Progress in Polymer Science 133 (2022) 101590

Contents lists available at ScienceDirect

Progress in Polymer Science

journal homepage: www.elsevier.com/locate/progpolymsci

Clickable polysaccharides for biomedical applications: A comprehensive review

Mohsen Khodadadi Yazdi[a], S. Mohammad Sajadi[b], Farzad Seidi[a], Navid Rabiee[c], Yousef Fatahi[d,e], Mohammad Rabiee[f], C.D. Midhun Dominic[g], Payam Zarrintaj[h], Krzysztof Formela[i], Mohammad Reza Saeb[i,*], Sidi A. Bencherif[j,k,l,m,**]

[a] Jiangsu Co–Innovation Center for Efficient Processing and Utilization of Forest Resources and International Innovation Center for Forest Chemicals and Materials, Nanjing Forestry University, Nanjing 210037, China
[b] Department of Nutrition, Cihan University-Erbil, Erbil, Kurdistan 625, Iraq
[c] School of Engineering, Macquarie University, Sydney, New South Wales 2109, Australia
[d] Department of Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[e] Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[f] Biomaterial group, Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
[g] Department of Chemistry, Sacred Heart College (Autonomous), Kochi, Kerala 682013, India
[h] School of Chemical Engineering, Oklahoma State University, 420 Engineering North, Stillwater, OK 74078, United States
[i] Department of Polymer Technology, Faculty of Chemistry, Gdańsk University of Technology, Narutowicza 11/12, Gdańsk 80-233, Poland
[j] Department of Chemical Engineering, Northeastern University, Boston, MA, United States
[k] Department of Bioengineering, Northeastern University, Boston, MA, United States
[l] Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, United States
[m] Sorbonne University, UTC CNRS UMR 7338, Biomechanics and Bioengineering (BMBI), University of Technology of Compiègne, Compiègne, France

ARTICLE INFO

Article history:
Received 3 April 2022
Revised 31 July 2022
Accepted 8 August 2022
Available online 9 August 2022

Keywords:
Polysaccharides
Click chemistry
Bioorthogonal reactions
Biomaterials
Drug delivery
Tissue engineering
Wound healing

ABSTRACT

Recent advances in materials science and engineering highlight the importance of designing sophisticated biomaterials with well-defined architectures and tunable properties for emerging biomedical applications. Click chemistry, a powerful method allowing specific and controllable bioorthogonal reactions, has revolutionized our ability to make complex molecular structures with a high level of specificity, selectivity, and yield under mild conditions. These features combined with minimal byproduct formation have enabled the design of a wide range of macromolecular architectures from quick and versatile click reactions. Furthermore, copper-free click chemistry has resulted in a change of paradigm, allowing researchers to perform highly selective chemical reactions in biological environments to further understand the structure and function of cells. In living systems, introducing clickable groups into biomolecules such as polysaccharides (PSA) has been explored as a general approach to conduct medicinal chemistry and potentially help solve healthcare needs. De novo biosynthetic pathways for chemical synthesis have also been exploited and optimized to perform PSA-based bioconjugation inside living cells without interfering with their native processes or functions. This strategy obviates the need for laborious and costly chemical reactions which normally require extensive and time-consuming purification steps. Using these approaches, various PSA-based macromolecules have been manufactured as building blocks for the design of novel biomaterials. Clickable PSA provide a powerful and versatile toolbox for biomaterials scientists and will increasingly play a crucial role in the biomedical field. Specifically, bioclick reactions with PSA have been leveraged for the design of advanced drug delivery systems and minimally invasive injectable hydrogels. In this review article, we have outlined the key aspects and breadth of PSA-derived bioclick reactions as a powerful and versatile toolbox to design advanced polymeric biomaterials for biomedical applications such as molecular imaging, drug delivery, and tissue engineering. Additionally, we have also discussed the past achievements, present developments, and recent trends of clickable PSA-based biomaterials such as three dimensional printing, as well as their challenges, clinical translatability, and future perspectives.

* Corresponding author.
** Corresponding author at: Department of Chemical Engineering, Northeastern University, Boston, MA, United States.
E-mail addresses: mrsaeb2008@gmail.com (M.R. Saeb), s.bencherif@northeastern.edu (S.A. Bencherif).

https://doi.org/10.1016/j.progpolymsci.2022.101590

1. Introduction

Some thermodynamically favorable processes, such as the transformation of diamond to graphite, occur so slowly that they can be considered impossible. While many processes with a broad range of reaction rates are accomplished in nature, many biological processes must occur at higher rates, requiring the utilization of enzymes [1]. Chemical interference is highly prohibited in complex inter- and intracellular environments, while many industrial-scale processes lack this orthogonality. In fact, many natural processes, especially those which are carried out in living systems, benefit from bioorthogonality, i.e., fast reaction rates, high selectivity, mild reaction conditions, non-toxic solvents (e.g., water), and high yield, eliminating the need for complex separation processes [2,3]. Achieving these unique features can only be possible through the optimization of several reaction parameters [4–6]. Chemical reactions in living systems have inspired many chemists and materials scientists to develop more efficient and environmentally friendly chemistries to create new molecules. However, no revolutionary advances were made in the field until 2001/2002 when the Nobel laureate chemist Barry Sharpless introduced the concept of click chemistry [7]. Sharpless and co-workers discovered that when copper is used as a catalyst, the Huisgen 1,3-dipolar cycloaddition could be carried out much faster with high regioselectivity. Click chemistry mimics nature and was designed to generate substances quickly and reliably by joining small modular units. This innovation has excited the scientific community as a whole and has prompted chemists to look for new click reactions [8].

Many fields of science, especially polymer chemistry and chemical biology, have been profoundly influenced by click chemistry [9–11]. Click reactions enable the creation of well-defined polymer architectures with tunable physicochemical and/or biological properties. Furthermore, click chemistry has revolutionized our understanding of cell biology by allowing researchers to perform chemistry inside living systems. For example, ultra-high-resolution images of cells and tracking of biomolecules are now possible with the aid of the click chemistry toolbox [12]. Polysaccharides (PSA) and their conjugates (glycoconjugates) are ubiquitous biomacromolecules that play critical roles in living organisms [13]. For example, hyaluronic acid (HA) represents an important class of PSA

