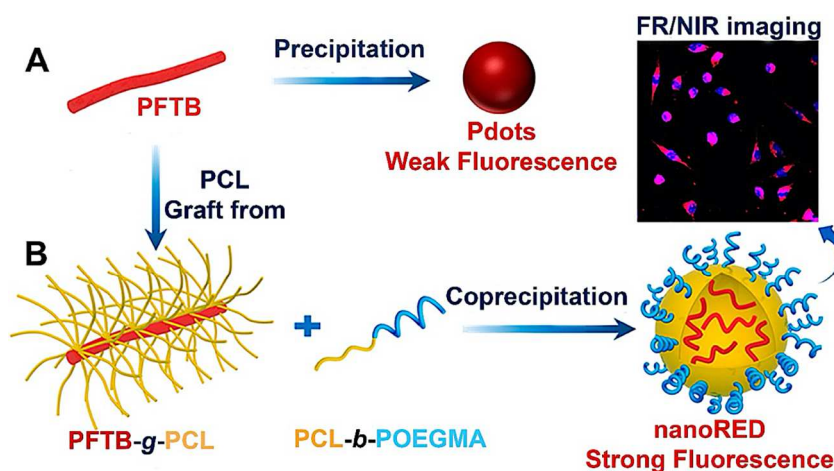


Fig. 21. Schematic depicting the synthesis of fluorophores based on semiconducting materials. (A) Precipitation of PFTB results in semiconducting polymer dots (Pdots) with weak fluorescence and relatively low quantum yield. (B) Hydrophobic PCL brushes were grafted from PFTB to create a bottlebrush copolymer with a conjugated backbone; coprecipitation of PFTB-g-PCL with amphiphilic PCL-*b*-POEGMA, which was created via ATRP, results in nano-assemblies with strong fluorescence. In the nanoRED nano-assemblies, conjugated PFTB and PCL segments are hydrophobic and constitute the inner core while hydrophilic POEGMA segments form a corona around the inner core and stabilize them in the aqueous environment. Reprinted with permission from [413]. Copyright 2016 American Chemical Society.

example, hydrophilic brush polymers were grafted to Eu^{3+} -doped luminescent HAp nanorods using an SI-O-ATRP strategy [419]. PTH was utilized as photocatalyst to graft a copolymer of 2-methacryloyloxyethyl phosphorylcholine (which contains zwitterionic side chains and itaconic acid) from the HAp nanorod surface. The obtained water-dispersible nanorods can be utilized in drug delivery and bioimaging applications. Because of the metal-free ATRP strategy, the modified nanorods possess good biocompatibility with no copper residue. Moreover, *in vitro* studies showed that these nanoplateforms can be successfully internalized by cells, which was confirmed by optical imaging (Fig. 22) [419].

Lanthanide-doped upconverting NPs (UCNPs) have been utilized in bioimaging, theranostics, and image-guided PDT [421]. Water-dispersible hairy UCNPs were prepared by an SI-photoATRP strategy in which poly(acrylic acid) (PAA) brushes grow from the UCNP surface [319]. Green light emission was observed after irradiation by NIR laser at 980 nm, indicating its potential application in luminescent bioimaging. On the other hand, particle-brush hybrid materials, based on heavy metal (oxides), can be utilized to improve the compatibility and blood circulation time of contrast agents. Hairy NPs can be used to enhance the resolution.

In vivo imaging can also be used for fundamental studies of the biodistribution of NPs or their clearance from the body. For example, nanomaterials of various shapes and types may be used in formulation of delivery platforms, imaging probes, and scaffolds. It is important to understand how they affect the human body in both the short and long term.

For example, while carbon nanomaterials such as graphene and carbon nanotubes (CNTs) are important in designing conductive scaffolds for tissue engineering, their biodistribution and clearance from the human body remains a challenge because they ultimately detach from degradable scaffolds. In order to trace CNTs *in vivo*, conductive nanocomposite fibers based on biodegradable poly(lactic-co-glycolic acid) (PLGA) and multi-walled carbon nanotubes (MWCNTs) were electrospun and used for fabrication of nerve guide conduits for a rat model [422]. Carboxyl-functionalized MWCNTs (MWCNT-COOH) were immobilized with BiBB followed by SI-ATRP to produce PGMA-grafted MWCNT. Epoxy moieties on PGMA further reacted with ethylenediamine to create amine functional groups, which were used for conjugation of fluorescein-5-isothiocyanate (FITC), a popular fluorescence-labelling reagent [422].

6.3. Theranostic platforms

Theranostic platforms that combine imaging probes and therapeutics in one delivery system are attracting much interest in the biomedical field of image-guided therapy [403]. Conjugated polymers with interesting electrical and optical properties can play an important role in drug delivery, bioimaging, and theranostic platforms [423]. Because of the intrinsic, tunable optical properties of semiconducting polymers that enable their use in PTT, they are appealing candidates for making nano-theranostic platforms for cancer [424]. However, their dispersibility in water and their interactions with biological systems should be improved, for example via PEGylation, for effective utilization *in vivo*.

Polyfluorene (PF) is an important conjugated polymer with interesting electrical and optical properties, like photoluminescence. Bottlebrush copolymers based on a PF backbone and homogeneous polymer brushes were synthesized to be used as theranostic platforms, as illustrated in Fig. 23 [377]. The PF backbone containing alternating azido and hydroxyl functional groups was utilized for click ligation of PCL, via grafting-to, and via installing ATRP initiation sites for OEGMA polymerization, respectively. The resulting amphiphilic macromolecules with alternating heterobrushes were loaded with DOX to create unimolecular micelles.

Gold nanostructures, benefiting from optical properties and loading capacities, are another member of theranostic platforms. AuNPs were decorated with hydrophilic PEG brushes and hydrophobic poly(2-nitrobenzyl acrylate) (PNBA) to make amphiphilic photo-responsive AuNPs [425]. The photosensitivity of this theranostic platform originates in o-nitrobenzyl (O-NB) moieties on hydrophobic polymer brushes, which may be clipped off upon light irradiation. The synthesized amphiphilic systems self-assemble to plasmonic vesicles that can be internalized by cells. As shown in Fig. 24, AuNPs were embedded in the hydrophobic shell of plasmonic

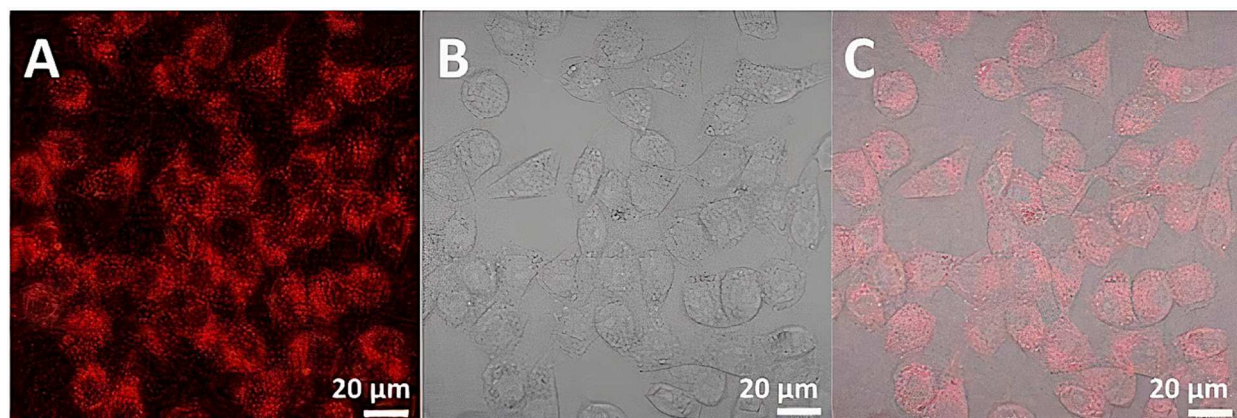


Fig. 22. Microscopy images displaying cell internalization of brush polymer-modified luminescent HAp nanorods. (A) The surface of Eu^{3+} -doped HAp nanorods were grafted with a copolymer; HeLa cells were incubated with these modified nanorods ($100 \mu\text{g mL}^{-1}$); after 3 h, fluorescent images were taken using a 405 nm laser, which show cell internalization of nanorods. (B) Images were taken under similar conditions but under bright field. (C) Images from (A) and (B) were merged together. Reprinted by permission [419]. Copyright 2017 Elsevier.

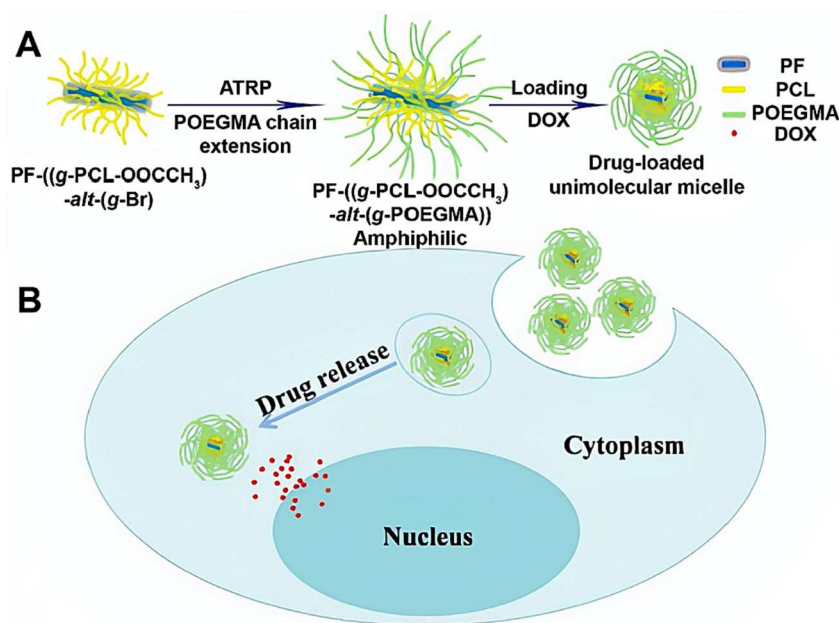


Fig. 23. Schematic describing the synthesis and cellular uptake of a theranostic system based on unimolecular micelles. (A) The conjugated backbone of polyfluorene (PF) was grafted with hydrophobic PCL brushes through a grafting-to methodology, followed by grafting hydrophilic POEGMA brushes from the PF backbone via ATRP; the amphiphilic bottlebrush copolymer includes alternating hydrophilic and hydrophobic heterobrushes and a conjugated hydrophobic backbone; this bottlebrush copolymer self-assembles in the presence of a hydrophobic anticancer drug to create a core-shell structure in which DOX is embedded in the hydrophobic core while the hydrophilic corona bestows the theranostic platform with stability in biological fluids, enhanced blood circulation time, and better cellular uptake. (B) After cell internalization, DOX molecules can be released to trigger cell apoptosis; in addition, optical properties of the PF backbone allow PTT by absorbing light and converting it to thermal energy to increase the internal temperature of cancer cells; moreover, the fluorescent property of PF enables imaging of cancer cells. Reproduced by permission [377]. Copyright 2018 Royal Society of Chemistry.

vesicles, while drug molecules were trapped inside the vesicle cavity. Upon irradiation, O-NB linkages are cleaved, resulting in transformation of PNBA to PAA, i.e., a hydrophobic to hydrophilic transition. This leads to disassembly of vesicles and release of DOX molecules, which corresponds to photo-responsive drug release. Additionally, this theranostic platform enables dual-modality imaging [425].

Copolymer brushes grown from imaging probes such as magnetic NPs enable loading of various hydrophilic and/or hydrophobic drug molecules [426]. In most of these nano-theranostics, hydrophilic segments (especially those based on OEG/PEG) are used to enhance the water dispersibility and circulation half-life of nano-theranostics [317]. An SI-ATRP strategy was used to make nano-theranostics based on magnetic mesoporous silica nanospheres (MMSNs) [317]. The surface of MMSNs were pretreated with APTES to create amine functionalities, followed by tethering of ATRP initiator (BiBB). The SI-ATRP of *tert*-butyl acrylate monomers resulted in poly(*tert*-butyl acrylate) (PtBA) brushes grown from MMSN surfaces. The PtBA brushes were hydrolyzed in the presence of p-toluenesulfonic acid to obtain pH-sensitive PAA brushes. Experimental results showed that the obtained nano-theranostics can be internalized by cancer cells; in addition, they can be used as CAs in MRI and for pH-responsive delivery of DOX molecules to HeLa cancer cells [427]. This strategy can be used for other imaging probes as well (QDs, fluorescent dyes, etc.) [428].

Some theranostic platforms do not carry therapeutics; rather, they kill cancer cells via generating harmful radical species like reactive oxygen species (ROS) or increasing temperature locally, i.e., hyperthermia. For example, a theranostic nanoplatform was manufactured based on lead sulfide (PbS) coated by gadolinium (Gd) complexes for simultaneous PTT and dual-modality imaging, i.e., CT imaging and MRI [429]. Interesting optoelectronic properties of PbS, such as strong NIR absorption and good X-ray absorption, indicate its potential utilization in PTT, and as a CA for CT and fluorescent imaging [430]. Furthermore, Gd(III) complexes are the most common CA used in MRI [431]. PbS NPs were modified by PDMAEMA polycation brushes, using SI-ATRP. Photothermal conversion of NPs was as high as 31 %, indicating they can perform well in PTT of cancers. Both *in vitro* and *in vivo* studies confirmed excellent performance of nano-theranostics in CT scanning and MRI. *In vivo* studies in a mouse model confirmed the effective accumulation of nanoscale theranostics in the tumor site because of the enhanced permeability and retention (EPR) effect [429]. A nanoscale targeted DDS with excellent pH-responsiveness was developed for breast cancer [432]. An SI-ATRP strategy was utilized to graft PEGMA brushes from the surface of bimetallic Prussian blue analogs (PBA) to induce pH-responsive behavior. Furthermore, immobilization of AS1411 aptamers that target nucleolin receptors, a protein overexpressed on the surface of breast cancer cells, enhances the targeting capability and uptake of this DDS. pH-triggered disassembly and endosomal escape of the obtained biocompatible nano-theranostic platform resulted in efficient intracellular delivery of DOX.