Abbreviations: 2-APBA, 2-acetylphenyl boronic acid; 2-FPBA, 2-formylphenyl boronic acid; 3D, three dimensional; 4-arm PEG-N_3, azide-functionalized four-armed polyethylene glycol; 4-arm PEG-TCO, trans-cyclooctene-functionalized four-armed polyethylene glycol; β-HHz, β-hydroxy hydrazides; β-CD, β-cyclodextrin; AAC, azide-alkyne cycloaddition; Ac_4ManNAz, tetraacetylated N-azidoacetyl-D-mannosamine; Ac-β-CD, acetalated β-cyclodextrin; ADIBO, azadibenzocyclooctyne; ADIBO-Chol, ADIBO modified cholesterol; ADIBO-DSPE, ADIBO modified distearyl phosphatidyl ethanolamine; ADIBO-PEG_4-NOTA-^{64}Cu, azadibenzocyclooctyne (ADIBO) and 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA) dually functionalized 4-unit polyethylene glycol labeled with ^{64}Cu radioisotope; ADSC, adipose-derived mesenchymal stem cells; Ag, silver; AGA, automated glycan assembly; AHA, oxidized hyaluronic acid; AI, artificial intelligence; AIBN, azobisisobutyronitrile; Alg, alginate; Alg-Nor, norbornene-functionalized alginate; Alg-Tz, tetrazine-functionalized alginate; Alkylated-PEG, alkylated polyethylene glycol; Alkyne-β-CD, alkyne-functionalized β-cyclodextrin; Aminooxy-PEG-aminooxy, aminooxy-terminated polyethylene glycol (i.e., aminooxy group at two ends); AMP, antimicrobial peptides; AMR, antimicrobial resistance; ANR, double-layer-coated gold nanorods; AO-4-arm PEG, four-armed aminooxy-polyethylene glycol; Au, gold; Azide-Dex-PA, azidedextran polyampholyte; Azide-DOX, 5-azidopentanehydrazide-functionalized doxorubicine; BCN, bicyclo[6.1.0]non-4-yne; BDNF, brain derived neurotrophic factor; BMP2, bone morphogenetic protein-2; BMP4, bone morphogenetic protein-4; BMSC, bone marrow mesenchymal stem cells; BP, bisphosphonate; BSA, bovine serum albumin; CAPAC, click activated protodrugs against cancer; CBT, cyanobenzothiazole; Ce6, chlorin e6; c-FLIP, cellular FLICE-like inhibitory protein; CHO, Chinese hamster ovary; Chol, cholesterol; CMC, carboxymethyl chitosan; CMT, controlled morphology transformation; CNS, central nervous system; CnS, chondroitin sulfate; CnS-furan, furan grafted chondroitin sulfate; CnS-HS, thiolated chondroitin sulfate; Col 1, collagen type I; COS, chitooligosaccharides; CRP, controlled radical polymerization; CS, chitosan; CS-HS, thiolated chitosan; CS-N_3, azide-functionalized chitosan; Cu(II) sulfate, copper(II) sulfate; CuBr, copper(I) bromide; CuAAC, copper(I)-catalyzed azide-alkyne cycloaddition; Cx43, connexin 43; Cy3-PNA, cyanine3 fluorescent dye-labeled antiPNA21; Cy5, cyanine5 dye; Cys, cysteine; D-Cy5, cyanine5-labeled dendrimers; DA, Diels–Alder; DBCO, dibenzylcyclooctyne; DCC, dynamic click chemistry; D-Cys, dendrimer-cysteine conjugate; D-Dexa, dendrimer-dexamethasone conjugate; DDS, drug delivery systems; D-Ene, dendrimer-pentenoic acid; Dex, dextran; Dex-ADIBO, azadibenzocyclooctyne-modified dextran; Dex-DBCO, dibenzylcyclooctyne-modified dextran; Dex-N_3, azide-functionalized dextran; DHHC, dihydroxyphenyl/hydrazide bifunctionalized hydroxyethyl chitosan; Dhvar-5, a synthetic antimicrobial peptide; DIFO, difluorinated cyclooctyne; DLQ, DBCO-modified low molecular weight heparin-quercetin conjugates; DMTMM, 4-(4,6-dimethoxy triazine)-4-methyl morpholine hydrochloride; DNA, deoxyribonucleic acid; DOX, doxorubicin; DR4/5, death receptors DR4 and DR5; DS, degree of substitution of ADIBO; DSPE, distearyl phosphatidyl ethanolamine; ECM, extracellular matrix; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EDG, electron-donating groups; EDTA, ethylenediaminetetraacetic acid; EGF, epidermal growth factor; EPC, endothelial progenitor cell; EPR, enhanced permeability and retention; ES, electrophilic substitution; EWG, electron-withdrawing groups; F127-maleimide, maleimide-functionalized Pluronic F127; FAK, focal adhesion kinase; FBS, fetal bovine serum; FDA, food and drug administration; G', storage modulus; GALA, a 30-residue fusogenic peptide (WEAALAEALAEALAEHLAEALAEALEALAA); GCS-NP, glycol chitosan nanoparticles; Gel-furan, furan-functionalized gelatin; GF, growth factors; Glycolipids, lipid-glycan conjugates; Glycoproteins, protein-glycan conjugates; GlycoRNA, ribonucleic acid-glycan conjugates; GNR, gold nanorod; GO, graphene oxide; GSH, glutathione; HAase, hyaluronidase; HA, hyaluronic acid; HA-acrylate, acrylated hyaluronic acid; HA-benzaldehyde, benzaldehyde-functionalized hyaluronic acid; HA-CBT, cyanobenzothiazole-modified hyaluronic acid; HA-CHO, aldehyde-functionalized hyaluronic acid; HA-Cys-MA, cystamine-methacrylate modified hyaluronic acid; HA-D-Cys, D-cysteine-functionalized hyaluronic acid; HA-furan, furan-functionalized hyaluronic acid; HA-furan-ADH, dually functionalized hyaluronic acid with furan and hydrazide; HA-furan-CHO, dually functionalized hyaluronic acid with furan and aldehyde; HA-*g*-AMA, 2-aminoethyl methacrylate grafted hyaluronic acid; HA-BP, bisphosphonate modified hyaluronic acid; HA-*g*-Cys-MA, cystamine-methacrylate grafted hyaluronic acid; HA-*g*-Lys-MTet, lysine-4-(4(dimethylamino)phenyl-tetrazole)-benzoic acid grafted hyaluronic acid; HA-*g*-Lys-Tz, lysine-tetrazole grafted hyaluronic acid; HA-GO, HA-conjugated graphene oxide; HA-*g*-OEG-DBCO, diarylcyclooctyne-modified oligo(ethylene glycol) grafted hyaluronic acid; HA-HS, thiolated hyaluronic acid; HA-HS-ADH, thiol and hydrazide-functionalized hyaluronic acid; HA-hydrazine, hydrazine-functionalized hyaluronic acid; HA-Lys-Tet, lysine-tetrazole modified hyaluronic acid; HA-MA, methacrylated hyaluronic acid; HA-maleimide, maleimide-functionalized hyaluronic acid; HA-Tz, tetrazine-functionalized hyaluronic acid; HECS, hydroxyethyl chitosan; HIF-1α, hypoxia-inducible factor-1α; HOMO, highest occupied molecular orbital; HP-PEG, hyperbranched polyethylene glycol; HTL-HCl, DL-Homocysteine thiolactone hydrochloride; iEDDA, inverse electron demand Diels–Alder; IEG, iterative exponential growth; IL-2, interleukin-2; IPN, interpenetrating polymer network; JR2EK-Az, azide-functionalized JR2EK peptide; L929, mouse fibroblast cells; LMWH, low molecular weight heparin; LUMO, lowest unoccupied molecular orbital; Lys, lysine; MAA, methacrylic acid; MAA-*g*-CS, methacrylic acid grafted chitosan; mAb, monoclonal antibody; MAL-PEG-MAL, dimaleimide poly(ethylene glycol); MAL-PPO-PEG-PPO-Mal, poly(propylene oxide)-*b*-poly(ethylene oxide)-*b*-poly(propylene oxide) bismaleimide; MDa, megadalton; MeOH, methanol; MES, 2-morpholinoethane sulfonic acid; MGE, metabolic glycoengineering; MITCH, mixing-induced two-component injectable hydrogels; MMP2, matrix metalloproteinase 2; mPEG, methoxy polyethylene glycol; mPEG-*b*-PPLG, methoxy polyethylene glycol-*b*-poly(γ-propargyl-L-glutamate); MSC, mesenchymal stem cell; MTD, maximum tolerated dose; Mw, molecular weight; N_2, nitrogen; $N_2H_4.H_2O$, hydrazine hydrate; N_3-HA, azide-modified hyaluronic acid; N_3-*s*-TRAIL, azide-modified TRAIL-bound MMP 2 sensitive peptide; N_3-HGP21, Cy3-labeled antisense miR-21 PNA probes loaded onto HA-GO; NAC, N-acetyl-L-cysteine; NaN_3, sodium azide; Nb, norbornene; Nb-Tz, norbornene-tetrazine; NEDDA, normal electron demand Diels–Alder; NHS, N-hydroxysuccinimide; NIR, near-infrared; NK, natural killer; NP, nanoparticles; O_2, oxygen; $^1O_2$, singlet oxygen; O-CnS, oxidized chondroitin sulfate; o-NB, ortho-nitrobenzyl; OSA, oligosaccharide; PBAE, poly(β-amino ester); PDT, photodynamic therapy; PEG, polyethylene glycol; PEGDA, poly(ethylene glycol) diacrylate; Photo-DIBO, cyclopropenone-masked dibenzocyclooctyne; PhTAD, N-phenyltriazolinedione; PLGA, poly(lactic-*co*-glycolic acid); PLL, poly-L-lysine; PLL-SH, thiol-functionalized poly-L-lysine; PLL-*g*-CS, poly-L-lysine grafted chitosan; PNBA, poly(o-nitrobenzyl acrylate); PNA, peptide nucleic acid; PNS, peripheral nervous system; PPLG, poly(γ-propargyl-L-glutamate); Proteoglycans, heavily glycosylated proteins; PSA, polysaccharides; PTK2, protein tyrosine kinase 2; QSI, quorum sensing inhibitor; Qu, quercetin; RAFT, reversible addition fragmentation chain-transfer polymerization; rDA, reverse Diels–Alder; RFP, riboflavin phosphate; Rfv, riboflavin; RGD, arginyl-glycyl-aspartic acid; RNA, ribonucleic acid; RNAi, ribonucleic acid interference; ROS, reactive oxygen species; Se, selenium; Semi-IPN, semi-interpenetrating polymer network; SHA, salicylhydroxamic acid; siHSP70, heat shock protein 70-targeting siRNA; SPAAC, strain-promoted azide-alkyne cycloaddition; SQ3370, TCO-modified DOX; SQL70, tetrazine-modified HA; SSD, silver sulfadiazine; SuFEx, sulfur(VI) fluoride exchange; TA, tetra-aniline; TAD, 1,2,4-triazoline-3,5-dione; TCO, trans-cyclooctene; TE, tissue engineering; TFA, trifluoroacetic acid; t_{gel}, gelation time; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Tri-Adam, adamantyl trimers; Tri-β-CD, β-cyclodextrin trimers; TsCl, 4-toluene sulfonyl chloride; TsC-No, thiosemicarbazide-functionalized nopoldiol; Tz, tetrazine; UV, ultraviolet; Van, vanillin; VEGF, vascular endothelial growth factor; ZnPc, zinc phthalocyanine.

as it constitutes a major component of the extracellular matrix (ECM) and has been extensively exploited for biomaterials design [14–22]. Conjugates of PSA or oligosaccharides (OSA) with proteins (glycoproteins and proteoglycans), lipids (glycolipids) and RNA (glycoRNA) constitute the glycome of cells. These glycoconjugates are made through dynamic glycosylation, the process by which sugar molecules are added to proteins, lipids, or RNA. Glycosylation is usually carried out using glycosyltransferases residing in the Golgi apparatus [23]. Glycosidases catalyze the opposite reaction—glycosidic linkage hydrolysis (bond cleavage) [24]. Glycoconjugates cover the cell surface or are secreted to the intercellular microenvironment. Moreover, in living organisms, intra- and intermolecular interactions are mediated by glycoconjugates. The critical roles played by glycoconjugates make glycome composition an important biological cue in defining overall health and disease conditions. Very recently, with the aid of click chemistry, glycoRNAs (RNA-glycan conjugates) were found on the outermost surfaces of cellular membranes of various cell types in mammals [25].

The synthesis of PSA, unlike protein translation and DNA replication, is not a template-driven process, facilitating the formation of a large variety of OSA/PSA molecules [26]. While only 17 monosaccharides are present in mammals, a vast number of PSA with various compositions (different monosaccharides), glycosidic linkages, and chain conformations can be created. For example, unlike proteins, PSA produced by various organisms may differ in molecular structure (e.g., chain length). Despite their biocompatibility, the utilization of PSA in the biomedical field has been limited due to their shortcomings in reproducibility and bioactivity. Accordingly, many chemical modification strategies have been developed for engineering synthetic PSA, or improving the functionalities, physicochemical properties, and biological activities of naturally-derived PSA [27]. However, most strategies require harsh reaction conditions or toxic reagents, whereas click chemistry enables the chemical modification, grafting and crosslinking of PSA to occur under mild reaction conditions, which can be carried out *in vivo*. Furthermore, bacteria or living cells can be utilized as bioreactors for the synthesis of clickable PSA [28,29]. Clickable PSA (i.e., PSA functionalized with clickable groups) make chemical reactions possible on the plasma membrane as well as in the intracellular space, ECM, and vessels carrying biological fluids (blood and lymph) [30].