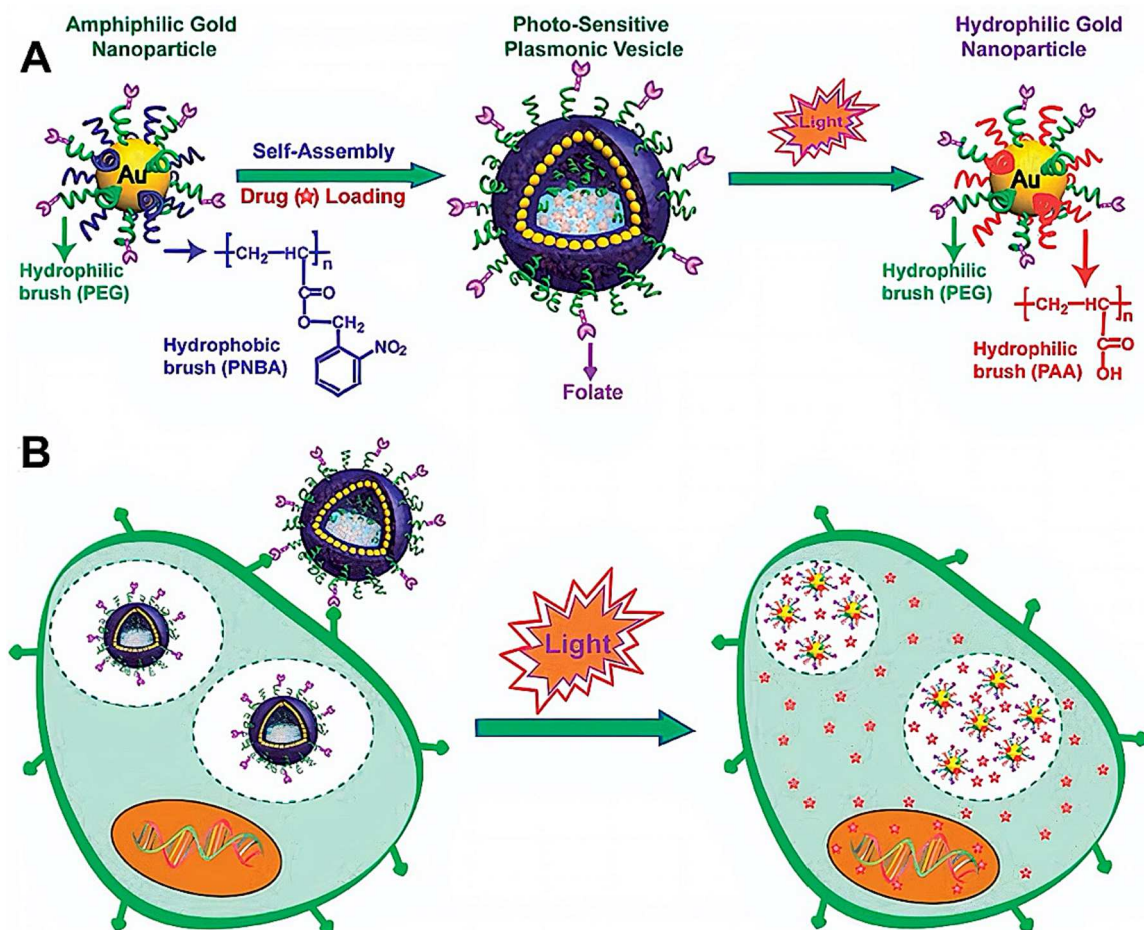


Fig. 24. Schematic highlighting the synthesis and mechanism of action of theranostic nanoplatform based on amphiphilic and photo-responsive AuNPs. (A) AuNPs decorated with hydrophilic PEG brushes and light-sensitive poly(2-nitrobenzyl acrylate) (PNBA) brushes self-assemble to plasmonic vesicles, in which a nanogel is isolated from the surrounding aqueous environment by a hydrophobic layer containing AuNPs and PNBA. Both the internal nanogel and external polymer corona are based on PEG brushes while the middle layer of vesicle is based on light-sensitive and hydrophobic PNBA. PEG brushes are end-functionalized by folate moieties to target folate receptors on cancer cells. (B) After cell internalization of these nano-assemblies, light irradiation results in *o*-nitrobenzyl (*O*-NB) moiety cleavage, which results in a hydrophobic to hydrophilic transition followed by disassembly of vesicles; DOX release can be regulated via light-induced disassembly of vesicles; in addition, AuNPs with interesting optical properties can be used for imaging or PTT. Reproduced by permission [425]. Copyright 2018 Royal Society of Chemistry.

6.4. Tissue engineering

Tissue engineering aspires to use cells, biomaterials, and bioactive molecules to regenerate damaged or lost tissue. Biomaterials play crucial roles in tissue engineering as they are responsible for delivery of cells and bioactive molecules, and they can support cell adhesion and growth, *in vitro* and *in vivo* [433]. However, most synthetic materials such as metals, ceramics, and synthetic polymers suffer from limitations like hydrophobicity and bioinertness. Most of these challenges can be addressed through modification of the interface properties of tissue engineering constructs [434]. Coating with hydrophilic polymers is a general strategy to enhance the biocompatibility of various forms of hydrophobic materials. However, effective coatings usually require robust covalent linkages between the underlying substrate and hydrophilic polymers with a controlled thickness. SI-ATRP not only permits high-density, covalent attachment of polymer chains *in situ*, but it allows control over the thickness and chemical composition of grafted brushes. This has led to great opportunities for SI-ATRP in surface modification of tissue engineering constructs. Furthermore, its incorporation with click chemistry enables conjugation of bioactive molecules (synthetic peptides, proteins, and growth factors) as a generic post-polymerization modification strategy under mild conditions. Moreover, as discussed earlier in Section 5, regardless of click reactions, conjugation of bioactive molecules can be mediated by strong multi-/polyvalent physical interactions. This section provides an overview of various applications of ATRP in tissue engineering, including cell separation, tuning of biomaterial–cell interfaces, and surface modification of medical implants and scaffolds.

6.4.1. Cell separation/isolation

Cell delivery constitutes an important part of tissue engineering in situations where cell isolation and culture are important before transplantation. Separation of target cells is usually carried out via cell modification with fluorescent antibodies, which can adversely affect cell behavior [435]. Thus, development of cell separation techniques that do not require cell modification is of great importance

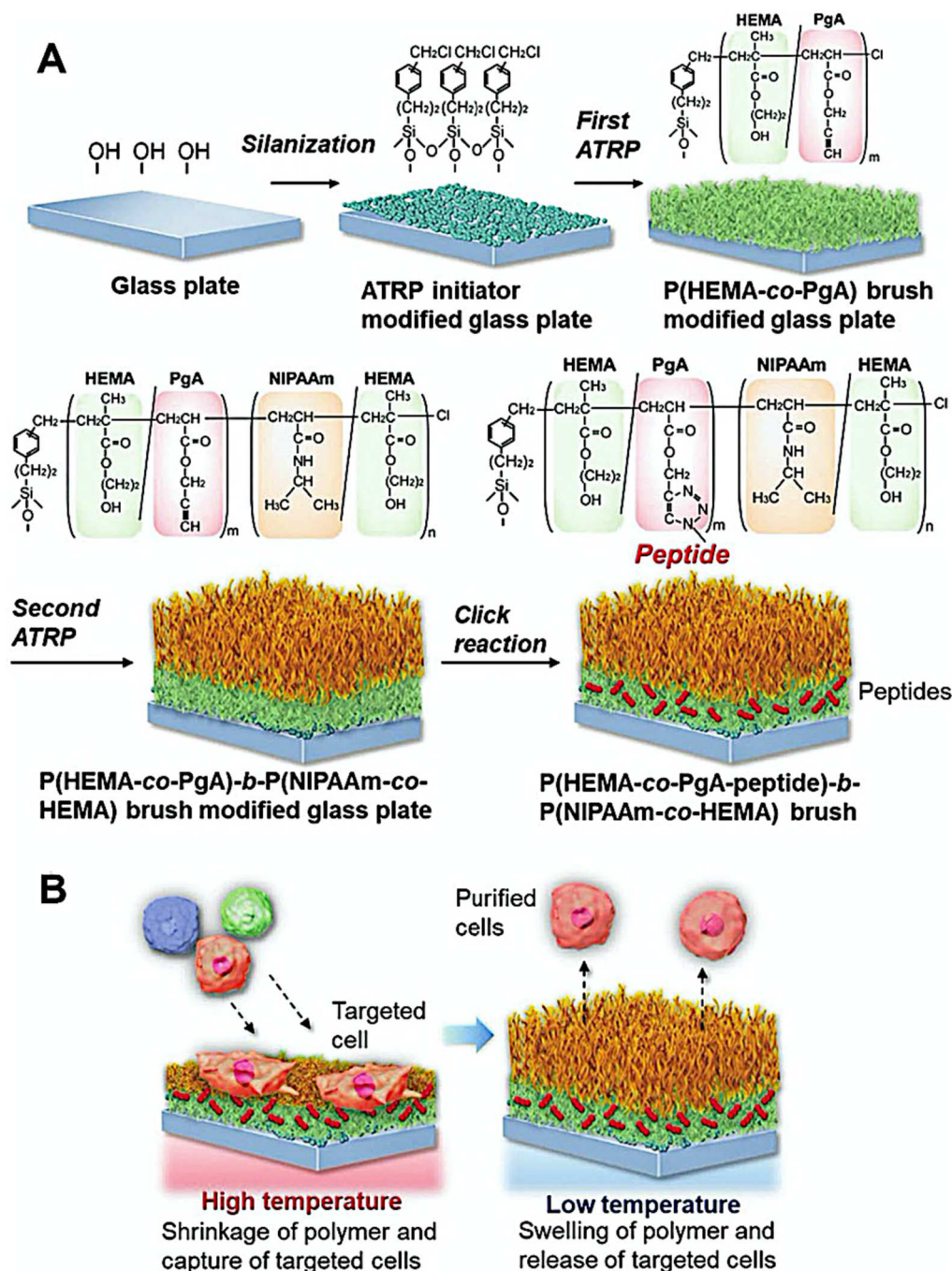


Fig. 25. Schematic illustrating the manufacturing process of polymer brush-modified glass for cell separation. (A) First layer (green) was manufactured through ATRP of HEMA and propargyl acrylate (PgA) to yield P(HEMA-co-PgA) copolymer brushes on glass surface; after that, the second layer (brown) was synthesized through copolymerization of NIPAAm and HEMA via an ATRP methodology to yield thermosensitive copolymer brushes, P(NIPAAm-co-HEMA); CAPs were conjugated with PgA side chains through click ligation. (B) Schematic illustration of cell separation mechanism of the manufactured double-layer structure on glass substrate. Reproduced by permission [436]. Copyright 2021 Royal Society of Chemistry.

in tissue engineering.

Application of smart polymers, such as thermosensitive PNIPAAm, that respond to external stimuli via property alternation, have attracted much attention for making modified surfaces for cell separation [437]. In PNIPAAm-modified thermoresponsive surfaces (e. g., in cell culture flasks), the dehydration of PNIPAAm chains below the lower critical solution temperature (LCST) results in chain hydration followed by cell detachment. Since the temperature-dependence adhesion/detachment behavior differs between various cell lines, this phenomenon enables cell separation without requiring cells to be modified [438]. Moreover, pendant ionic groups on PNIPAAm chains intensify this phenomenon because of the presence of ionic groups on the cell membrane [439]. Using SI-ARGET ATRP, Nagase *et al.* grafted glass substrates with thermoresponsive copolymer brushes based on anionic poly(N-isopropylacrylamide-co-N-*tert*-butylacrylamide-co-acrylic acid), or P(NIPAAm-co-tBAAm-co-AA) [439]. Human aortic smooth muscle cell (SMC) attachment was higher on thermoresponsive surfaces modified by these anionic brushes. Furthermore, *in vitro* experiments confirmed that temperature alternation provides a facile route to separate the SMCs from human umbilical vein endothelial cells (HUVECs). These results showed that a thermosensitive surface modified with PNIPAAm brushes containing ionic groups can be used for cell separation in cardiac tissue engineering [439]. However, hydrophilic to hydrophobic transformation of polymer chains or ionic groups may not be enough for efficient cell separation in all applications.

In a recent paper, brushes of thermosensitive PNIPAAm and a cationic polymer were grafted onto glass using two RDRP strategies, including RAFT and ATRP, in two steps [440]. This unique combination allows for controlled cell attachment and detachment via adjustment of surface cationic properties. Shrinkage or expansion of PNIPAAm brushes occurs when the temperature oscillates above or below the LCST, respectively. This results in cationic polymer brushes being either concealed or exposed, altering the surface charge. Accordingly, the electrostatic interactions between cells and mixed polymer brushes change, resulting in adjustable and selective cell adhesion.