Although several review articles have focused on the applications of click chemistry for PSA, they are usually limited to a few examples of PSA or click chemistry strategies and/or do not cover their biomedical applications [31–33]. For example, in a recent review article by Deng and co-workers, the application of click chemistry was centered only on alginate and its biomedical applications [34]. However, most of the other review articles do not focus on PSA; instead, they summarize the concept of click chemistry and its applications. For instance, Kaur and co-workers described click chemistry-assisted bioconjugation and probes for bioimaging, as well as the growing impact of click chemistry on drug discovery [35]. However, they did not focus on many other biomedical applications such as tissue engineering (TE), which are now discussed in this review article. Additionally, they did not introduce other clickable functional compounds such as clickable PSA. Another recent review article discussed the applications of metal-free click reactions in the field of cancer theranostics [36]. However, they did not focus on PSA or other biomedical applications beyond cancer. Furthermore, another review article published by Agrahari and co-workers thoroughly discussed the applications of copper(I)-catalyzed 1,3-dipolar cycloaddition (CuAAC) click chemistry in glycoscience [37]. While they highlighted bioorthogonal click reactions as a powerful tool for synthesizing various glycoconjugates for several applications (e.g., modern drug development and biosensing), alternative click reactions and the use of PSA were not included. Other review articles have focused on a few specific click reactions in the context of PSA and their specific application [38–40]. In light of available literature, this review article provides a comprehensive overview of key click reactions followed by an in-depth discussion of the potential, challenges, and opportunities of clicked PSA-based biomaterials for various biomedical applications.

2. Click chemistry toolbox

Huisgen 1,3-dipolar cycloaddition was reported in 1965, in which azides were utilized as dipolar reagents [41]. However, this chemistry did not gain much attention because the reaction required high temperature to proceed, and the resulting product is a mixture of regioisomers. Nearly 37 years later, a catalytic version of Huisgen 1,3-dipolar cycloaddition was introduced independently by Meldal and Sharpless [7,42]. The copper(I)-catalyzed version of Huisgen 1,3-dipolar cycloaddition gained ground due to its regioselectivity (i.e., it produces only the 1,4-regioisomer) and mild reaction conditions. In fact, the CuAAC reaction of azides and alkynes is associated with fast reaction rate even at room temperature. The features of the CuAAC reaction, accompanied by naturally occurring chemical reactions, inspired Sharpless to introduce the concept of click chemistry. Click reactions are a class of high yield chemical reactions that can be performed under mild conditions. Moreover, the scope of click reactions is wide and they possess a modular nature [43]. A click reaction requires a high thermodynamic force ($>$ 20 kcal/mol) to enable high selectivity toward a single product with high rate [43]. While reversible carbonyl bonds are ubiquitous in nature (amide heteroatom linkages in proteins), click chemistry usually results in irreversible formation of carbon-heteroatom linkages [44].

Despite the merits of CuAAC, Cu(I) ions can be cytotoxic to mammalian cells even at low concentrations (i.e., $<$ 500 μM). Accordingly, many researchers looked for catalyst-free versions of CuAAC, and making reagents less stable was recognized as an efficient strategy to address this challenge. It was found that the stability of cycloalkynes (the cyclic analog of an alkyne) is greatly reduced when fewer than 10 carbon atoms are in the ring, an effect stemming from the geometric constraints of triple bonds [45]. Strain energy reduces nearly exponentially as the number of carbons increases from 3 to 10 in angle-strained cycloalkynes [46]. Cyclooctyne, cyclononyne, and alkynes of higher carbon atoms are stable and can be prepared and isolated with relative ease, contrary to cycloalkynes with fewer carbon atoms. Cyclooctyne is the smallest isolable cycloalkyne that reacts with azides in a copper-free environment at room temperature. This strain-promoted azide-alkyne cycloaddition (SPAAC) was introduced by Bertozzi and co-workers in 2004 [47]. While copper catalyst decreases reaction enthalpy from 24 to 11 kcal/mol, the SPAAC lowers it to only 18 kcal/mol, indicating a much lower reaction rate of SPAAC compared to CuAAC [9]. However, introducing electron-withdrawing groups (EWG) on the cyclooctyne ring and electron-donating groups (EDG) on the azides increases click reaction kinetics by affecting the frontier orbitals in reacting molecules, i.e., the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). It was found that exocyclic substitution of EWG, such as difluorinated cyclooctyne (DIFO), greatly enhances the reactivity of cyclooctyne derivatives, making it feasible for glycobiology and *in vivo* bioimaging [48,49]. In addition, it was discovered that endocyclic heteroatom substitution interrupts the intrinsic hydrophobicity of the cyclooctyne moiety, improving its biological applications. Hydrophilicity can be further enhanced by installing exocyclic hydrophilic groups such as methoxy [50]. However, increasing the hydrophilicity results in decreased reaction rate, exhibiting a trade-off between kinetics and hydrophilic-

ity [51,52]. Because of the biocompatibility of the SPAAC approach, a wide spectrum of cyclooctyne derivatives have been developed during the last two decades to enhance the rate of click reactions. However, laborious synthesis of reagents is the major drawback of the SPAAC approach, which has driven researchers to search for alternative click reactions.

Thiol X click reactions are broadly classified into radical-mediated (thiol-ene and thiol-yne) and base/nucleophile-mediated (e.g., thiol-Michael addition, thiol-epoxy, thiol-isocyanate, thiol-halogen) thiol-X click reactions [53]. Thiol-X reactions have a long history in organic synthesis. Thiol-X click reactions are highly efficient, green, and selective, yielding a product under mild conditions. Radical-mediated are the most utilized subclass of thiol-X click reactions and require ultraviolet (UV) exposure to proceed [54]. The modular aspect of thiol-Michael addition makes this click reaction highly robust in materials synthesis, from small molecules to complex polymeric systems [55].

Diels–Alder (DA) reaction is a [4+2] cycloaddition with high selectivity that couples a diene and a dienophile (usually an alkene), resulting in a cyclohexene adduct with high stability [56]. The wide scope of the DA reaction, which includes hetero DA reactions, enables heterocyclic six-membered rings (usually containing nitrogen or oxygen (O_2) heteroatoms) and even heteroatom–heteroatom bonds [57]. The reversibility of DA reactions at relatively high temperatures (50–150°C) enables the design of polymeric materials that can be self-healed by temperature enhancement [58]. Furan and maleimide derivatives are the most popular substituted alkenes and dienes for DA reactions. These moieties react via DA click reaction to make thermoreversible adducts. The reverse reaction (rDA) breaks the adduct down to its reagents, and this phenomenon enables the creation of self-healing polymers [59].

Dynamic click chemistry (DCC) based on the DA reaction is very important to designing innovative materials [60,61]. It broadens the scope of reversible (but weak) physical bonds in making smart materials possessing appropriate mechanical properties. Similar to the DA reaction, where a click reaction occurs between furan and maleimide, an electrophilic substitution (ES) click reaction includes the reaction between furfuryl and 1,2,4-triazoline-3,5-dione (TAD) derivatives such as N-phenyltriazolinedione (PhTAD), which serve as reactive dienophiles [62,63]. The ES click chemistry has been utilized to make self-healing polymers [64]. Polymer science has benefited from ultrafast click chemistry based on TAD derivatives [65].

Oxime ligation denotes the condensation reaction between carbonyl (aldehyde or ketone) groups and nucleophiles, which proceeds in mild acidic aqueous solutions [43]. In fact, carbonyl condensation (e.g., aldol reaction) produces hydrazone, imine, and oxime bonds. The hydrolytic stabilities of the obtained hydrazone and oxime are superior compared to imines, making them suitable for physiological conditions [66]. Oxime ligation is a chemoselective reaction which has been widely utilized to synthesize novel polymer structures and multifunctional biomacromolecule constructs [67,68]. However, synthesis of molecules containing aldehyde or aminooxy functionalities is a relatively difficult and laborious process.

A breakthrough in click chemistry and bioorthogonal reactions was made by introduction of the inverse electron demand DA reaction (iEDDA) [69]. iEDDA is the reaction between tetrazines and strained dienophiles. In the normal electron demand DA reaction (NEDDA), EWG and EDG are introduced into the dienophile and the diene, respectively (opposite to iEDDA). The reaction between the electron-deficient diene (having an EWG group) with reduced LUMO, and the electron-rich dienophile with enhanced HOMO, results in an accelerated rate of reaction [70]. Exceptional reaction rate, unparalleled orthogonality, and high biocompatibility have made iEDDA a unique click reaction for chemical biology [70].

Sulfur (VI) fluoride exchange (SuFEx) is another click reaction that was introduced in 2014 [71]. This click chemistry transformation is based on the capability of silicon centers to exchange S–F bonds for S–O bonds [72]. SuFEx reactions can be utilized to make synthetic polymers [73,74].

In 2017, a spontaneous amino-yne click reaction was introduced which not only offers the benefits of standard bioorthogonal reactions but also possesses additional advantages such as the ubiquity of amines, reaction spontaneity, and stimuli-responsive cleavability of the resulting products [75,76]. Activated alkynes (e.g., ester or sulfone activated ethynyl), having electron-withdrawing groups, can undergo spontaneous reactions with amines at room temperature. This new type of click chemistry is an excellent tool not only for bioconjugation but also for click polymerizations [75,77]. Conventional strategies for click polymerization allow bifunctionalized clickable monomers such as activated diynes or diamines to react effectively with high reaction rates at relatively low temperatures [78]. However, amino-yne click polymerization offers additional merits such as the generation of regio- and stereoregular polymers containing stimuli-responsive linkages, a feature that allows introduction of dynamic structures such as degradable linkages within a polymer network [79].

Light-triggered click reactions, which combine the advantages of click chemistry with photochemical processes, have recently gained much interest [39]. Diffusion of Cu(I) ions and heat transfer implications can impose limitations on conventional click reactions (e.g., CuAAC and SPAAC) such as heterogeneous reaction rate, which results in a nonuniform material. In fact, the gelation process may initiate in the boundaries before the central regions. Gelled boundaries have lower mass and heat transfer coefficients indicating complications in Cu(I) inflow and heat flow in or out of central regions. Uniform light irradiation throughout the material allows excellent spatiotemporal control over light-triggered reactions, resulting in a homogeneous click reaction and a construct of uniform texture [80,81].