Cell specificity and separation efficacy can be further enhanced via immobilization of targeting ligands (e.g., antibodies and nanobodies) through physical interactions or post-polymerization modification strategies [441]. Since end functionality can be controlled by CRP methods, the end functionalities can be further utilized for conjugation with bioactive molecules, for example via click reactions or carbodiimide chemistry. Moreover, higher graft density of polymer brushes created via SI-ATRP, corresponds to higher numbers of active sites for post-polymerization modifications, indicating high-density immobilization of target biomolecules like cell-affinity peptides (CAPs). For example, in one study, a glass surface was modified with a double-layer structure of two block copolymers, which were synthesized using a two-step SI-ARGET ATRP and were used for cell separation [436]. As schematically illustrated in Fig. 25A, after silanization, the ATRP initiator was grafted onto the glass substrate, followed by copolymerization of HEMA and propargyl acrylate (PgA) monomers to obtain the first copolymer layer (green segment in Fig. 25). In this figure, the brown layer is a thermosensitive copolymer, p(NIPAAm-co-HEMA), that enables cell separation. The hydrophilic HEMA residues in both layers inhibit non-specific cell attachment as the temperature increases beyond the LCST. PgA residues on the inner layer enable CAP conjugation via CuAAC click chemistry. These CAPs enhance the specificity of the cell-separation process. It was observed that at physiological temperature (i.e., 37 °C), HUVECs attach to the modified surface, in contrast to SMCs and fibroblasts (Fig. 25B). Moreover, when the temperature falls below 20 °C, HUVECs detach from the surface [436].

Research already mentioned in this section confirmed that the composition of polymer brush has a profound effect on the selectivity and efficacy of the cell separation process. Accordingly, a generalized conclusion may be that modified surfaces with a composition gradient in one direction can be used for separation of various cell types. On the other hand, a gradient in polymer brush thickness can also affect the cell separation process. This was recently confirmed by a paper in which PEGMA brushes with thickness gradient resulted in a gradient change in both hydrophilicity and endothelial cell adhesion [244]. As the thickness can be controlled via the SI-ATRP process, the thickness gradient, and thus cell adhesion, can be finely tuned.

Stimuli-responsive polymer brush-modified surfaces have also been used in the fabrication of cell sheets and bioseparation processes [442,443]. Cell sheets, which can be transplanted without the requirement of tissue scaffolds, are a promising technology in tissue engineering and regenerative medicine (TERM) [444]. Monolayer cell sheets are usually created in a culture flask with thermosensitive polymer-modified surfaces. On the other hand, stimuli-responsive polymer brush-modified particles (e.g., silica) enable an efficient and selective separation of various biological products (e.g., biomarkers, proteins, nucleic acids) as well, a process known as bioseparation [445]. Regardless of cell transplantation, delivery of bioactive molecules obtained from the bioseparation process is another pillar of TERM.

6.4.2. Plasma membrane modification

A living cell is one of the most complex chemical systems on Earth. Mammalian cells are separated from the external environment by a semipermeable membrane decorated with various types of biomacromolecules, including glycoproteins, glycolipids, and glycoRNA [446]. These biomacromolecules endow the cell's membrane with the utmost importance to cellular functions and behavior. Cells continually interact with their environment through their surfaces. Biological receptors on the plasma membrane interact with signaling molecules in their microenvironment and transduce them to the intracellular microenvironment. Some of these biomacromolecules behave like strain sensors, detecting mechanical cues in their vicinity, and they may also exert sub-nanonewton forces on the extracellular matrix (ECM), for example during cell migration.

In some cases, cells for TERM are encapsulated or embedded in biodegradable hydrogels (e.g., microgels and cell-laden hydrogels) that mimic their native ECM, to protect them against harsh environmental conditions [447]. However, these are biodegradable scaffolds that do not affect the microstructure of individual cell membranes significantly. On the other hand, cell membranes may be modified with various biological entities (e.g., antibodies) to induce further functionalities, such as installing chimeric antigen receptors on T cells for immunotherapy. Some of these strategies exploit the cells' own biosynthetic machinery via an artificial genetic

code that is intentionally delivered into the cells. Although there is a forest of various biomacromolecules on the plasma membrane of cells, it is not very dense, indicating that numerous sites are available for tethering or chemical grafting, both on the phospholipid bilayer and on biomacromolecules (e.g., reactive handles on amino acid sequences) without the worry of steric hinderance. Various chemical handles (e.g., amine, thiol) present on plasma membrane proteins (see section 5.1.1) can be exploited for covalent cell modification. For example, the surface of red blood cells (RBCs) was chemically grafted with a bioconjugate via amine functional groups [448]. In addition, phosphonate groups on the plasma membrane can be used to functionalize a cell surface reversibly [449]. On the other hand, electrostatic interactions between the plasma membrane and polyelectrolytes can be used to coat cells with polymers [450].

Cell membranes can be further modified artificially (membrane engineering) via rafting synthetic (macro)molecules, especially synthetic polymer brushes, using cytocompatible SI-CRP strategies. In fact, ATRP can be leveraged for more than just the cell separation via stimuli-responsive surfaces that was discussed in the previous section. Although most ATRP techniques are carried out under environmental conditions lethal to living cells, thanks to recent achievements in cytocompatible ATRP methodologies, it is now possible to use ATRP for surface modification of living cells. Radical concentration is low in ATRP strategies, but they may still be cytotoxic to living cells; accordingly, in some cases shielding strategies may be required before initiating controlled polymerization. For example, SI-ARGET ATRP was used to grow polymer brushes from yeast cell surfaces [360]. A polydopamine (PDA) coating was applied onto the plasma membrane; this layer not only serves as ATRP macroinitiator but also protects the cell from harmful radicals during polymerization, as PDA benefits from radical-scavenging features. This paper used the material-independent nature of mussel-inspired chemistry for coating cells with PDA. In another study, human erythrocytes or RBCs were modified by polymers with free-radical-scavenging properties using ATRP, to protect cells against oxidative stresses [451]. ATRP was used to polymerize N,N-dimethylacrylamide into poly(N,N-dimethylacrylamide) (PDMA). Antioxidant properties were introduced into PDMA by post-polymerization modification with a (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) derivative that shows intrinsic antioxidant properties. NHS end-functionality enables cell attachment via carbodiimide chemistry (amide bond formation). This is a grafting-to strategy to attach well-defined PDMA chains with antioxidant properties to the surface of RBCs, via carbodiimide chemistry.

On the other hand, bioorthogonal click chemistry is another appealing strategy to install clickable oligo-/polymers onto plasma membrane using metabolic glycoengineering (MGE). This strategy has been previously used to install clickable artificial receptors on cancer cells for targeting [452]. However, to the best of our knowledge, it has not yet been exploited for cell modification with polymer brushes. After installing clickable oligo-/polysaccharides onto plasma membranes of cells, an ATRP initiator containing complementary clickable groups can be used to install ATRP initiation sites onto the cells under biologically relevant conditions. After that, a cytocompatible ATRP methodology can be used to grow polymer brushes from the ATRP initiation sites. For example, cytocompatible Fe⁰-mediated ATRP has been previously used to grow polymer brushes in the presence of mammalian cells without affecting their viability [453]. This strategy does not require cell pre-modification using protective polymer coatings that may adversely affect cell behavior. Coating layers may shield various types of plasma membrane receptors, resulting in diminished signal transduction. They may also disrupt the transport of molecules and/or ions across the plasma membrane, which is harmful for cells.

6.4.3. Surface modification of medical devices

Tissue engineering constructs should support cell adhesion, spreading, growth, and proliferation. Cell–biomaterial interactions at the interface greatly affect the performance of these constructs [454]. Materials used to construct tissue scaffolds affect nearby tissue via various mechanisms (e.g., biodegradation-induced tissue ingrowth), and surface-modification strategies such as immobilization of bioactive molecules (e.g., RGD peptides) also affect their performance significantly, *in vivo*. Patterning the substrate and scaffolds also affects cells' behavior and their interactions with biomaterials [455]. With these factors in mind, ATRP can affect the cell–biomaterial interface through surface modification of implants or scaffolds (i.e., creating a uniform/non-uniform layer of brush (co)polymers), creation of patterned surfaces (i.e., grafting ATRP initiator in predefined areas of substrate or scaffold as in lithographic techniques), and immobilization of bioactive molecules (e.g., high density grafting of affinity peptides on dense polymer brushes that are made via ATRP) or bioconjugates (e.g., synthetic polymer–protein/PSA conjugates). The effects of some of these factors and the role of ATRP on the manufacturing process are discussed in this section.

Creation of gradients in polymer brush length or chemical composition can be used to tune cell adhesion on the surface of implants. SI-ATRP not only enables grafting of densely packed brush (co)polymers from surfaces of various chemistries, length scales, and shapes, but it also gives control over brush thickness [244]. This thickness gradient can be utilized to modulate adhesion of various cells, including cancer cells and immune cells. Recent works have revealed that cell functions and activities are impacted by the thickness of the grafted polymer brushes. SI-ATRP was carried out to make PHEMA brushes to study the effect of brush thickness on immune cell attachment [456]. It was revealed that murine macrophages showed better activity (viability, differentiation, and migration) on thicker brushes. In other words, brush thickness affects the interactions of immune cells with the surface-modified material, which dictates the material's immunogenicity.

Adhesion of different cell lines can be adjusted via the thickness of PNIPAAm brushes that were synthesized using SI-ATRP [457]. In this work, a glass surface was modified with an ATRP initiator containing APTES species, followed by growing of PNIPAAm brushes from the glass surface. It was revealed that the mechanism responsible for this phenomenon originates from enhanced production of ECM glycoprotein, fibronectin, and a cytoskeletal protein, vinculin [457]. In another study, zerovalent copper (Cu⁰)-mediated SI-ATRP was used as a versatile and oxygen-tolerant methodology to grow polymer brushes from various surfaces under ambient conditions [458]. A copper plate was used in combination with an initiator-modified substrate. PMPC brushes were grafted from large organic substrates of different morphologies (e.g., microporous cellulose sheets and PBA), with high speed and using a tiny volume of reagents. PMPC is a zwitterionic and biodegradable polymer which shows bioinertness, lubrication properties, and resistance against

non-specific protein adsorption [459]. SI-ATRP was used for grafting various polymer brushes with controlled length on iron-coated plates to adjust cell–substrate interactions [453]. In the cytocompatible zerovalent iron-mediated ATRP, Fe^0 acts as both a reducing agent and a catalyst. Furthermore, the ATRP can be carried out in both organic and aqueous media.

The bioactivity of tissue engineering constructs is crucial for their practical applications. Various bioactive molecules, such as growth factors (GFs), have been immobilized on tissue engineering constructs to enhance cell adhesion and facilitate tissue regeneration. However, achieving stable immobilization that maintains the bioactivity and sensitivity of these biomacromolecules poses a challenge. Covalently immobilizing GFs onto the surface of scaffolds typically leads to reduced GF sensitivity and bioactivity. Nevertheless, a recently published paper demonstrated a novel approach. They used SI-ATRP-induced polymer brushes based on poly (glycidyl methacrylate) (PGMA) for covalent *in situ* immobilization of GFs, all while preserving sensitivity and bioactivity [460]. The PGMA brushes containing reactive epoxy side chain groups enabled robust immobilization of bone morphogenetic protein-2 (BMP-2) without compromising its bioactivity.

Poly(ether ether ketone) (PEEK) is a synthetic polymer with high chemical, thermal, and biological stability, low friction coefficient, high mechanical properties, and bone-mimicking mechanical properties [461]. PEEK is an FDA-approved material for making bone tissue engineering constructs but suffers from hydrophobicity and limited bioactivity. However, surface modification of PEEK can enhance the osseointegration and biocompatibility of PEEK implants. CRP strategies, including ATRP, have been widely utilized in surface modification of PEEK [462]. Various polymer brushes (e.g., PNIPAAm) were grafted from PEEK surface via SI-ATRP to improve biological properties or induce stimuli-responsiveness [463]. In a recent work, the surface of PEEK microparticles were modified by SI-ATRP to create a soft corona around a hard PEEK core that better fit with its surrounding tissue [340]. The carbonyl groups on PEEK chains that are on the surface of PEEK microparticles were chemically reduced to hydroxyl functionalities using sodium borohydride. After that, an ATRP initiator was esterified with hydroxyl groups on the surface of the microparticles. Four types of monomers were grafted from the numerous initiation sites on these microparticles to create hairy microparticles, and their performance was assessed. Moreover, they used various ATRP strategies including photoATRP, ARGET ATRP, and ICAR ATRP based on monomer types. Osteoblast adhesion and proliferation was improved on the PDMAEMA-grafted microparticles. Thus, these microparticles can be used in formulating implants or as compatibilizers to regulate implant–tissue interactions. They can also be used as additives in formulation of scaffolds and injectable hydrogels for bone tissue engineering, as these hairy particles better disperse in solvents and hydrogels.

In some cases, ATRP has been used to modify the metal implants that are widely used in bone tissue engineering. Although, titanium (Ti)-based implants are commonly utilized in orthopedics and dentistry, more improvement is required to enhance their osseointegration. Osseointegration originating from cell–biomaterial interactions can be improved via immobilization of binding motifs, such as the synthetic 15-amino-acid biomimetic peptide sequence (P15) derived from a region of the alpha (α)-1 chain of collagen I [464]. Thus, effective immobilization of P15 peptide on Ti-based alloy can improve osteoconductivity and matrix mineralization. Copolymer brush, based on acetylenyl methacrylate (AEMA) and DMAEMA, was grafted from the Ti surface via SI-ATRP, as illustrated in Fig. 26 [280,465]. Ketone functionalities on AEMA residues of the copolymer enable post-polymerization modification, i. e., conjugation of the aminoxy functional group on P15 to ketone groups through the oxime click chemistry [466]. *In vitro* investigations revealed significantly enhanced cell viability of Ti-copolymer-P15, compared to neat Ti or Ti plates with randomly adsorbed P15 (Ti-P15). In addition, more tightly packed cells, enhanced osteoblast differentiation, and increased osteoblast mineralization were observed on Ti-copolymer-15 compared to neat Ti or Ti-P15 substrates. In fact, densely grafted polymer brushes with numerous active sites for oxime ligation enable more effective immobilization of P15 peptide on the Ti surface [280,465].