On the other hand, combining the concept of click chemistry with controlled radical polymerization (CRP) provides a vast playground for designing and manufacturing novel multifunctional materials with well-defined architectural complexity and unparalleled functional specificity [82,83]. The initiators, monomers, crosslinkers, and postmodifiers can be clickable [84]. On the other hand, DCC, which enables reversible click reactions under mild conditions, is an invaluable tool in materials science. Iminoboronate and salicylhydroxamic–boronate are among the most important reversible click chemistries [85].

The idea of non-covalent click chemistry was introduced recently to highlight the importance of structural selectivity in the association of two molecules [86]. In contrast to the selective conjugation of two clickable molecules in covalent click chemistry, the selective association of two molecules makes non-covalent click chemistry distinct from colloidal self-assembly, which is triggered by non-specific physical interactions such as electrostatic and hydrophobic interactions [87]. There are several specific macromolecular interactions in nature, such as biotin–avidin and receptor–ligand interactions, that fulfill non-covalent click chemistry requirements [88,89]. The specific hydrogen bonding between two complementary nucleobases in DNA structure, which allows high density data storage, is another type of non-covalent specific interaction [90]. Non-covalent click chemistry will enhance our ability to design more robust sensing nano-platforms for molecular recognition, well-structured nano-assemblies, and unprecedented sense-and-treat nanodevices [91,92].

Moreover, multiple click functional groups that can proceed orthogonally closely mimic natural processes. A sequence of click re-

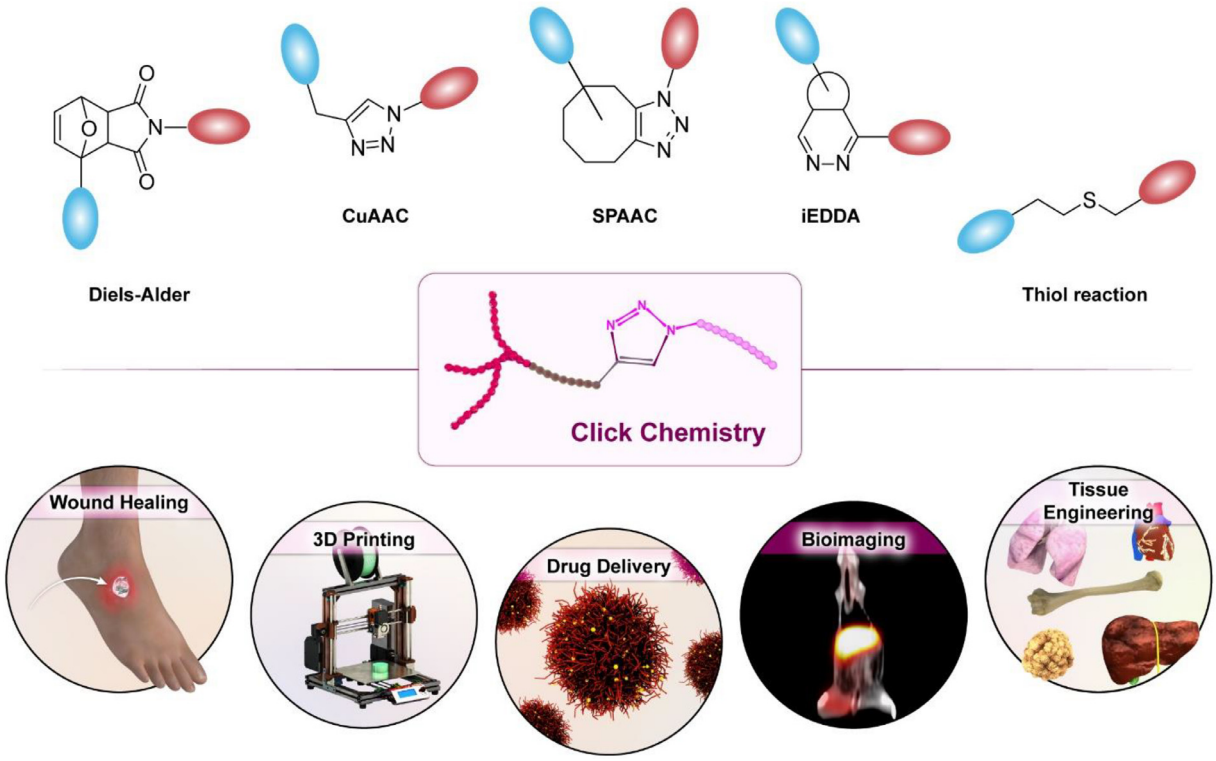


Fig. 1. Schematic illustrating how click chemistry could be applied to several biomedical applications. Click chemistry refers to a group of reactions (e.g., DA, CuAAc, SPAAC, iEDDA, thiol reaction) that are easy to perform, relatively fast, and highly efficient. Click chemistry provides an excellent platform in the biomedical arena and has found increasing applications.

actions can be carried out without interference to make multifunctional systems. On the other hand, orthogonal click reactions can be utilized to make sequence-regulated synthetic polymers [73]. For example, they can be utilized in iterative exponential growth (IEG) for preparation of sequence-defined polymers without utilization of protecting groups [93]. The concept of sequence-defined polymers may further fuel the metabolic glycoengineering (MGE) and synthetic OSA/PSA fields as illustrated in Fig. 1, which depicts important click reactions and their potential biomedical applications.

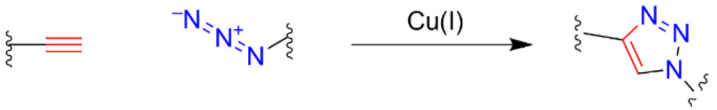
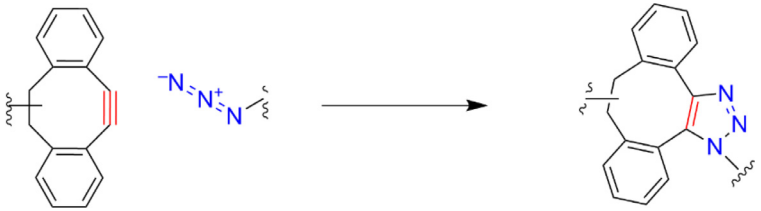
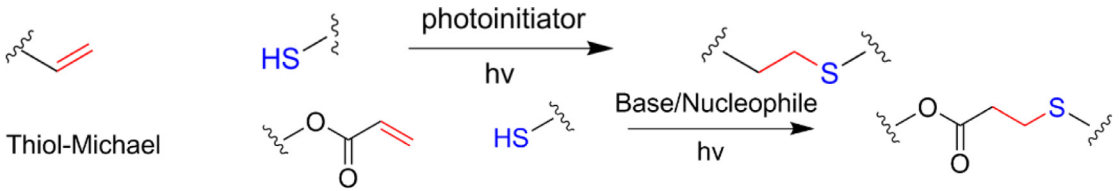
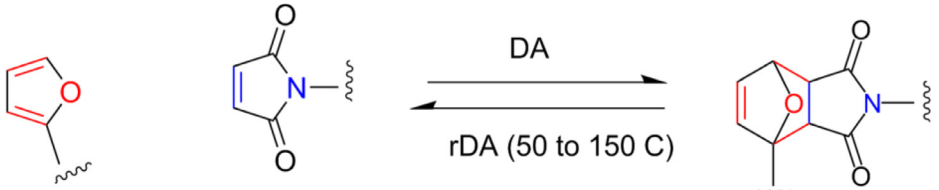
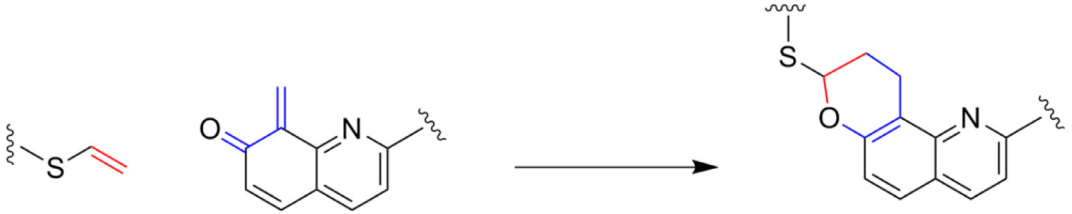
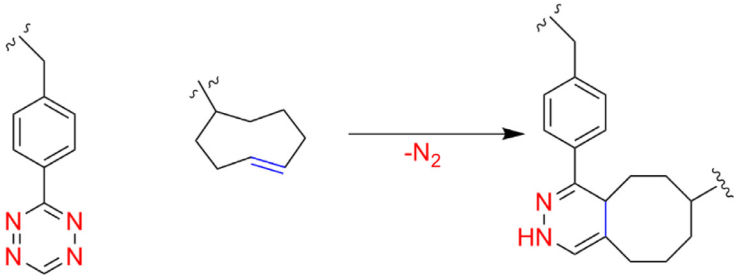
Although the utility of click chemistry for a number of biomedical applications has recently been described, it is usually limited to a few PSA and reports do not always dive into the wealth of available click chemistry strategies, their interactions with complex biological systems, or their wide range of applications within the biomedical field and healthcare industry [94,95]. This review article provides a comprehensive overview of key click reactions (Table 1), both conventional and emerging, followed by an in-depth discussion of clicked PSA-based materials for various biomedical applications, as well as their potential, challenges, clinical translatability, and future perspectives.