This polymer brush-mediated immobilization of biomolecules using ATRP can also be applied to proteins that promote bone cell

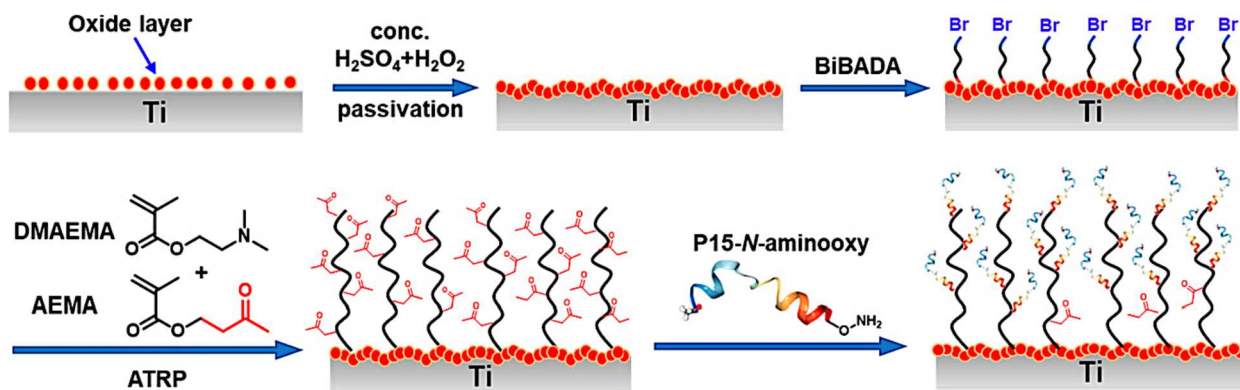


Fig. 26. Schematic describing a multi-step functionalization procedure for the fabrication of a short peptide-grafted titanium (Ti) surface. First, piranha solution etching of Ti discs increases the number density of hydroxyl groups and enhances surface roughness. Next, the initiator, 12-(2-bromoisobutyramido)dodecanoic acid (BiBADA), is immobilized on Ti surface via hydrogen bonding (between hydroxyl groups on Ti surface and carboxylic acid functional groups on BiBADA) and hydrophobic interactions (aliphatic chain of BiBADA). Copolymerization of acetylenyl methacrylate (AEMA) and DMAEMA via SI-ATRP results in copolymer brushes. AEMA residues on the copolymer are rich with ketone functionalities that can be used to conjugate aminoxy-containing P15 peptide via an oxime ligation reaction. Reprinted with permission [465]. Copyright 2019 American Chemical Society.

adhesion [281]. When biomaterials contact structures of the blood stream, such as heart valves, thrombus or blood coagulation may occur at the surface of biomaterials, and this is a concern in clinical applications of medical devices and implants. It is believed that this biomaterial-induced thrombus is associated with non-specific protein adsorption on the surface [467]. Thus, development of hemocompatible surfaces to resist against thrombus formation is valuable for utilization of biomaterials in vascular tissue engineering [468]. Grafting polymer brushes from biomaterials that contact the blood stream has been used as a solution to address this challenge and create an antithrombotic surface [469]. It was revealed that densely grafted hydrophilic polymer brushes can inhibit thrombi on the surfaces of vascular medical devices [470]. In this work, neutral and highly hydrophilic PDMA brushes were grafted from polystyrene particles using SI-ATRP. It was observed that, although there is protein adsorption on brush-modified surfaces, the amount and type of adsorbed proteins is significantly influenced by brush graft density, which also affects polymer chain conformation on the surface. This brush-grafting strategy was also used to modify poly(dimethylsiloxane) (PDMS) surfaces for cardiovascular tissue engineering [471]. PDMS is a hydrophobic and biocompatible polymer that is frequently used in medical devices such as pacemakers and contact lenses. The researchers grafted hydrophilic PHEMA brushes from the PDMS surface using SI-ATRP. The resulting hemocompatible surface showed antibiofouling properties; moreover, platelet adhesion was significantly reduced. Bioactive gelatin macromolecules were conjugated to PHEMA brushes to enhance surface bioactivity and trigger endothelial cell adhesion.

In biological systems like the human body, water lubrication mechanisms play a crucial role in joints. For example, extremely low friction coefficients are observed in body joints such as the knee. Bottlebrush-like macromolecules in cartilage are responsible for this remarkably low coefficient of friction [473]. By taking inspiration from this structural superlubricity, it is possible to design the low-friction surfaces that are required in tissue engineering constructs with moving parts, as in artificial joints [473,474]. In this context, tethering polyelectrolyte or hydrophilic polymer brushes that retain large amounts of water molecules can significantly reduce the friction coefficient via water lubrication mechanisms.

As illustrated in Fig. 27, an ATRP initiator containing a catechol anchoring group was used to trigger SI-ATRP of hydrophilic polymer brushes from diamond-like carbon (DLC) film for utilization as lubricant for articular cartilage [472]. In fact, this work utilizes mussel-inspired chemistry, which allows adhesion under wet conditions, to attach the ATRP initiator physically and robustly to the DLC surface. In addition, it mimics the bottlebrush-like structure of glycoproteins, which is exploited to make extremely low-friction surfaces in body joints. This dual biomimicry strategy has enabled researchers to develop excellent biological lubricants. Tribological evaluation of PSPMA brush-modified DLC film showed ultralow friction coefficients in aqueous and biofluids, that protect against aging. While water is not a good lubricant because of its low viscosity, confinement of water molecules (or preserving them under exogenous stresses) in polymer chains (e.g., polymer brushes or aqueous polymer solutions) can address this challenge and make extremely low-friction surfaces based on water-containing systems. PSPMA brushes extend in water, resulting in a low friction coefficient, while they collapse under dry or high ionic strength conditions, resulting in a higher friction coefficient. Alternation in conformation of PSPMA brushes results in friction-responsive behavior of the construct.

A cartilage matrix-like structure was manufactured based on hydrophilic polymer brushes grown from a hydrogel subsurface, which closely mimics the structure of natural articular cartilage [343]. While the stiff hydrogel layer enables bearing and/or damping of applied loads, polyelectrolyte brushes grown from the hydrogel subsurface (i.e., tens of microns beneath the surface) give the bilayer structure high lubricity. Effective covalent attachment of polymer brushes into the hydrogel network was attained via grafting polymer

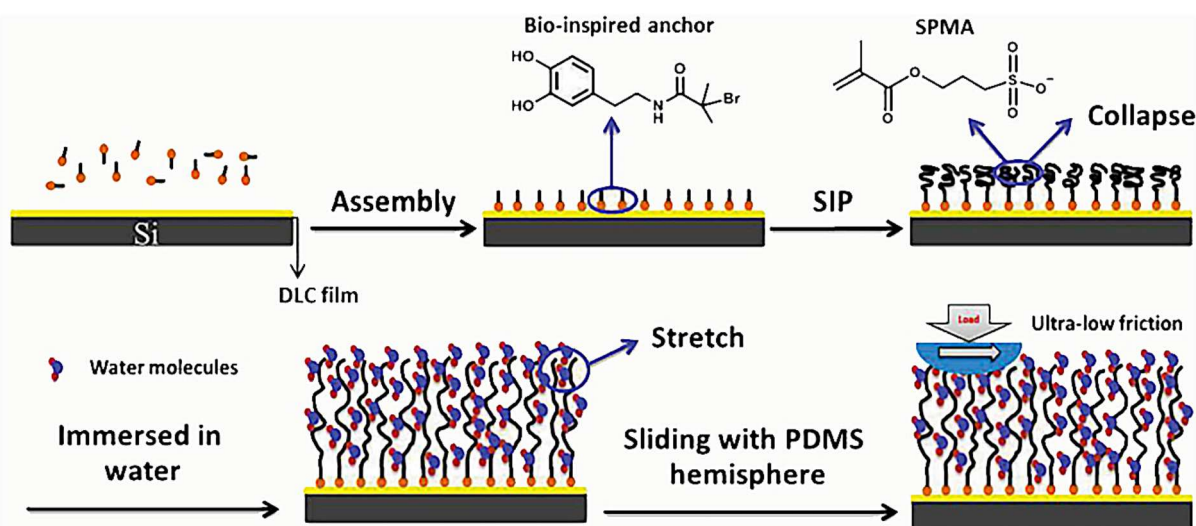


Fig. 27. Schematic describing the fabrication process of diamond-like carbon (DLC)-based lubricants for cartilage tissue engineering. DLC film was grown on silicon substrate; an ATRP initiator containing catechol functionality was immobilized on DLC through physical interactions; SI-ATRP was used to grow poly(3-sulfopropyl methacrylate potassium salt) (PSPMA) brushes from the DLC layer; polymer brushes collapse under dry conditions and they extend in the presence of water molecules; this water-swollen PSPMA layer causes extreme reduction in friction coefficient at interface. Reproduced by permission [472]. Copyright 2014 Wiley-VCH.

brushes from initiators embedded in the hydrogel network, as schematically illustrated in Fig. 28.

The tough hydrogel is a copolymer of acrylic acid, acrylamide, and 2-(2-bromoisobutyryloxy) ethyl methacrylate (a polymerizable ATRP initiator–modified monomer) dually crosslinked by covalent crosslinkers (endowing the hydrogel with high mechanical strength) and metal complexation (introducing self-healing features into the hydrogel). Subsurface-initiated ATRP (SS-ATRP) was used to graft zwitterionic poly(2-(methacryloyloxy) ethyl) dimethyl-(3-sulfopropyl) ammonium hydroxide (PSBMA) and anionic PSPMA polymer brushes from embedded ATRP initiators, after immersion of tough hydrogels in a solution containing monomers. Because of mass transfer limitations, polymerization initially starts from ATRP initiators on the hydrogel surface and in the immediate subsurface regions (i.e., just a few tens of microns beneath the hydrogel surface). In a similar work, a tough, double-network hydrogel (DNH) was modified by Cu^0 -mediated ATRP from monomers serving as initiation sites embedded throughout the DNH to create polymer brushes [475]. Tissue scaffolds are an indispensable part of tissue engineering. In this context, various biomaterials like natural and synthetic polymers have been widely applied to make tissue scaffolds [433,476]. For *in vivo* applications, the biocompatibility, biodegradation, and interfacial interactions between scaffold and biological systems should be considered when designing the scaffold. In another study, polymer brush–modified CS NPs were employed as a biolubricant for the treatment of osteoarthritis (OA) [477]. Brushes composed of zwitterionic PMPC were grafted from initiator-modified surfaces of CS NPs using SI-ATRP. Additionally, a non-steroidal anti-inflammatory drug was encapsulated within the brush-modified NPs. The resulting nanoplateforms exhibited exceptional lubrication properties and demonstrated an effective anti-inflammatory response.

Silk fibroin (SF) is a biocompatible, naturally-derived protein with numerous applications in biomedicine [478]. However, its hydrophobicity necessitates functionalization of SF-based scaffolds to improve their suitability for physiological applications. Surface modification of SF via grafting hydrophilic polymer brushes is a solution to this problem. In this context, ATRP was used to graft hydrophilic and zwitterionic polymer brushes from SF films [479]. Contrary to previous studies, in which plasma treatment was utilized to enhance the number density of hydroxyl groups, in this work, surface treatment was carried out using two strategies: photocatalyzed oxidation of the SF surface and modification by reaction with ethylene oxide. Polymer brush density was higher, and a

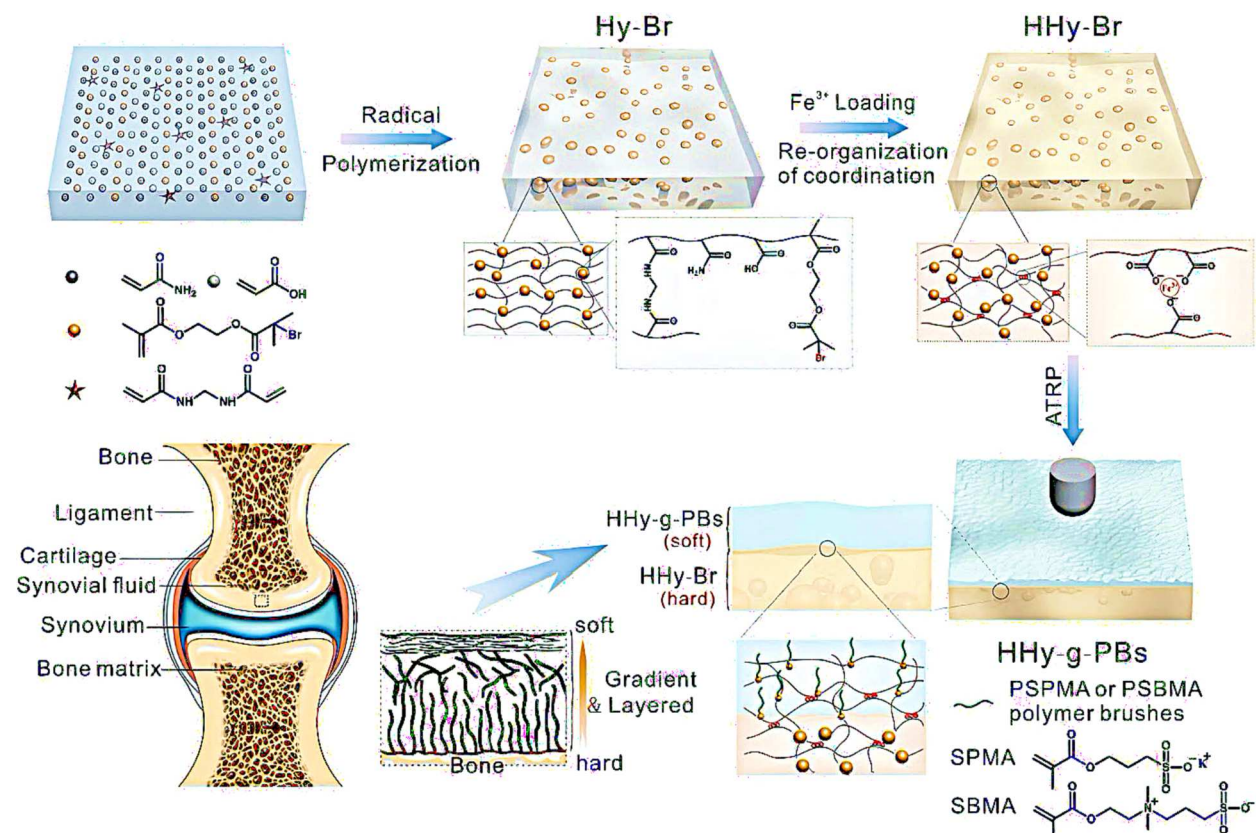


Fig. 28. Schematic displaying the manufacturing process of cartilage matrix-like hydrogels. The tough hydrogel was manufactured based on radical polymerization of acrylic acid and acrylamide as comonomers in the presence of N,N' -Methylenebisacrylamide as crosslinker, and a methacrylate monomer containing ATRP initiation sites, i.e., α -bromoisobutyryl species. Chemical crosslinking and metal complexation (i.e., ferric ions) of carboxylic acid side groups results in creation of a tough hydrogel. ATRP of 3-sulfopropyl methacrylate potassium (SPMA) and 2-(methacryloyloxy) ethyl) dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA) results in growing polyelectrolyte polymer from the hydrogel subsurface. This tough-to-soft transition in the hydrogel interface closely mimics the properties of articular cartilage at the bone interface. Reproduced by permission [343]. Copyright 2020 Wiley-VCH.

more uniform coating was obtained, by the photocatalyzed oxidation procedure which increased the density of hydroxyl groups. BiBB, the ATRP initiator, was covalently attached to the SF surface via chemical reaction with hydroxyl groups. Two acrylate-based monomers containing PEG and zwitterionic pendant groups were grafted from the SF surface by SI-ATRP.

Surface modification by polymerization of PEGMA, a neutral macromonomer, and [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfo-propyl)ammonium hydroxide, a charged monomer, resulted in polymer brush-modified SF films. It was observed that brush-modified SF performed better with higher hydrophilicity zwitterionic pendant groups than with PEG pendant groups. Protein adsorption using bovine serum albumin (BSA) showed that PEG-brushes grafted from the SF surface performed better compared to PEG chains that were grafted to SF. Zwitterionic polymer brush-modified SF showed superior performance in non-specific protein adsorption compared to PEG brush-modified surfaces. This reduction in attachment was also observed with human mesenchymal stem cells (hMSCs) such that hMSC attachment order was unmodified > PEG brush-SF > zwitterionic brush-SF. Controlling cell and protein adsorption is important for implanted scaffolds as non-specific protein adsorption and biofouling should be prevented [479].

Poly-L-lactic acid (PLLA) is a biodegradable and biocompatible polyester with wide applications in biomedicine. However, because of inertness it possesses limited cell adhesion, and modifications (e.g., immobilization of biomolecules) are normally required before utilization in medicine. Alternatively, polymer brushes can be grafted onto PLLA substrates (e.g., films and nanofibers) to increase their hydrophilicity and bioactivity. SI-ATRP was used to graft poly(ethyl acrylate) (PEA) brushes from PLLA films as shown in Fig. 29 [480].

Fibronectin, an important constituent of ECM, undergoes organization into nanofibrils at the PEA interface, which is a kind of protein remodeling induced by materials [481]. However, PEA suffers from non-degradation under physiological conditions which may prevent its utilization as base material. PEA brush-modified PLLA constructs combine the biodegradability and mechanical

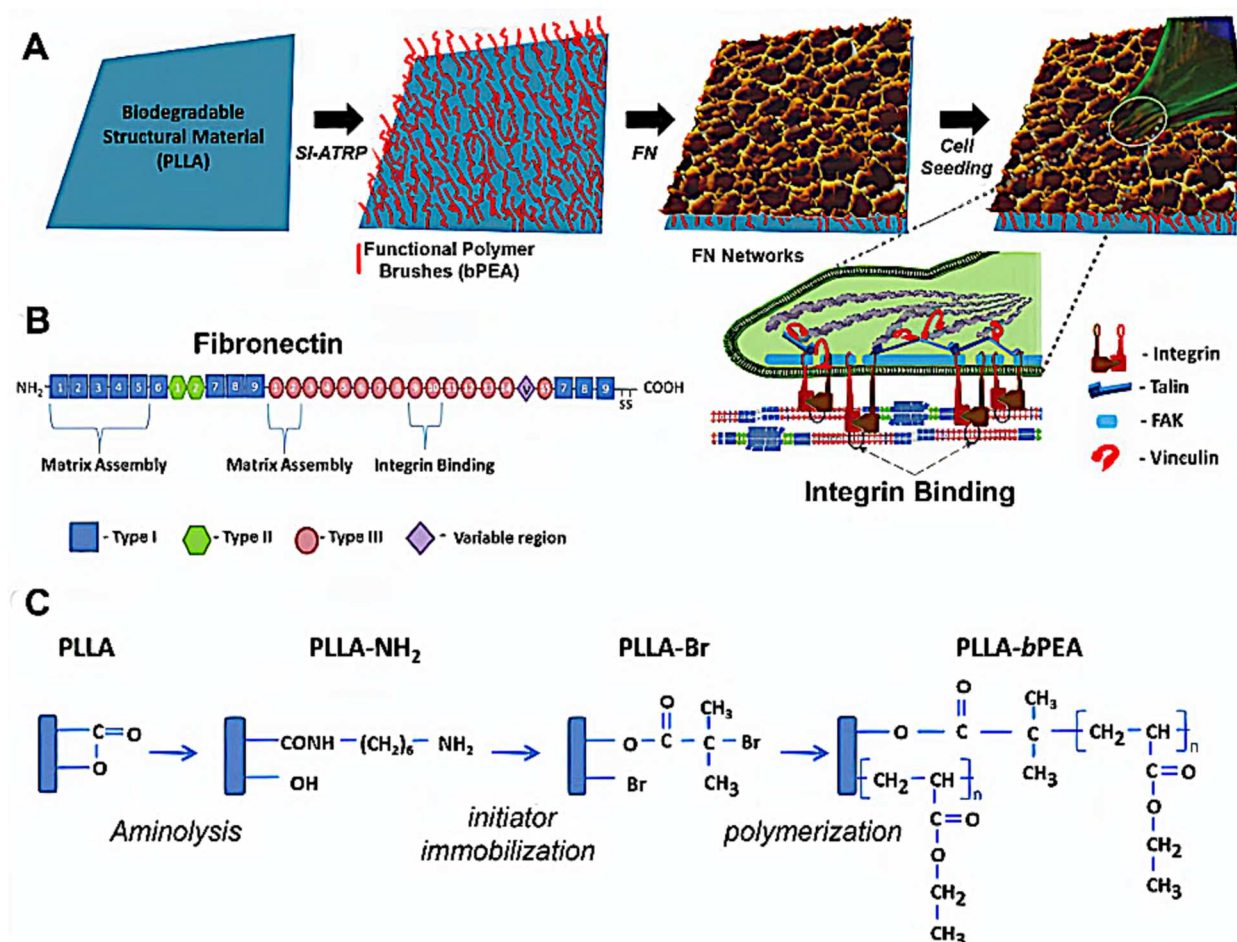


Fig. 29. Schematic depicting the conversion of a bioinert PLLA material to a bioactive state via an SI-ATRP strategy. (A) SI-ATRP grows poly(ethyl acrylate) brushes (bPEA) from bioinert PLLA surface; fibronectin (FN) remodeling forms a network similar to physiological conditions which allows for cell attachment and spreading. (B) FN assembly on PEA results in formation of nanofibrils in which integrin binding sites are exposed, allowing the cells to build up larger focal adhesions (FA). This integrin-mediated cell adhesion mechanism enables better cell attachment and spreading on PEA brush-modified surface. (C) Synthetic route for preparation of PEA brush-modified PLLA surface using the SI-ATRP methodology. Reproduced under the Creative Commons CC BY license [480]. Copyright 2019 Wiley-VCH.

properties of PLLA substrate with the fibronectin fibrillogenesis-inducing features of PEA in one construct. Although under physiological conditions the PEA brushes (bPEA) may detach from the PLLA substrate in the long term, renal clearance can remove relatively low molecular weight polymer chains [482]. Focal adhesion size is higher for PEA and PLLA-bPEA compared to neat PLLA, which confirms enhanced cell–biomaterial interactions. The transformation from bioinert to bioactive is highly interesting for tissue engineering constructs [480]. This strategy can be used for other biodegradable polymers such as PLGA in nano or higher scales for *in vivo* applications, for which hydrophobic/bioinert surfaces can switch to hydrophilic/bioactive through the grafting of short polymer brushes. HAP is an important mineral and constitutes one of the main mineral components of bone. However, aggregation of HAP nanocrystals (HAP NCs) can prevent its effective distribution in polymer nanocomposites, ultimately limiting its wide utilization in bone tissue engineering [483]. Grafting polymer brushes on HAP NC surfaces is a very effective method of improving the interactions of HAP NCs with polymer matrices to prevent their aggregation [278]. For example, the SI-ATRP strategy, using 2-bromo-2-methylpropionic acid, was used to graft various polymer brushes (i.e., PDMAEMA, polyacrylonitrile, and PMMA) onto NC surfaces.

This section discussed the importance of ATRP in the modification of various natural and synthetic biomaterials. While base biomaterials may be extracted from natural resources or may be created using other polymerizations routes, ATRP is used to modify their surface properties to regulate their performance *in vivo*. However, in some cases, the base biomaterials can be synthesized using an ATRP strategy.

6.4.4. Antifouling and antibacterial surfaces

Antibiofouling and antibacterial surfaces certainly fall into the category of surface modification strategies via ATRP; however, we preferred to discuss them in a separate section because of their importance. Antifouling surfaces are important in a wide variety of applications, including surfaces of marine vehicles (e.g., vessels and submarines), purification membranes, packaging, and implantable medical devices [484]. Indeed, biofouling originating from microorganism and/or cell adhesion can lead to many complications, including infection. Functionalization of a surface with hydrophilic polymers is a robust strategy to prevent biofouling on biomaterials [485]. The hydrated barrier layer formed between surface and environment is responsible for this phenomenon. Recently, zwitterionic polymers have attracted much interest for making antifouling surfaces [486]. Zwitterionic polymer brushes can be grafted using SI-ATRP strategies to create antifouling surfaces. In one study, zwitterionic polymer brushes based on sulfobetaine vinylimidazole monomers, which inherit antibacterial properties from imidazolium and sulfonate groups, were grafted on a silicon surface [487]. They showed superior antibacterial properties compared to poly(vinylimidazole) brushes. In another work, zwitterionic poly[2-(methacryloyloxy)ethyl choline phosphate] (PMCP) brushes were grafted from hydrophobic PCL substrates, via an SI-ATRP strategy to

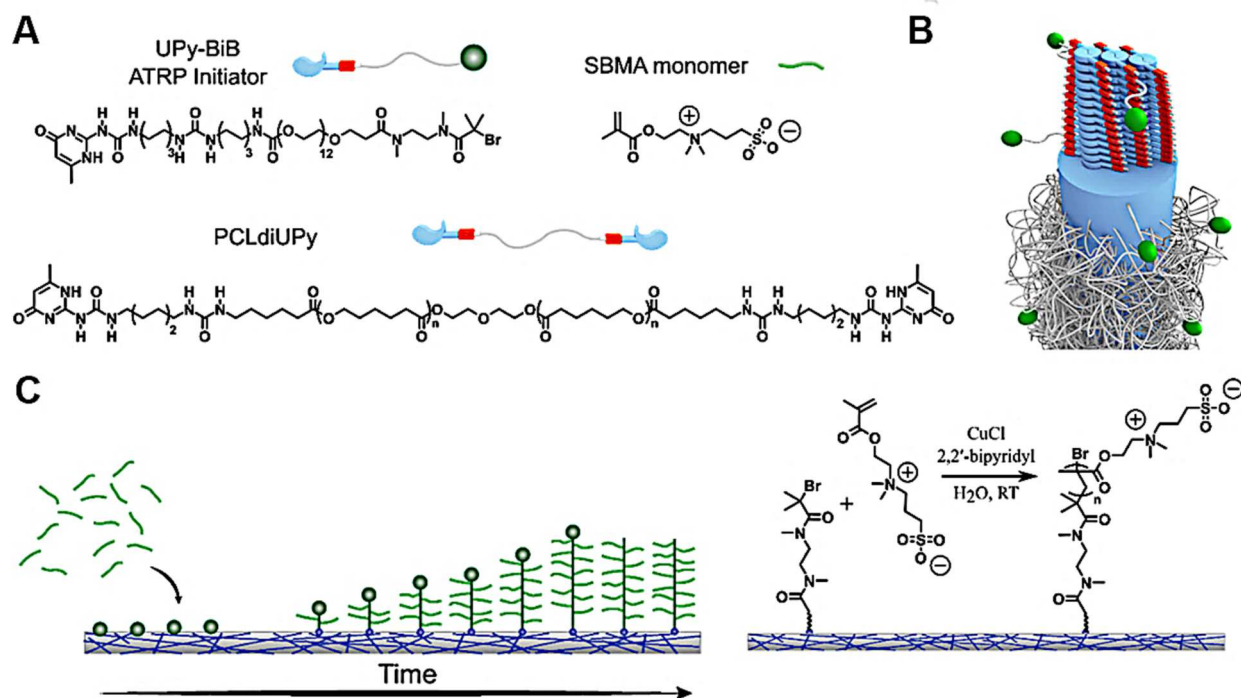


Fig. 30. Schematic describing the synthetic approach for the design a zwitterionic brush-modified supramolecular material. (A) Polycaprolactone modified with two UPy end-functionalities (PCLdiUPy) serves as base supramolecular material; an ATRP macroinitiator containing UPy and BiB end-functional groups (UPy-BiB). (B) Self-assembly of UPy species in PCLdiUPy, via hydrogen bonding, results in fibrillar assemblies in which double UPy moieties and PCL chains constitute the core and shell, respectively. (C) Sulfobetaine methacrylate (SbMA) monomers polymerize from ATRP initiation sites on the UPy-BiB macroinitiator embedded throughout the supramolecular matrix to yield zwitterionic PSbMA brushes. Reproduced under the CC-BY-NC-ND license [488]. 2020 American Chemical Society.

adjust their fouling properties and hydrophilicity [52]. The PMCP brushes not only significantly enhance the hydrophilicity of PCL but can also prevent non-specific adsorption of proteins, a trait that stems from the zwitterionic characteristics of polymer brushes. Moreover, cell attachment was promoted, and cell differentiation toward bone cells was triggered.