3. Clickable polysaccharides: manufacturing process

The manufacturing processes of clickable PSA can be classified into three groups: (a) *in situ* fabrication of clickable-PSA using MGE strategies, (b) chemical modification of naturally derived PSA, and (c) construction of artificial OSA/PSA bearing clickable groups from scratch. The manufacturing process for the fabrication of clickable PSA to undergo CuAAC and SPAAC was previously reviewed by Elchinger and co-workers [31]. However, the scope of their review was limited to the chemical modification of PSA with azides and alkynes bearing functional groups. Moreover, the authors did not discuss their current and potential applications, which are all covered in the current review article. Additionally, the modification of PSA using various click reactions, including CuAAC, metal-free cycloaddition, DA and iEDDA, oxime ligation, and thiol-Michael addition reaction, was discussed in a review article by Meng and co-workers [33]. While they thoroughly discussed the strategies for the synthesis of clickable PSA, they did not discuss their applications or consider alternative click reactions, which are covered in the current review article.

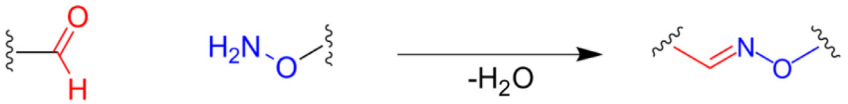
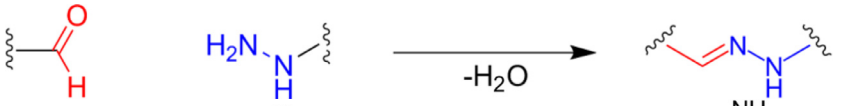
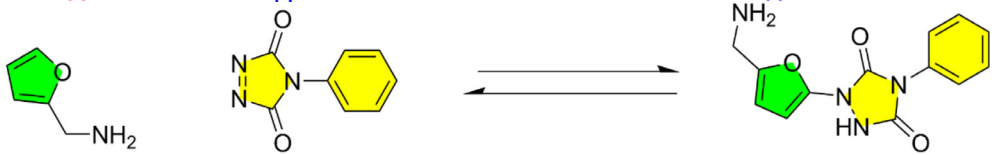
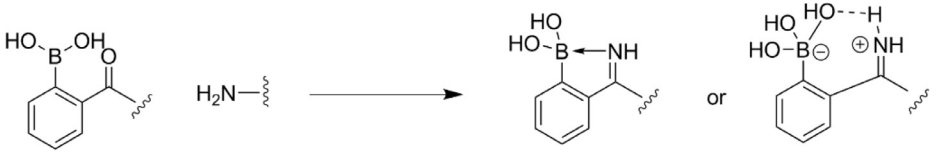
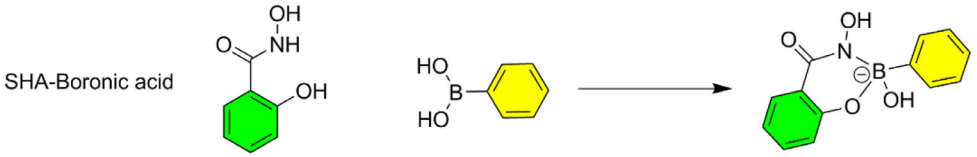
MGE involves the incorporation of unnatural building-blocks (synthetic monosaccharides not found in nature) bearing unnatural chemical functionalities (e.g., azido sugars), into glycoconjugates via the biosynthetic pathways of mammalian cells or other organisms [100,101]. In other words, this approach modulates the glycosylation process via manipulation of cellular metabolism and can be used in various biomedical fields [102]. In this approach, unnatural sugars bearing clickable groups (e.g., tetraacetylated N-azidoacetyl-D-mannosamine, $Ac_4ManNAz$) are added to the culture medium which also contains glucose and other necessary biomolecules [103]. Subsequently, clickable monosaccharides are embedded into the glycoconjugates produced by the cells [49,104,105]. Bioorthogonal click chemistry provides scientists with an invaluable tool to explore and understand biology in living organisms. For instance, it could be used to study glycan function by attaching fluorophores outside or inside cells, conduct noninvasive imaging of glycans on the cell surfaces, and monitor the dynamics of glycan biosynthesis [106,107]. Therefore, understanding and optimizing the lifetime of clickable groups on the cell surface is paramount for a successful outcome. The internalization pathway of clickable cell-surface glycans by cells requires some time to go through a complex biological process, moving from the endoplasmic reticulum to the Golgi, and then traveling to various destinations within the cell, including the lysosomes and the cell surface. Varki and co-workers have described that the stability of clickable groups depends on several factors, including chemical kinetics,

Table 1
An overview of established click reactions with an emphasis on their reaction pathways, advantages, and shortcomings.

Click reaction	Mechanism	K (M^{-1} S^{-1})	Comments, advantages, and limitations	Refs.
CuAAC		10-100 (10-100 μM Cu(I))	Selective formation of stable 1,2,3-triazole ring; efficient, versatile, and fast reaction; cytotoxicity of Cu(I)	[42]
SPAAC		0.17-0.96	Catalyst free; thiol attack susceptibility of some octyne derivatives	[96]
Thiol-X			Subgroups: 1) Radical-mediated thiol-ene/yne, 2) Nucleophile/base-mediated thiol-X; simplicity, high selectivity and efficiency, no byproduct, biocompatibility, fast, O_2 and water tolerant; harmful UV irradiation and/or cytotoxic photo-initiator	[54] [53]
DA			Diene-dienophile [4+2] cycloaddition to produce a stable fused bicyclic adduct; reversibility at elevated temperatures; high selectivity, wide scope, thermoreversibility, water insensitivity, no byproduct	[56, 97]
Hetero DA		0.0015	Heteroatom–heteroatom formation	[44]
iEDDA		210-2,800,000 (37°C in PBS)	High rate even under physiological conditions; gradual isomerization of TCO; nitrogen byproduct	[98]

(*continued on next page*)

Table 1 (*continued*)

Click reaction	Mechanism	K (M^{-1} S^{-1})	Comments, advantages, and limitations	Refs.
Oxime ligation (aldehyde/ketone condensation)		0.001	Neutral to basic pH of medium; creation of physiologically stable bonds; room temperature reaction; catalyst free	[66]
Hydrazone				
ES-Click			Reversible click reaction	[64]
Boronic acid–based DCC	Iminoboronate 	9 (for 2-APBA and TsC-No functionalities) 955 (for 2-FPBA and β-HHz)	Reversible click reaction	[85]
	SHA-Boronic acid 			
SuFEx		0.18	Catalyst free; O_2 and water tolerant; high conversion	[99]
Amino-yne			Spontaneous and catalyst free; ubiquitous raw materials; cleavability of created bonds (stimuli-responsiveness)	[75]
Non-covalent click	biotin–(strept)avidin association	10^7	Very high rate	[86]

their position on the cell surface, since they may be gradually recycled through membrane turnover, and their potential degradation in the lysosomes (i.e., catabolism) [108]. Therefore, the half-life of clickable groups should be long enough to allow sufficient time for optimal reaction conditions. For instance, a half-life of 15 min was observed for the internalization of fluorescent-labeled sialoglycoconjugates on the surface of Chinese hamster ovary (CHO) cells [48]. Assuming that the half-life of clickable sialoglycoconjugates before fluorescent labeling is a little over 15 min, this leaves a few minutes for imaging sialoglycoconjugates on the surface of cells before the fluorescent signal deteriorates significantly. Notably, internalization of clicked glycoconjugates was also observed for other cell types such as cytotoxic T cells and human endothelial progenitor cells (EPCs) [109,110].

Another approach for *in vivo* incorporation of clickable monosaccharides into PSA structures utilizes delivery vehicles such as nanogels or injectable hydrogels to carry synthetic monosaccharides to target locations such as tumor tissue [111,112]. This strategy drives the cells to produce clickable glycans that may become part of glycoproteins, glycopeptides, or glycoRNAs. More interestingly, transmembrane glycoproteins bearing clickable groups can serve as artificial receptors that can be targeted using complementary clickable groups, with exceptional selectivity compared to targeting biological receptors. To elaborate, traditional targeted drug delivery systems (DDS) rely on differences in physiological factors (e.g., pH, O_2 level, and enzymatic activities) between tumor and surrounding healthy tissue or specific cancer biomarker expression, which suffers from various limitations such as scarce and nonuniform distribution of targeted receptors [113–116]. However, artificial receptors based on click chemistry can address the challenges and shortcomings associated with current targeted delivery platforms targeting specific biological receptors (e.g., folate) that are overexpressed on the surface of cancer cells, but also present on healthy cells [117]. This approach can be used to edit the surface of extracellular vesicles with clickable azide-bearing moieties for further chemical modifications using bioorthogonal click chemistry [118]. Clickable groups, in glycoconjugate structures, serve as receptor-like chemical groups (i.e., bioorthogonal chemical receptors) which can be targeted through complementary clickable groups. As shown in Fig. 2, Lee and co-workers made a two-shot tumor-targeting system which utilizes MGE and click chemistry to target cancer cells more efficiently [112]. The first injection contains glycol chitosan nanoparticles (GCS-NP) loaded with Ac_4ManNAz. The effect of enhanced permeability and retention (EPR) aids the GCS-NP to accumulate in the tumor site via passive targeting. After cellular uptake, MGE enables the installation of numerous azide functionalities on cancer cells (Fig. 2a). The second shot includes bicyclo[6.1.0]nonyne (BCN)-modified GCS-NP (Fig. 2b). These NP were also conjugated with a photosensitizer (chlorine e6, Ce6) to improve the outcomes of photodynamic therapy (PDT). It is worth mentioning that noncanonical amino acids can also be incorporated into a predefined protein in live mammalian cells using a click chemistry approach [119].

The second class of clickable PSA includes those which are chemically modified by click-containing molecules on their side chains or end groups. Introduction of clickable groups in naturally derived PSA may be carried out through single or multiple steps. Usually, chemical modifications are required prior to introducing clickable groups on PSA chains. Several popular strategies for the chemical modification of PSA consist of converting a hydroxyl group to carboxylic acid or amine, introducing a carboxymethyl group on a pyranose ring, or opening the pyranose ring to give rise to an aldehyde functionality. For example, to graft β-cyclodextrin (β-CD) onto alginate chains, a two-step approach, including chemical oxidation and reductive amination, was carried out to affix alkyne residues to alginate [120]. The introduction of clickable end-functionalities on OSA/PSA, resulting in telechelic oligomers, allows their sequential modification and further reaction. For instance, mono and bifunctionalized telechelic OSA/PSA oligomers with clickable residues usually undergo click reactions under mild conditions and can be used to fabricate homopolymers and block copolymers. In a recent review article, the applications of bifunctional clickable linkers (dihydrazide and dioxyamine) for the fabrication of block PSA were discussed [32]. These clickable linkers, enabling the synthesis of diblock PSA-based macromolecules via hydrazone and oxime ligation click chemistries, resulted in materials with preserved properties of the parent PSA and new features such as unique self-assembly behavior.

The third class of clickable PSA is based on artificial OSA/PSA, such as using the automated glycan assembly (AGA) strategy [121]. However, the regio- and stereochemistry of the glycosidic linkage pose important challenges for the total synthesis of glycans [122]. One of the major goals in total synthesis of PSA is to investigate the structure–activity relationship [123]. Click chemistry enables the regio- and chemoselective modification of PSA. However, click chemistry has not yet enabled the total synthesis of well-defined PSA bearing pendant clickable groups. Therefore, designing well-defined clickable OSA/PSA could further advance the field and have great potential in future biomedical applications.

In many applications, clickable PSA are manufactured for further functionalization. These pre-click modifications can be utilized for grafting or crosslinking purposes. For example, in order to make dextran (Dex)-*g*-poly(o-nitrobenzyl acrylate) (PNBA) copolymers, pre-click modification of Dex and PNBA was carried out to install alkyne and azide groups, respectively [124]. CuAAC enabled grafting of PNBA onto the Dex main chain. Copper(I) bromide (CuBr) was utilized as the source of Cu catalyst for the reaction. After the click reaction, excess copper ions were removed using ethylenediaminetetraacetic acid (EDTA). Besides EDTA, other metal-chelating agents such as sodium citrate and nitriloacetic acid are useful for removing cytotoxic metal ions. PNBA is a light-sensitive, hydrophobic polymer, while Dex constitutes the hydrophilic segments. Self-assembly of the copolymer in the solution creates dextran-coated hydrophobic PNBA-based cores. Light sensitivity enables precise control over release of the encapsulated drugs.

Clickable OSA are interesting building blocks for making architecturally complex biopolymers with multifunctional properties and specific functions. These intermediates can be conjugated with other synthetic or naturally derived polymers. Moreover, other small molecules (e.g., dopamine) with specific functionalities and bioactivities can be embedded in their molecular structure. OSA can be prepared by bond cleavage (depolymerization) of naturally derived PSA or by organic synthesis pathways such as AGA. For example, chitooligosaccharides (COS), possessing reactive aldehyde end-functional groups, are unique derivatives of natural chitosan (CS) which can be modified using clickable functional groups. In contrast to CS, these commercialized, lower molecular weight OSA can dissolve in water at neutral pH. COS exhibit antitumor, antioxidant, anti-inflammatory, immunostimulatory, antibacterial, antifungal, and hypocholesterolemic biological activities [125,126]. Reductive aldehyde end groups and primary amine side groups provide rich functional sites for versatile modification of COS with various clickable groups such as alkene, alkyne, thiol, tetrazine, azide, and hydrazide. However, because of the amine groups' importance to COS bioactivity, reductive aldehyde groups are better candidates for chemical modification. Moussa and co-workers mounted various clickable groups on the reactive aldehyde end of COS to fabricate various clickable OSA to make diblock COS-*b*-polyethylene glycol (PEG) copolymers [127]. This is a potent strategy for using OSA in the structure of graft and block copolymers. On the other hand, reductive amination using aniline is a robust strategy for preparing clickable COS [128].

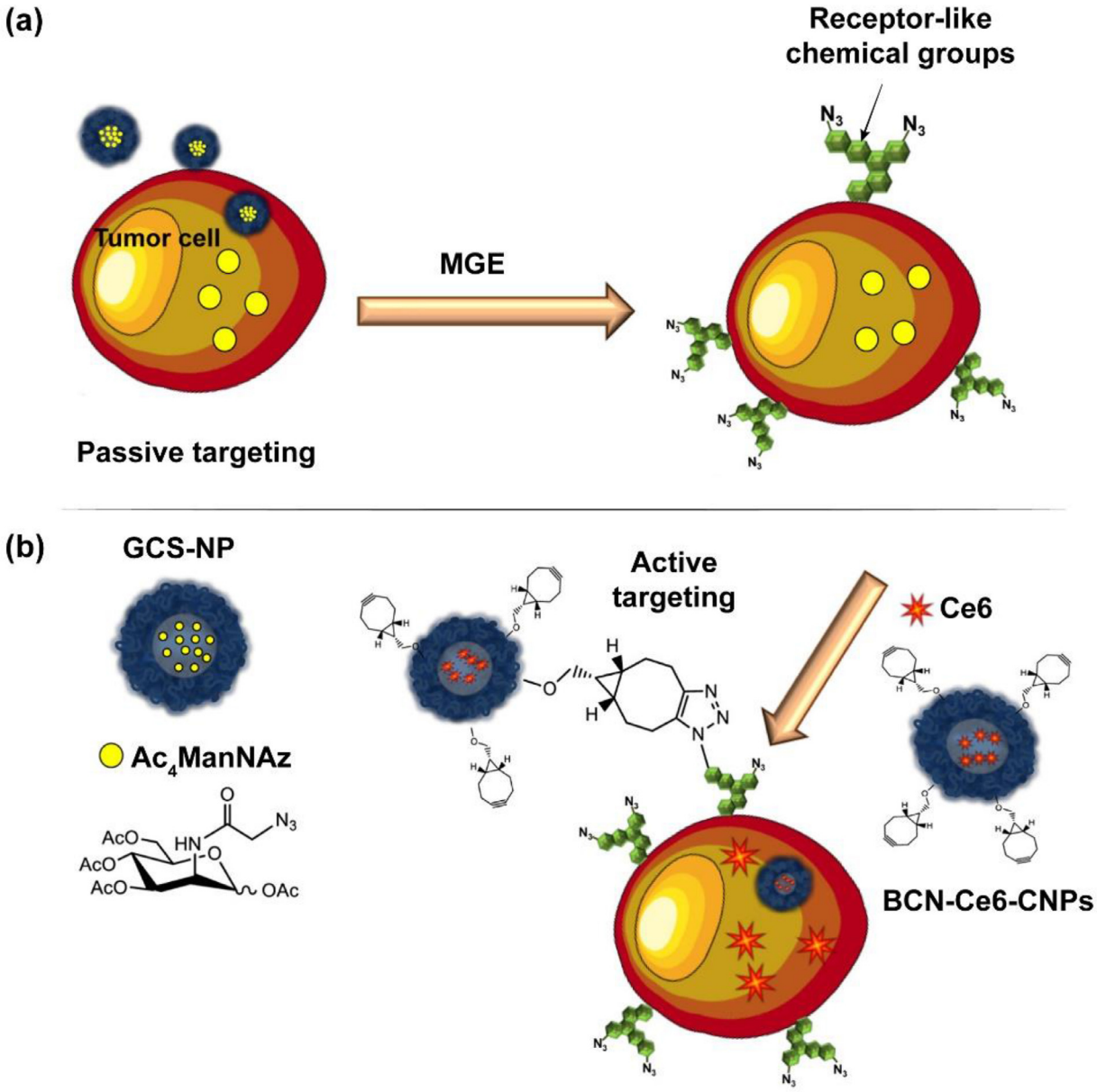


Fig. 2. Schematic illustrating the mechanism of action of a two-shot tumor-targeting system. (a) Passive targeting of GCS-NP into tumor cells by the EPR effect and the intracellular delivery of Ac_4ManNAz. MGE-mediated clickable glycoconjugates on cancer cells serve as receptor-like chemical groups. (b) Ce6-loaded and BCN-modified GCS-NP selectively target the artificial receptors on tumor cells via bioorthogonal click chemistry, leading to the intracellular delivery of Ce6 for enhanced PDT. [112], Copyright 2014. Adapted with permission from the American Chemical Society.

Thiol-ene click chemistry was used to prepare a series of cationic peptidopolysaccharides with antimicrobial properties [129]. In this case, methacrylated cationic antimicrobial peptides (AMP) were grafted onto a thiol-functionalized dextran backbone.

Host–guest interactions are an important class of physical interactions that can be exploited to make supramolecular hydrogels. Host and guest species can be installed onto PSA chains via click chemistry methods. For example, β-CD can be chemically modified using a clickable group before grafting to the backbone of another clickable PSA. Alkyne-modified pullulan was clicked using azide-bearing β-CD through CuAAC [130]. On the other hand, Dex was modified with adamantane, guest molecules for β-CD, using a similar CuAAC reaction. Accordingly, β-CD and adamantane groups on the pullulan and Dex can interact strongly to make a supramolecular hydrogel. Pullulan has a higher degradation rate while Dex chains endow the hydrogel with more flexibility. In other research, Dex chains were functionalized with two ene-bearing molecules including 6-maleimidohexanoic acid and 5-norbornene-2-carboxylic acid [131]. A condensation reaction between the carboxylic acid of these molecules and the hydroxyl groups of Dex results in ester linkage formation. Maleimide and norbornene-functionalized Dex chains are clickable PSA that can undergo further thiol-X (thiol-Michael or thiol-ene) reactions using thiol-containing molecules such as N-acetyl-L-cysteine (NAC).