SI-ATRP was used to modify supramolecular biomaterials with zwitterionic poly(sulfobetaine methacrylate) brushes, as illustrated in Fig. 30 [488]. Polymer brushes based on sulfobetaine methacrylate (SbMA) grow from ureidopyrimidinone (UPy)-based macro-initiators that were embedded in UPy-modified polycaprolactone (PCLdiUPy) as the base supramolecular material. It was revealed that the antifouling property of PSbMA is dependent on sulfobetaine content. Moreover, it was concluded that the translation of this strategy to electrospun nanofibers is possible.

In a recent paper, poly[N-(2-methacryloyloxyethyl)pyrrolidone] (PNMEP) was grown from a silica surface via SI-ATRP [489]. The obtained thermoresponsive PNMEP brushes were confirmed to be living, as they undergo copolymerization with oligo(ethylene glycol) methyl ether methacrylate monomers. In fact, PNMEP chains better preserve their livingness compared to PNIPAAm, which make them superior polymers for the making of more complex thermoresponsive structures. The LCST of PNMEP (LCST > 51 °C) is higher than that of PNIPAAm (LCST ~ 31 °C). In addition, *in vitro* studies confirmed the antifouling properties of polymer brushes.

Antibacterial coatings are also important to prevent infection in medical devices, medical implants, and wound dressings. In fact, antibacterial dressings are highly valuable in treatment of wounds. In this context, many antibacterial agents (e.g., antibacterial peptides, silver NPs, and graphene-based materials) have been incorporated in the formulation of wound dressings [490]. Cationic polymers possess inherent antibacterial properties since they can induce plasma membrane rupture in bacteria. For example, the surface of GO nanoplatelets was modified with cationic poly[2-(*tert*-butylaminoethyl) methacrylate] (PTA) brushes to introduce antibacterial properties [338]. Both PTA and GO showed antibacterial properties, which were inherited in GO-g-PTA as well. After this, polyvinyl alcohol (PVA)/GO-g-PTA nanocomposite fibers were made using the electrospinning method [491]. Although PTA exhibits broad-spectrum antibacterial activity, it shows low cytotoxicity against cells, which is highly favorable. The grafting density and antimicrobial efficacy of GO-g-PTA made via ATRP was higher than GO-g-PTA made by FRP, which was similarly incorporated in electrospun nanofibers. Moreover, the nanocomposite nanofibrous scaffolds showed significant antimicrobial properties.

It is challenging to develop coating materials that are biocompatible yet also exhibit anti-infective properties. To endow substrates with antibacterial properties while preserving cytocompatibility, various biodegradable polyester brushes with inherent antibacterial properties were grown from substrate surface [492]. Grafted polymers, including PPEGMA, PHEMA (nonionic), and poly([2-methacryloyloxyethyl] trimethyl ammonium chloride) (PMETA) (cationic, with an antibacterial effect) were characterized to evaluate their antibacterial properties. Cationic PMETA can induce an antibacterial effect, while nonionic PHEMA and PPEGMA induce anti-adhesive properties to the substrate materials. Cationic polymer brushes based on PMETA outperformed other brushes, in terms of anti-infectious properties. This strategy for growing PMETA brushes was successfully translated to 3D-printed scaffolds based on PLA/aliphatic ester blends. The obtained results showed that there is a specific length for PMETA brushes in 3D scaffolds where the trade-off between cytocompatibility and antibacterial properties reaches an optimum [492].

6.4.5. Tissue engineering scaffolds

This section highlights the importance of ATRP for making base biomaterials that can be utilized in making tissue engineering constructs. In other words, ATRP is used to synthesize polymers that constitute major parts of tissue engineering constructs or wound dressings.

Native ECM has a fibrous and porous structure that allows mass transfer of nutrients, signaling molecules, and waste materials. Moreover, the special architectural and compositional features of ECM endow soft living tissue with a unique strain-stiffening behavior that cannot be replicated by conventional synthetic hydrogels. However, it was discovered that special types of bottlebrush copolymers can closely mimic the peculiar mechanical properties of soft tissue [493]. To bridge the gap between mechanical properties of ordinary hydrogels and soft tissues, an ATRP strategy was used to make a linear-bottlebrush-linear (L-BB-L) architecture [494]. The middle PDMS bottlebrush segment was made by an ATRP strategy; then end chains were modified to serve as initiation sites for ATRP. The obtained PDMS macroinitiator with dual initiation sites underwent another ATRP to grow PMMA chains from the bottlebrush polymer, resulting in the L-BB-L architecture. These solvent-free elastomers were observed to closely mimic the strain-stress behavior of different soft tissues like brain and adipose [494].

A recent innovation in the design of bottlebrush polymers involves creating graft copolymers with mechanical characteristics resembling those of natural tissues [46]. Utilizing CRP techniques such as ATRP and RAFT, these architecturally distinct copolymers were developed. The resulting elastomer exhibits exceptional softness, yet maintains robust strength and resilience, accompanied by a high damping factor – attributes that conventional bottlebrush polymers struggle to achieve. Furthermore, the properties of these unique graft copolymers can be fine-tuned by selecting appropriate monomers, rendering them suitable for a range of soft tissue engineering applications, spanning from skin tissue to brain-related applications.

An injectable hydrogel, formulated using a triblock copolymer featuring an L-BB-L architecture comprising hydrophilic and thermosensitive blocks, was developed by Vashahi *et al.* [495]. In their work, a combination of ATRP and RAFT techniques was used to synthesize these complex structural polymers. The resulting hydrogel effectively emulates the mechanical properties of soft tissues. This attribute can be attributed to the presence of compact bottlebrush segments, which contribute to low viscosity in solution, enabling easy injectability. Moreover, the incorporation of thermosensitive segments leads to the hydrogel transitioning into a gel-like state at 37 °C. *In vivo* experiments conducted on a rat model demonstrated favorable biocompatibility and minimal inflammation associated with the hydrogel.

6.5. Biosensing

Biosensors are highly valuable tools in medical diagnosis, monitoring of health conditions, and point-of-care devices. In early stages of infections, the concentration of various biomarkers is as low as picomolar to sub-femtomolar. However, conventional biosensors can only measure down to picomolar concentrations [496]. This highlights the importance of chemical amplification strategies for detecting biomarkers with ultralow concentrations. SI-CRP provides a robust strategy for chemical signal amplification that is highly valuable for ultrahigh-sensitivity biosensing applications [53]. A signal amplification strategy using ATRP is a very promising tool to make ultrasensitive biosensors to detect target DNA, RNA, or gene biomarkers. Through this procedure, a single initiation trigger would result in polymer chain growth via an SI-ATRP strategy.

Sun *et al.* managed to fabricate a highly selective and ultrasensitive electrochemical biosensor for detection of target DNA (t-DNA) at sub-attomolar levels [53]. In their approach, a gold electrode was chemically modified with hairpin DNA probes containing azide end-functionality as depicted in Fig. 31. Addition of t-DNA, even at extremely low concentration, results in hybridization of t-DNA and hairpin DNA probes, resulting in duplex DNA and exposure of the azido functional groups.

A propargyl-functionalized ATRP initiator (propargyl 2-bromoisobutyrate, PBIB) was then conjugated to a gold surface via a CuAAC click reaction to form the initiation site for eATRP. Numerous electroactive ferrocenylmethyl methacrylate (FMMA) probes, linked together to create polymer chains on a gold electrode, significantly amplified the electrical signals, detectable using a voltammetry technique. Therefore, combining eATRP with electrochemical sensors provides a robust strategy to detect ultralow concentrations of chemicals. In addition, the created polymer chain can be detached from the electrode surface via temperature increase to denature (i.e., unzip) the duplexed DNA. In other words, the biosensor can be reused after heat treatment and washing.

A similar methodology was utilized for detection of tobacco mosaic virus RNA, where photoATRP was used in combination with click chemistry, for signal amplification [497]. As with the previous work, a combination of click reactions and SI-CRP was utilized for detection of nucleic acids. A similar strategy was also used to develop an ultrasensitive electrochemical biosensor for detection of miRNA-141, a cancer biomarker [498]. In this study, hairpin DNA probes were modified with thiol functionalities, in contrast to the previous works. An ATRP initiator containing two bromoisobutyrate functional groups and one disulfide linkage was grafted onto AuNPs via Au-S bonds. Hairpin DNA probes were grafted onto glassy carbon electrodes (GCEs) followed by conjugation to modified AuNPs. The AuNP-modified GCE surface with immobilized ATRP initiators (on the AuNPs) served as initiation site for AGET ATRP.

Genetic-level detection of cancer biomarkers can significantly enhance our ability to diagnose cancers at early stages and monitor the effectiveness of cancer therapies via noninvasive methods. For example, cytokeratin fragment antigen 21-1 (CYFRA 21-1) is a

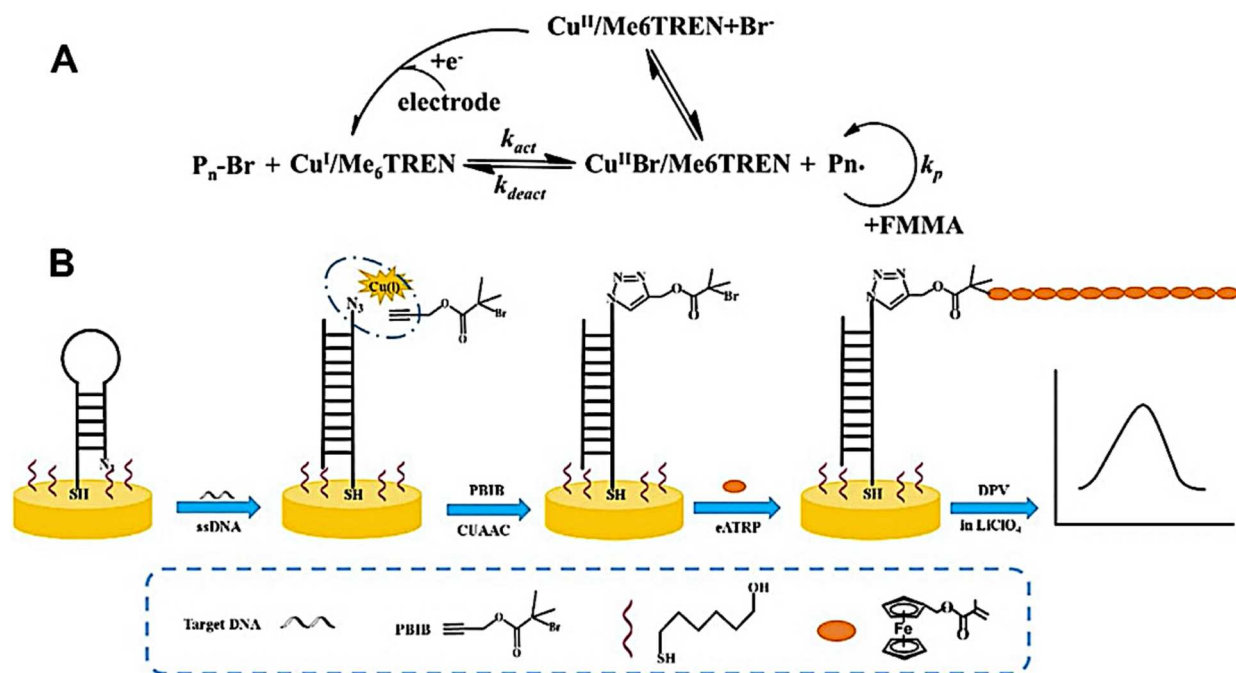


Fig. 31. Schematic depicting the mechanism of action of an ultrasensitive biosensor for the detection of DNA. (A) Mechanism of eATRP of electroactive ferrocenylmethyl methacrylate (FMMA) monomers on gold electrode surface. (B) Schematic representation of mechanism of function of biosensor: hairpin DNA was grafted onto gold surface via thiol end-functional groups; this DNA also contains an azido functional group at its other end, close to the electrode surface and unexposed. The presence of target DNA (t-DNA), even at very low concentration, results in DNA hybridization and creation of duplex DNA, leaving azido functional groups exposed to click ligation with the propargyl functional group on the ATRP initiator. After immobilization of the ATRP initiator, FMMA monomers attach via eATRP to create polymer chains, which correspond to current and voltage changes in cyclic voltammograms. Reproduced by permission [53]. Copyright 2018 Elsevier.

specific biomarker closely related to non-small-cell lung cancer [500]. However, the low concentration of target DNA limits the applicability of conventional biosensors because of their relatively low sensitivity level. Thus, chemical amplification strategies, especially polymerization, can significantly enhance the sensitivity and limit of detection (LOD) of biosensors [501]. ATRP and its modified versions, especially eATRP and photoATRP, are highly effective polymerization-based strategies for signal amplification in biosensing of nucleic acids. Through this strategy, biosensor sensitivity can be enhanced by increasing the length or graft density of the polymer chains grafted from the electrode's surface. Grafting density can be increased via utilization of a macroinitiator (e.g., polymer), grafted onto the electrode surface, that bears numerous initiation sites as side chains. This indicates a dual signal amplification procedure. Using this strategy an ultrasensitive biosensor was manufactured for detection of CYFRA 21–1, as shown in Fig. 32 [499].

The same group also immobilized a thiolated peptide nucleic acid (PNUA), which forms duplexes in the presence of t-DNA, onto gold electrodes. Zirconium–phosphate–carboxylate chemistry was used to attach HA to PNUA/DNA heteroduplexes, followed by creating numerous ATRP initiation sites on HA via carbodiimide chemistry. The polymer chain grafted via eATRP from HA-mounted

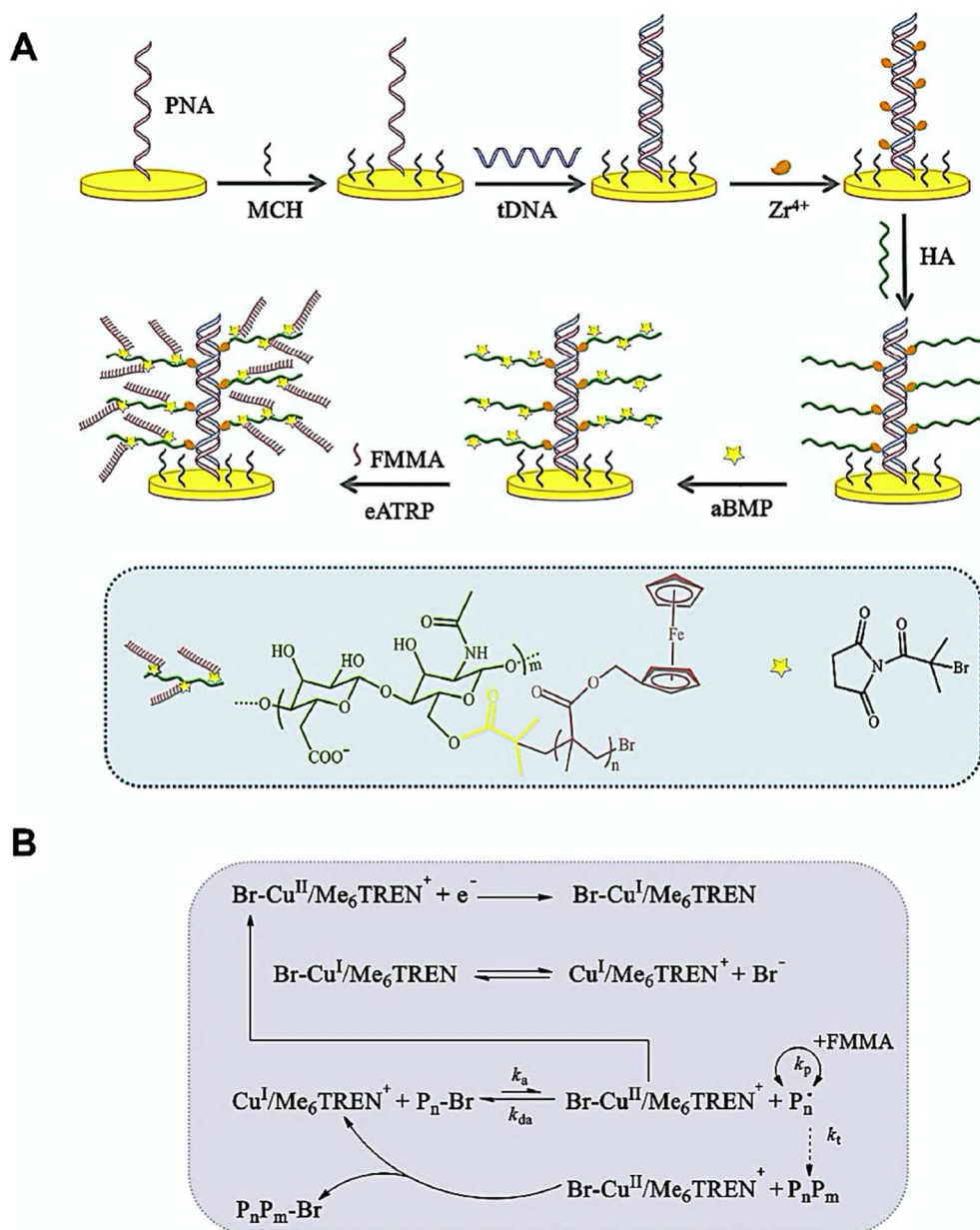


Fig. 32. Schematic illustrating a DNA biosensor via eATRP and polysaccharide dual signal amplification strategy. (A) Working principle of ultrasensitive DNA biosensor based on dual amplification strategy using polysaccharides and eATRP. (B) Mechanism describing the eATRP signal amplification approach for the detection of DNA. Reprinted by permission [499]. Copyright 2019 Elsevier.

initiation sites results in signal amplification. This dual amplification strategy performed 1000-fold better compared to NP-based signal amplification and can detect t-DNA as low as 9.04 aM with high selectivity. A similar dual amplification strategy, based on eATRP and DNA-templated AgNP, was also used previously to make ultrasensitive biosensors for nucleic acids, where the lower detection limit for t-DNA was 4.725 aM [503].

Detection and/or measuring of various molecules or parameters (temperature, biomarkers, NPs) are invaluable clues for evaluating health conditions, and they require special devices [504]. For example, fluorescent nanothermometers can measure the exact temperature of the intracellular environment [505]. Numerous biomolecules are involved in physiological processes inside living cells and measuring them is highly important not only for fundamental biological research but also for diagnosis of the physiological conditions inside individual cells. For example, microRNAs (miRNAs) are key regulatory molecules in biological processes (e.g., differentiation) and their dysfunction can result in many diseases, including cancer [506]. However, measuring such biomarkers at the intracellular or ECM levels is much more challenging because of diversity, chemical interference, and ultralow concentration [507]. Sensitivity of measuring techniques will be greatly improved using dual/multiple amplification strategies that enable measuring at the cellular level [499,503].

In a different study, magnetic nanobeads (MnB) in combination with eATRP and strand-displacement amplification (SDA) strategies were used to make an ultrasensitive electrochemical sensor for detection of miRNA-21, as shown in Fig. 33 [502]. MnB were modified with hairpin DNA (MB-H1) that enabled hybridization with miRNA-21, i.e., enabling a pre-purification process for miRNA. Furthermore, signal amplification based on eATRP and SDA drastically enhanced the sensitivity of the obtained biosensor, permitting anti-interference detection of miRNA-21 at the cellular level.

In a recently published study, photoATRP was used as a signal amplification strategy enabling highly sensitive detection of disease-related DNA [508]. In this approach, PTH served as a photocatalyst to initiate the polymerization of FMMA. The resulting biosensor demonstrated an impressive limit of detection (LOD) as low as 79 aM. Similarly, another research effort utilized photoATRP for the detection of target DNA, which is key for disease screening [509]. The naturally derived, metal-free photocatalyst rose bengal (RB) was utilized, which becomes excited upon exposure to blue light, subsequently triggering FMMA polymerization. The achieved biosensor

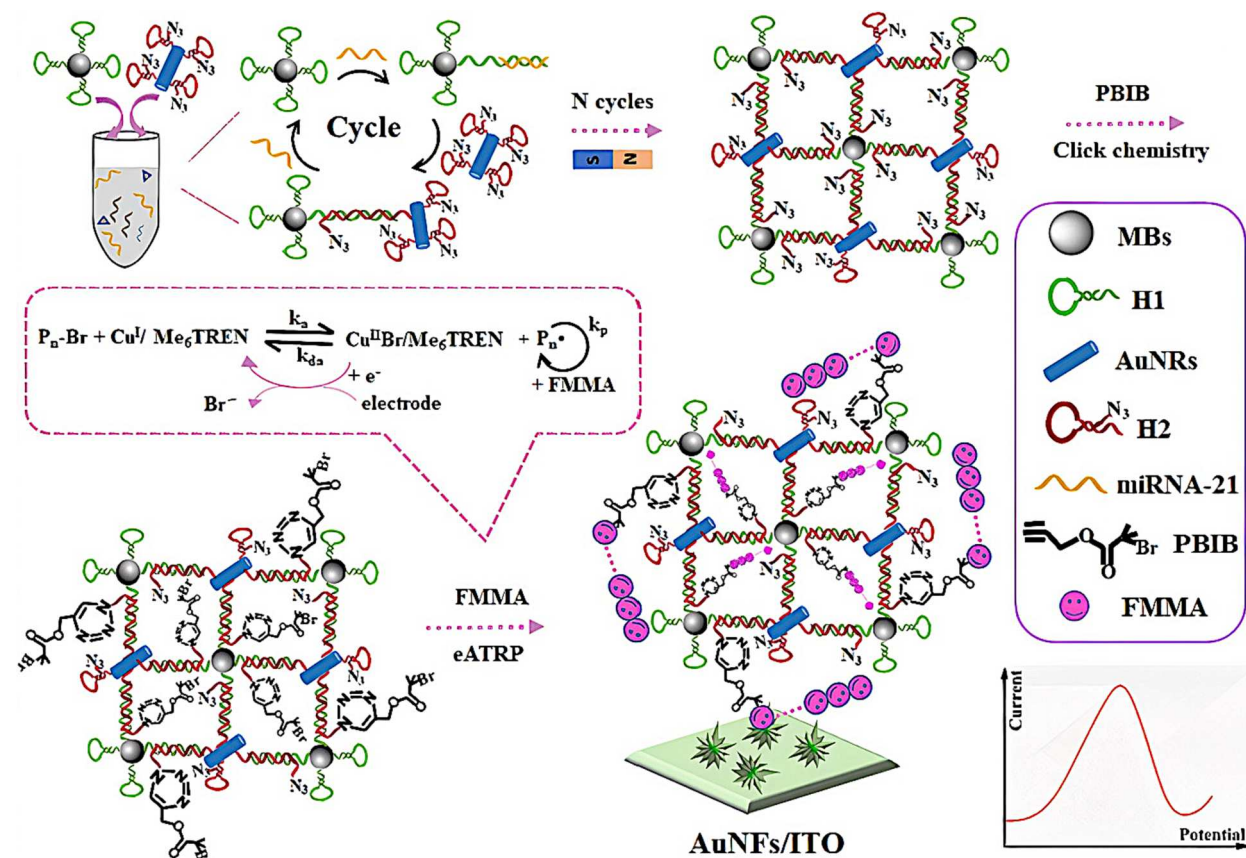


Fig. 33. Schematic displaying an ultrasensitive electrochemical biosensor with multiple amplification strategies. Magnetic nanobeads (MnB) were decorated with hairpin DNA and gold nanorods (AuNRs) with azido-functionalized hairpin DNA. DNA hybridization with miRNA-21 results in DNA hybridization for both nanomaterials, resulting in a 3D network with numerous azido groups. An alkylated ATRP initiator, PBIB, is then clicked with these azido groups, leaving BiB moieties appropriate for eATRP of FMMA which corresponds to a current-voltage curve in CV experiments. Reprinted with permission from [502]. Copyright 2021 American Chemical Society.

exhibited strong performance across a wide range of DNA concentrations ($1\text{--}10^5$ fM) and a remarkable LOD as low as 0.115 fM. Furthermore, an innovative signal amplification strategy was implemented in the creation of an ultrasensitive biosensor designed for the early detection of microRNA-21, a key biomarker associated with lung cancer [510]. In this unique approach, ATRP-mediated signal amplification harnessed ferritin, a non-toxic water-soluble protein, as the activator or catalyst, while a nitroxyl-based free radical acted as the monomer. The estimated LOD for this biosensor was as low as 6.03 fM.

6.6. Alternative applications

Organ-on-a-chip (OOC) devices are important in fundamental biological studies and the drug development process [511]. Most OOC devices are manufactured from bioinert materials such as glass and PDMS, which limits their application to real-world problems due to limited cell attachment. Therefore, coating the channels with hydrophilic polymer brushes, with or without immobilized bioactive molecules, can be considered a general strategy to address such challenges. Parts of the microchannels and/or cavities in OOCs can be modified by special types of polymer brushes to control cell attachment, growth, proliferation, and differentiation. Cell behavior on these surfaces is regulated by the polymers' chemistry, architectural features, gradients, and patterning. Additionally, ATRP can be exploited to create tissue-mimicking structures in OOC devices. For example, bottlebrush structures can be used to replicate body joint structures in OOC devices, and SS-ATRP from hydrogel membranes can be used to mimic the structure of cilia in OOC devices [343,512].

In addition, the behavior of cells can be influenced by polymers that respond to external stimuli. For instance, surfaces modified with stimuli-responsive polymer brushes can serve as nanoactuators. These actuation mechanisms may be triggered by internal chemical stimuli or external factors like heat or light irradiation. Furthermore, actuation or oscillation can arise from oscillating chemical reactions, such as the Belousov–Zhabotinsky (BZ) reaction [513]. (Co)polymer brushes containing components that catalyze a BZ reaction (e.g., ruthenium complexes) can undergo self-oscillation in response to chemical waves, as depicted in Fig. 34 [514]. Notably, SI-ATRP-mediated grafting of polymer brushes can facilitate chemical wave propagation, analogous to waveguides that guide electromagnetic or sound waves. Chemical wave propagation proves advantageous for scenarios requiring chemical signal detection or transmission, such as biosensors. Within living systems, chemical signals play pivotal roles in processes such as wound healing, immunological responses, and cellular signaling (e.g., paracrine and endocrine signaling) [515]. Consequently, mimicking the propagation of chemical signals in artificial systems, such as OOC devices, represents a promising avenue of research for enhancing human-on-a-chip (HoC) devices.

Growing well-defined polymers at biointerfaces, such as in the vicinity of cells, may enable the construction of the next generation of artificial ECM-mimicking materials. In these materials, cells or microorganisms could select monomers from a solution of different monomers [273]. This cell- or organism-instructed synthesis of polymers can be beneficial in several fields, for example, the isolation and detection of bacteria [516].