AMP are an important part of the innate immune system. In fact, all organisms benefit from small cationic peptides with amphiphilic properties that show antibacterial characteristics [132]. In addition, these ubiquitous small-molecule antibiotics induce little to no antimicrobial resistance (AMR) [133]. Pyrolytic degradation, inappropriate pharmacokinetics, high hemolytic activities, and cytotoxicity to mammalian cells limit their application, but chemical modification and conjugation can circumvent these limitations. For example, PEG-based peptides were utilized as synthetic mimics

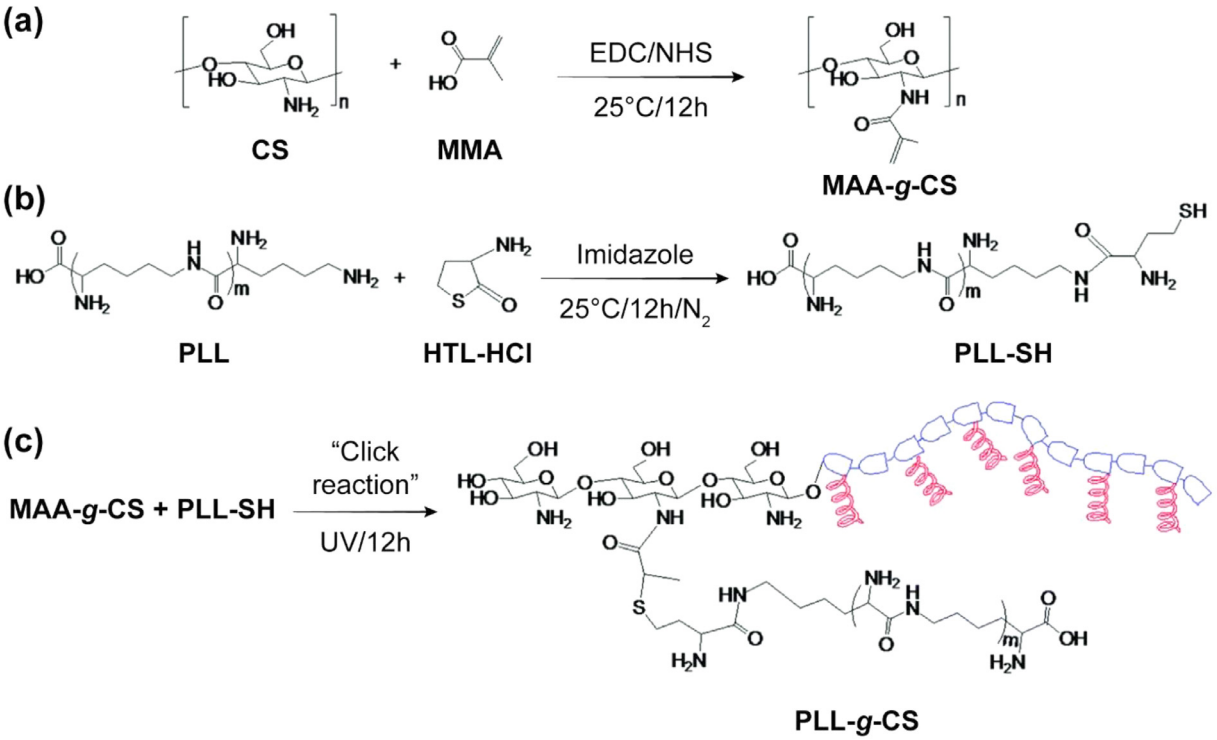


Fig. 3. Synthetic procedure for the synthesis of a cationic peptidopolysaccharide. (a) MAA was grafted onto CS via a carbodiimide-mediated coupling reaction. (b) Thiol end-functional groups were introduced on PLL under nitrogen gas (N_2). (c) Grafting PLL (depicted as red helices) onto the backbone of CS via UV-mediated thiol-ene click reactions. [137], Copyright 2014. Adapted with permission from the American Chemical Society.

of AMP for bacterial suppression [134]. A recent review article has thoroughly discussed the mechanisms of action of AMPs and introduced the idea to incorporate other macromolecules (e.g., synthetic polymers and biopolymers) possessing tunable bactericidal properties [135]. Bioorthogonal chemistry strategies, especially click chemistry, provide a robust and versatile method for selective and rational chemical modification and conjugation of AMP [133]. Peptidoglycans, which are small peptides attached to sugar molecules, constitute a rigid envelope surrounding the plasma membrane of most bacteria. Inspired by these natural copolymers, synthetic cationic peptidopolysaccharides have emerged as novel antimicrobial agents that directly affect the bacterial cytoplasmic membrane. For example, an AMP (CysHHC10) was selectively grafted to amino and hydroxyl (C6 position) functional groups of CS via a thiol-X click reaction [136]. C6-position-grafted CysHHC10 peptide showed superior antibacterial activity compared to C2-position-modified CS. Furthermore, the hemolytic activity and mammalian cell toxicity of both CS-grafted AMP was diminished, compared to free AMP. These AMP-*g*-CS were utilized to make antibacterial coatings via layer-by-layer assembly.

Su and co-workers designed a cationic peptidopolysaccharide that can effectively kill gram negative bacteria, gram positive bacteria, and fungi while being safe to mammalian cells [137]. As shown in Fig. 3, they grafted thiol end-functionalized poly-L-lysine (PLL), a cationic homopolypeptide, onto a methacrylic acid (MAA)-functionalized CS via a thiol-ene click chemistry strategy. The resulting PLL-*g*-CS copolymer, benefiting from the inherent antimicrobial properties of CS and PLL, exhibited strong antimicrobial activity.

Selective functionalization of PSA is vital to their effective use in medicine because the location of functionalization affects their properties [138]. In one study, the C6 position of curdlan was selectively modified with a cationic group using click chemistry [139]. The obtained lysine-"clicked" curdlan with high water solubility can be used as a gene delivery platform. It showed good endosomal escape capability, low cytotoxicity, and strong DNA binding ability.

It is known that the type and number of functional groups, and the molecular weight of the crosslinker affect the properties of the obtained hydrogel [140]. In order to elucidate this effect, bi- and trifunctional clickable crosslinkers were used for alginate hydrogel [141]. PEG crosslinkers with different chain lengths, containing 2 or 3 maleimide groups, were used to investigate the effect of crosslinkers on mechanical properties of the hydrogel. The results showed that the stiffness and storage modulus (G') is higher for the hydrogels crosslinked with PEG-triple click. On the other hand, crosslinker length affects the swell behavior of the hydrogel. The hydrogel was used as a drug delivery platform for vanillin. Amphiphilic CS derivatives were synthesized using a click chemistry approach [142].

It is worth mentioning that click chemistry can be utilized for surface treatment at nano-, micro-, and macroscales. For example, contact lens surfaces were treated by thiolated HA using thiol-ene click chemistry [143].

4. Clickable polysaccharides: properties

4.1. Rheological properties

Rheological properties of clickable PSA-based hydrogels are of primary importance in designing biomaterials for injectable hydrogels and bioprinting applications. Rheological properties of hydrogels include injectability, mechanical properties, and dynamic features (e.g., self-healing and degradation). For example, hydrogels with shear-thinning properties are important for fabricating bioinks [144,145]. Rheological properties of hydrogels are also important in simulating flow behavior in bioprinters and predicting how cavities will be filled *in vivo* [146]. Polymeric solutions often exhibit complex and peculiar fluid flow behaviors, especially in higher concentrations, due to a wide variety of inter/intramolecular

Table 2
Gelation time of PSA-based hydrogels formed via click chemistry.

Click reaction	Polysaccharide	Additives	Gelation time	Comments	Refs.
CuAAC	HA-hydrazine, HA-CHO or HA-benzaldehyde	Collagen, Cu(II) sulfate pentahydrate, stem cells	5 min	Functional groups ratio=1:1 Concentration=1-4%	[159]
SPAAC	Dex-ADIBO and Dex-N_3	Chondrocytes	1.1-10.2 min	Dex-ADIBO:Dex-N_3=1:1 t_{gel}=1.1 for DS=10 and concentration=10% t_{gel}=10.2 for DS=5 and concentration=5%	[160]
	HA-*g*-OEG-DBCO, 4-arm PEG-N_3	Chondrocytes	10-14 min	HA-*g*-OEG-DBCO = 10 mg/ml 4-arm PEG-N_3=0-2.5 mM	[161]
DA	CnS-furan, F127-maleimide, PEG-furan	BMP4, cells	75 sec	(CnS-furan + F127-maleimide):PEG-furan ratio=3:1	[162]
	HA-furan, HA-maleimide	Dexamethasone, cells	< 60 min	t_{gel} for various volume ratios of HA-furan:HA-maleimide: 34 min (1:2), 44 min (1:1), 47 min (2:1)	[163]
Thiol-ene	HA-acrylate, HA-HS		10 sec (Mw=2MDa, 20 mg/ml) to 2 min (Mw=0.1MDa, 10 mg/ml)	HA-acrylate:HA-HS volume ratio=1:1; t_{gel} decreases while increasing Mw or concentration	[164]
	HP-PEG (acrylated), HA-HS	Stem cells	50-100 sec	HP-PEG: HA-HS ratio=2.5-10%	[165]
Oxime ligation	Oxidized HA, AO-4-arm PEG		20 min	Oxidized HA:AO-4-arm PEG ratio=4.67; *in vitro* t_{gel}=1.6-115 min (for pH=4-7); *in vivo* t_{gel}=20 min (pH-independent)	[154]

interactions between neighboring chains [147]. Predicting flow behavior in such non-Newtonian fluids usually requires measurement of several rheological parameters, in contrast to Newtonian fluids, in which flow depends on viscosity. The elastic modulus and complex viscosity are usually measured using rheometry. Regardless of dynamic rheological features, *in situ* reactivity of injectable hydrogel components impose enhanced complexity to the rheology of mixing-induced two-component injectable hydrogels (MITCH). In other words, based on reaction kinetics under different environmental conditions, time-dependent rheological properties may be different. However, in this paper, only gelation time (Table 2) is independently investigated, and it is usually determined through rheological measurements.