In addition, conducting ATRP under biologically relevant conditions (e.g., aqueous solutions, low to moderate temperatures, and non-toxic reagents) is important for biomedical applications [143]. For instance, when creating PPCs, where proteins may serve as macroinitiators for SI-ATRP, it is crucial to perform ATRP in aqueous solutions to prevent alterations in the secondary and tertiary structures of proteins. To achieve these goals in these applications, prerequisites include designing water-dispersible catalysts that allow polymerizations at desirable kinetics (i.e., using appropriate ligand design), utilizing very low concentrations of transition metal catalysts, or employing metal-free strategies. Furthermore, performing ATRP within living organisms or conducting *in vivo* polymerizations can significantly advance our understanding of biological fundamentals as well as the technological evolution of medicine. While biological polymerization is pervasive in cellular and bacterial metabolism, artificial polymerization has rarely been carried out outside of MGE strategies that utilize the synthetic machinery of cells [517]. However, the development of mRNA-based vaccines and their remarkable success in providing immunity against COVID-19 have ignited hopes for *in vivo* artificial protein synthesis—an approach involving precision polymerization for delivering genetic codes and utilizing cellular synthetic machinery [518]. Moreover, achieving *in vivo* polymerization and even intracellular (i.e., *in cellulo*) polymerization is feasible using biocompatible polymerization strategies [519].

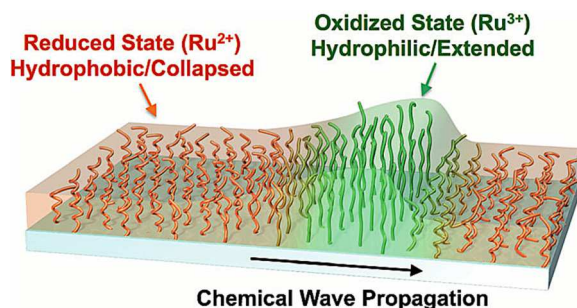


Fig. 34. Schematic depicting a chemical wave propagation along a substrate modified with grafted polymer brushes. These polymer brushes exhibit two oxidation states: in their reduced state, they are hydrophobic and contract as they lose water; in the oxidized state, they absorb water and extend. This cyclic contraction and extension behavior of the polymer brushes displays a resemblance to wave propagation [514].

The first instance of *in cellulo* polymerization was an UV-initiated FRP reported by Bradley *et al.* in 2019 [520]. Subsequently, it was discovered that polymerization within cells, tissues, and even animals can be triggered by endogenous stimuli, such as genetically modified enzymes. In a recent study, conjugated oligomers were polymerized in an animal model for bioelectronic applications [521]. This emerging field of intracellular polymerization holds the potential to modulate cellular behaviors, including cell growth or apoptosis. However, certain challenges, such as the delivery of reagents into cells, potential chemical interference of polymerization with other biological processes, the need for suitable triggers to initiate polymerization, and addressing the toxicity and removal of side products, must be tackled before these concepts can be translated into practical applications [517]. In such polymerizations, reactive intracellular species, redox species in the cytosol (e.g., glutathione), or cellular processes can be harnessed to initiate or mediate polymerization. Furthermore, the principle of orthogonal reactions can be employed to prevent chemical interference [522]. Additionally, established intracellular delivery systems (e.g., nanogels) can be utilized for the intracellular delivery of monomers. Chemical reactions that can be conducted under biological conditions (e.g., aqueous medium and 37 °C) with high yield (close to 100 %) and high specificity can address the challenges related to the toxicity/removal of residual reagents and side products. These criteria align well with the principles of click chemistry. By combining the concepts of click chemistry and orthogonality with CRP, exciting opportunities for new scientific and technological innovations emerge in both materials science and medicine [523]. In essence, the development of alternative 'controlled click polymerization' strategies could mark a revolutionary step towards creating future biomolecular materials *in vivo*, without relying on the synthetic machinery of cells [524]. We believe that exploring the utilization of sugar-based (co)monomers in ATRP strategies is a promising research avenue in the realm of *in vivo* CRP [123].

7. Conclusion and outlook

With over 25 years of continued research and development, ATRP is now a relatively mature and well-understood strategy to engineer advanced materials. However, moving forward, it could further be improved in terms of the reagents used, reaction conditions, and targeted applications. We believe that the potential of ATRP to design mission-oriented materials, especially in the biomedical arena, has not been fully exploited yet. In addition, leveraging its potential in materials design can further revolutionize the adoption of biomaterials in medicine. While we provided a comprehensive review on ATRP and its applications in the biomedical field, the current status and requirements for further development are now discussed. This section also offers a roadmap for future research while highlighting potential challenges and discussing how this technology could further help in the design of complex chemical and biological systems while pushing the boundaries of innovation.

7.1. The current status of ATRP technology

Based on extensive investigations, a relatively deep understanding of the ATRP mechanism is now available. Additionally, a wide variety of ATRP reagents are commercially accessible. ATRP has demonstrated versatility across various monomers, with numerous catalysts readily accessible to enhance the chemical activity and selectivity of the polymerization process. The evolution of ATRP has led to the development of new methodologies that require minimal catalyst concentrations or even function without a catalyst. Moreover, the incorporation of alternative transition metals, such as Fe, in ATRP catalytic systems allows for polymerization in environmentally friendly solvents, particularly aqueous media. This not only enhances the biocompatibility of these ATRP variants but is also achievable with current technology. Through innovative ATRP techniques that harness external stimuli, such as photoATRP and mechanoATRP, precise control over reaction conditions can be exerted with remarkable spatiotemporal resolution.

SI-ATRP has proven to be an exceptionally robust strategy for modifying material surfaces with varying chemistries across different length scales. This approach has enabled researchers to significantly modify material interface properties and interactions with biological systems, given its capacity for synthesizing high-density grafting of polymer brushes. Moreover, SI-ATRP offers a robust pathway for creating multifunctional inorganic–organic nanohybrids, versatile for a range of applications within the biomedical field. Furthermore, ATRP stands as one of the most versatile and effective strategies for producing advanced materials, including architecturally designed polymers and versatile bioconjugates with customized features and functions. These materials exhibit excellent mechanical behaviors and bioactivities, surpassing those attainable by conventional (bio)materials like synthetic polymers or natural proteins. ATRP-derived advanced materials can be customized to replicate unique mechanical or rheological characteristics found in native biomacromolecular structures, such as the stress–strain behaviors of soft tissues and the superlubricity of bodily joints. These sophisticated materials and constructs not only find utility in designing tissue engineering structures and DDS but also serve as invaluable tools for fundamental biological research and drug screening processes. By accurately emulating various aspects of biomacromolecular constructs within the human body, they provide a reliable *in vitro* representation. Furthermore, the incorporation of highly functional bioconjugates holds the potential to further push biomaterials toward practical real-world applications and clinical translation.

7.2. Current challenges for ATRP-derived materials for biomedical applications

ATRP has previously demonstrated its effectiveness as a robust approach for synthesizing well-defined polymers with precise structural features. However, to further expand the versatility of advanced polymers and hybrid materials prepared through ATRP methods, it is essential to broaden the range of monomers and application conditions. Special attention should be given to designing new catalytic systems that offer improved selectivity, activity, oxygen tolerance, and biocompatibility. Further development of catalysts or catalytic systems based on Fe and engineered enzymes, as well as catalyst-free and externally controlled systems, is necessary

to fabricate advanced functional polymers suitable for biomedical uses. These systems could potentially eliminate the need for complex and lengthy purification steps. Moreover, when designing biomaterials, it is advisable to employ environmentally friendly and non-toxic solvents, particularly water and ionic liquids. However, the challenge of developing ATRP strategies that operate under mild reaction conditions (such as aqueous environments and physiological temperatures) with appropriate kinetics, high yield, oxygen tolerance, and absence of side products still persists. This area of research requires further exploration by both researchers and industrial partners to design advanced polymers suitable for various biomedical applications.

While there are many advanced polymeric structures, such as dendrimers and bottlebrush polymers, that can be synthesized using ATRP, further research is needed to develop more architecturally complex polymers with specific goals. Such architectural complexity often leads to unique properties that could be beneficial for the biomedical field. Indeed, while the L-BB-L architecture has demonstrated the ability to replicate the stress-strain behavior of native biological tissues, there remains a vast realm for polymer chemists and materials scientists to explore how the manipulation of the architectural features of polymers can influence their physiochemical properties, biodegradability, and interactions with biological systems.

ATRP stands as a robust technique in modern organic chemistry, yet other complementary methods like RAFT, ROP, and click chemistry could greatly expand the potential for designing complex, multi-level macromolecular structures. ATRP methods are valuable for engineering advanced materials with flexible architectural designs and chemical compositions. The interactions and assembly behavior within these complex macromolecular structures should be thoroughly examined, as they possess the capacity to self-organize into elaborated hierarchical patterns. Such materials can exhibit dynamic (dissipative) self-assembly in response to environmental changes, such as shifts in temperature or pH *in vivo*. This dynamic behavior adds an extra layer of complexity to ATRP-derived materials, and its usefulness in dynamic physiological contexts should not be underestimated. This complexity can be harnessed to enhance our ability in designing DyDS or smarter tissue engineering constructs using adaptable ATRP-derived materials. However, this meticulous polymer design via ATRP must be coupled with advanced modeling, simulation techniques, multiscale computer simulations, and notably, artificial intelligence (AI) methodologies. For instance, the AlphaFold AI software, known for predicting protein structures using deep learning strategies, could be leveraged by researchers. Designing similar AI-based algorithms might be the most promising approach to address the challenges linked to the structure-properties relationship of these complex materials. Yet, given that synthetic polymers possess more chemical versatility than natural biopolymers, predicting the self-organization and hierarchy of synthetic polymers is more complicated and may incur higher computational costs.

Rationally blending two or more ATRP-derived macromolecular structures can lead to the formation of unique systems, further increasing the complexity of self-organized objects. These complex chemical systems, characterized by dynamic inter- and intra-molecular interactions, are poised to advance the technology landscape by enabling the design of adaptive materials. These materials could find applications ranging from smart delivery and theranostic platforms to adaptive tissue engineering. ATRP-derived complex molecular structures, characterized by their dynamic interactions, serve as foundational components for a diverse array of dynamic systems, showcasing inherent motility. Ultimately, these structures may even lay the groundwork for the development of novel forms of synthetic life. However, the strategic design of such complex chemical systems relies not only on CRP strategies for designing precisely defined advanced materials but also on AI-based methodologies enabling the prediction of their structures under both equilibrium and non-equilibrium conditions. These AI-driven strategies, rooted in the study of static and dynamic self-assembly, hold great potential to advance the fabrication of objects such as synthetic nanobots with programmable behaviors.

The structures—and, consequently, the properties—of ideal advanced polymers synthesized using ATRP for biomedical applications are not confined to customized static characteristics. In fact, the ideal design of sophisticated polymers for emerging biomedical applications requires the integration of functionalities and/or stimuli-responsive segments or connections that regulate the persistent behavior of polymers within environmental or biological systems. A future objective for CRP strategies, particularly ATRP, is the fabrication of advanced polymers with programmable attributes for encoding and decoding biological functions, such as time-dependent biodegradation. Programmable biodegradation of tissue implants or drug-eluting stents would eliminate the need for invasive methods such as surgery to remove their debris.

While biomacromolecules exhibit high functionality *in vivo*, conjugating them with well-defined synthetic polymers (i.e., BPCs) can further enhance their function and stability under physiological conditions. However, there has been limited research on the synthesis and characterization of BPCs beyond protein-polymer bioconjugates. An unexplored research avenue involves investigating conjugates of nucleic acids, OSA/PSA, and lipids with synthetic polymers. These functional bioconjugates hold the potential to advance the field by designing advanced biomaterials that imitate natural polymers yet possess multiple functionalities and increased stability. ATRP stands as a key tool for fabricating bioconjugates with accurately defined molecular attributes under gentle reaction conditions and suitable tolerance to oxygen and functional groups.

On the other hand, the development of ATRP strategies with minimal chemical interference in complex chemical and biological systems will bring us a step closer to achieving *in vivo* controlled polymerization. Future endeavors should be directed toward the advancement of such ATRP strategies. Furthermore, *in cellulo* ATRP represents a distinct research niche for polymer chemists and materials scientists aiming to develop new biomaterials. Polymers derived *in cellulo* can be conjugated to proteins or OSA/PSA, which are synthesized using cellular synthetic machinery. ATRP-based chemical signal amplification strategies have enhanced the sensitivity of electrochemical biosensors, and they are likely to remain a popular technique for engineering highly sensitive biosensors. These biosensors could play a crucial role in the early-stage detection of biomarkers for diagnosing various health conditions, including infectious diseases. However, further investigation in this field is necessary before these biosensors can be translated into real-world applications.

The tremendous potential of ATRP in designing well-defined polymers, bioconjugates, hybrid nanomaterials, and structures with modified surfaces indicates that there is still a need for extensive research. This suggests that high-throughput methodologies may play

an important role in accelerating the synthesis and evaluation of materials derived from ATRP. Progress in creating new ATRP techniques, like oxygen-tolerant ATRP, has significantly helped in the rapid synthesis and analysis of polymers. However, further research is required in this area to enable high-throughput production of ATRP-derived materials for use in biomedical applications. Finally, it is crucial to push ATRP-derived polymeric and advanced hybrid materials into applications within industries and clinical settings. Achieving clinical applicability will demand further research efforts to expand and make use of ATRP strategies that can create advanced materials with enhanced safety and performance.

CRedit authorship contribution statement

Mohsen Khodadadi Yazdi: Conceptualization, Writing – original draft. **Payam Zarrintaj:** Investigation. **Mohammad Reza Saeb:** Conceptualization, Supervision. **Masoud Mozafari:** Conceptualization, Methodology. **Sidi A. Bencherif:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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