Gelation time is primarily important for hydrogels used for TE, bioprinting, and delivery applications [148–153]. PSA and crosslinkers bearing clickable groups are coupled via click reactions to produce a highly hydrated, porous media. Generally, gelation time of such systems corresponds to click reaction kinetics-in other words, faster reaction kinetics results in shorter gelation time. Thus, one may expect that gel time is generally shorter for iEDDA-based injectable systems compared to CuAAC or SPAAC. However, several other parameters, including the concentration of initial reagents, steric hindrances, microenvironmental conditions (e.g., temperature, pH, enzymes, catalysts, light irradiation) and the density of clickable groups, also affect the gelation process, similar to other chemical reactions. Furthermore, *in vitro* and *in vivo* gelation time may be significantly different. For example, while subcutaneous injection of oxidized HA and 4-armed aminooxy-PEG results in gelation in 20 min for a relatively wide pH range (i.e., 4–10.5), *in vitro* gelation at body temperature shows significant pH dependence, varying from 30 min to more than 2 days [154]. This phenomenon indicates a long shelf life of this oxime-crosslinked injectable hydrogel *in vitro,* allowing for mixing of the two components before injection without worry of gelation. In fact, injectable hydrogels for which *in vivo* gelation time is shorter compared to *in vitro*, can be administered through a single syringe injection system, in contrast to traditional injectable systems requiring use of a double-syringe injector to prevent premature gelation [155].

On the other hand, for each click reaction, the reaction conditions should be considered to predict the real gel time of the system. For example, in the CuAAC, the rate of *in situ* catalyst production (i.e., Cu(I)) and its diffusion affect the overall gelation process. In other words, the diffusion mass transfer (usually a slow process) or the Cu(II) reduction process can play a critical role in the overall kinetics of the gelation process. This complication is addressed in the photo-click reactions in which a uniform initiation step is observed throughout the transparent raw materials for hydrogel fabrication. In photo-click reactions, light wavelength and intensity, initiator type and concentration, and transparency of materials to the irradiated light are important factors affecting reaction rate.

The gelation process may be affected by physical crosslinking, which is generally weaker but faster than chemical click crosslinking. For example, ionic crosslinking in clickable alginate hydrogels may result in creation of the first crosslinked network in a double-crosslinked hydrogel. The concept of non-covalent click chemistry has recently been introduced by Schreiber and co-workers to include specific physical interactions under the click chemistry umbrella [86]. Although there are differences between specific physical interactions (i.e., non-covalent) and bioorthogonal click chemistry (i.e., covalent), both chemistries represent a similar class of high-yielding chemical reactions that proceed rapidly and selectively in biological environments. Accordingly, non-covalent click chemistry should be considered as a subclass of click chemistry in a general context. Furthermore, as shown in Table 1, non-covalent click chemistry displays significantly higher reaction rates compared to conventional click chemistry, therefore reinforcing the arsenal of click chemistry as a powerful technique for fast and efficient covalent conjugation of molecular entities.

It is worth mentioning that crosslinking density and type of crosslinker significantly impact the rheological and mechanical properties of the hydrogel [156]. A biomimetic hydrogel with tunable gelation rate was developed based on thiolated hyaluronic acid (HA-HS) and thiolated chondroitin sulfate (CnS-HS) which can be crosslinked by poly(ethylene glycol) diacrylate (PEGDA) [157]. It was revealed that the kinetics of the thiol-ene click reaction between glycosaminoglycans and PEGDA change with degree of substitution (DS) of thiol and molecular weight of bifunctionalized PEG. Furthermore, it was observed that encapsulated mesenchymal stem cells (MSCs) remained viable during the gelation process of the hydrogel. MSC responses to matrix stiffness alteration was studied using focal adhesion kinase (FAK). FAK, known as protein tyrosine kinase 2 (PTK2), is a cytoplasmic tyrosine kinase that

plays a key role in cell adhesion and migration. It can also serve as a reversible molecular mechanosensor [158].

4.2. Mechanical properties

The mechanical properties of the native ECM greatly affect cell behavior (e.g., proliferation, migration, differentiation, and functions) and fate *in vivo*. This illustrates the importance of mechanical behavior, structural features, and biological cues of TE scaffolds on cells both *in vitro* and *in vivo*. In this regard, successful hydrogels for TE applications should mimic the dynamic mechanical features of the native ECM. The mechanical properties (e.g., elastic modulus) of the clickable PSA-based injectable hydrogels for TE should conform to surrounding tissues.

The mechanical properties of a hydrogel are mainly defined by intermolecular interactions between PSA chains. Physical interactions are reversible and occur at high rates, leading to association or dissociation of (macro)molecules. Factors such as steric hindrance can prohibit physical interactions, especially in bulky macromolecules. Such interactions are usually responsible for the fast-gelling properties of hydrogels and their dynamic behavior (e.g., self-healing).

On the other hand, chemical interactions like covalent bonds are responsible for enhanced mechanical properties such as higher modulus and stiffness. Covalent bonds are generally created at lower rates, but they are more robust. Moreover, increasing the density of crosslinking results in higher mechanical properties although with the expense of reduced dynamicity. Covalent crosslinking of hydrogels usually requires small molecules or macromolecules with two or more reactive functional groups. For example, dialdehyde molecules (e.g., glutaraldehyde) are used to crosslink polymers with pendant amino groups such as CS [166]. However, covalent crosslinking can occur between two polymer chains with pendant clickable groups, removing the need for exogenous crosslinkers that may potentially induce cytotoxicity.

Type and density of crosslinking greatly affect the mechanical properties of clickable PSA-based hydrogels, similar to other covalent crosslinking strategies. Increasing the number density of clickable groups on PSA chains results in enhanced crosslinking density, indicating improved mechanical properties. Creating double-crosslinked hydrogel networks can be used as a general strategy for enhancing mechanical properties of hydrogels, where click reactions can be responsible for creation of one or more crosslinked networks. Double-crosslinked systems may also benefit from both physical and chemical crosslinking strategies to adjust their gelation, toughness, and degradation behavior.

While ionic bonds are susceptible to chelating agents and physiological conditions, covalent bonds are stronger, and usually irreversible, endowing the hydrogels with durability and high mechanical properties [167,168]. Many researchers have tried to develop double-crosslinked hydrogels that benefit from the dynamic behavior of physical interactions and the robustness of covalent crosslinkers. Clickable polymers for making robust covalent bonds that remove the necessity of crosslinkers and that occur under mild reaction conditions are highly valuable for making mechanically robust but dynamic hydrogels. Double-crosslinking approaches which combine the beneficial aspects of physical interactions (faster but weaker association of macromolecules) with the merits of chemical interactions (slower but more robust conjugation) have garnered much attention in the biomaterials field. These double-crosslinked systems have a dynamic nature similar to the ECM. The ECM is dynamic both in composition and architecture during processes such as wound healing, tumor growth, and embryonic development. Furthermore, its composition varies in different tissues, from skin to muscles. Interestingly, the ECM may be much more dynamic in some creatures, such as sea cucumbers' skin [169]. These features have inspired materials scientists to look for biomimetic hydrogels that are mechanically robust while they benefit from dynamic characteristics [169]. Self-healing and stimuli-responsiveness have been the most studied dynamic behaviors in biomimetic hydrogels. Physical interactions, dynamic covalent bonds, and click reactions are the foundations of making dynamic hydrogels. Clickable PSA which can interact through physical interactions are interesting candidates to make double-crosslinked hydrogels having dynamic features. For example, introduction of clickable groups on alginate makes it suitable for fabricating double-crosslinked hydrogels with tunable mechanical properties [170]. In fact, controlled manufacturing of clickable alginate can result in modified PSA that benefits from ionic and covalent crosslinkers. The sequential physical and chemical crosslinking in this hydrogel enable a gradual enhancement in mechanical properties, i.e., the embedded collagen fibers are first allowed to self-assemble and later fixed when the covalent crosslinked network is created [170]. Oki and co-workers made maleimide-modified alginate-based microcapsules which are crosslinked using calcium ions and contain preosteoblastic cells [171]. Thiolated peptide molecules can diffuse by mass transfer operations into microcapsules, where they react with alginate chains via click chemistry. This *in situ* conjugation of biomimetic peptides to alginate affects the cells' behavior through altering of the mechanical properties of the microgel. Fibroblast proliferation is accelerated in the presence of alginate functionalized with arginyl-glycyl-aspartic acid (RGD) while RGD-free alginate had no detectable effect. Furthermore, bone morphogenetic protein-2 (BMP2) mimetic peptide–alginate conjugates triggered osteogenic differentiation of the encapsulated preosteoblasts.

4.3. Dynamic behavior and degradation

Making hydrogels with tunable physicochemical and mechanical properties is uniquely important for biomedical applications such as designing smart drug delivery platforms and novel, dynamic TE scaffolds. Furthermore, dynamic adjustment of these properties can further enhance their multifunctionality and adaptability. Dynamic tuning of properties stems from bond creation/cleavage at the intermolecular scale and assembly/dissociation at the intramolecular scale [172]. Physical bond dissociation results in phenomena like protein denaturization and melting in thermosensitive hydrogels such as agarose. On the other hand, cleavage of the covalent bonds results in depolymerization and degradation. For example, degradation of many natural polymers emanates from ester linkage hydrolysis or proteolysis of amide linkages, where the presence of water molecules (nucleophiles) trigger uncatalyzed or catalyzed bond cleavage. Moreover, other stimuli such as electromagnetic waves (UV light and gamma rays) can also trigger clipping of covalent bonds, known as photodegradation. This process depends on several factors such as the chemical bonds' energy and energy of the photons ($E = h\nu$) and light intensity. The presence of photocleavable linkers, such as nitroaryl groups (o-nitrobenzene), in the polymer backbone or side groups enable the creation of photodegradable polymers and hydrogels. Exposure of these photo-labile polymers to light induces polymer degradation, resulting in diminished mechanical properties and enhanced release of encapsulated species such as drug molecules. This approach can be used to make hydrogels with dynamic mechanical properties or to make photo-patternable hydrogels [173]. More interestingly, photo-click chemistry, which combines the merits of click chemistry with photochemical processes, allows the fabrication of photodegradable polymers with spatiotemporal control over the degradation profile [39]. This adjustable photodegradation is a highly valuable tool for on-demand release of therapeutics. For example, clickable HA (HA-HS) was

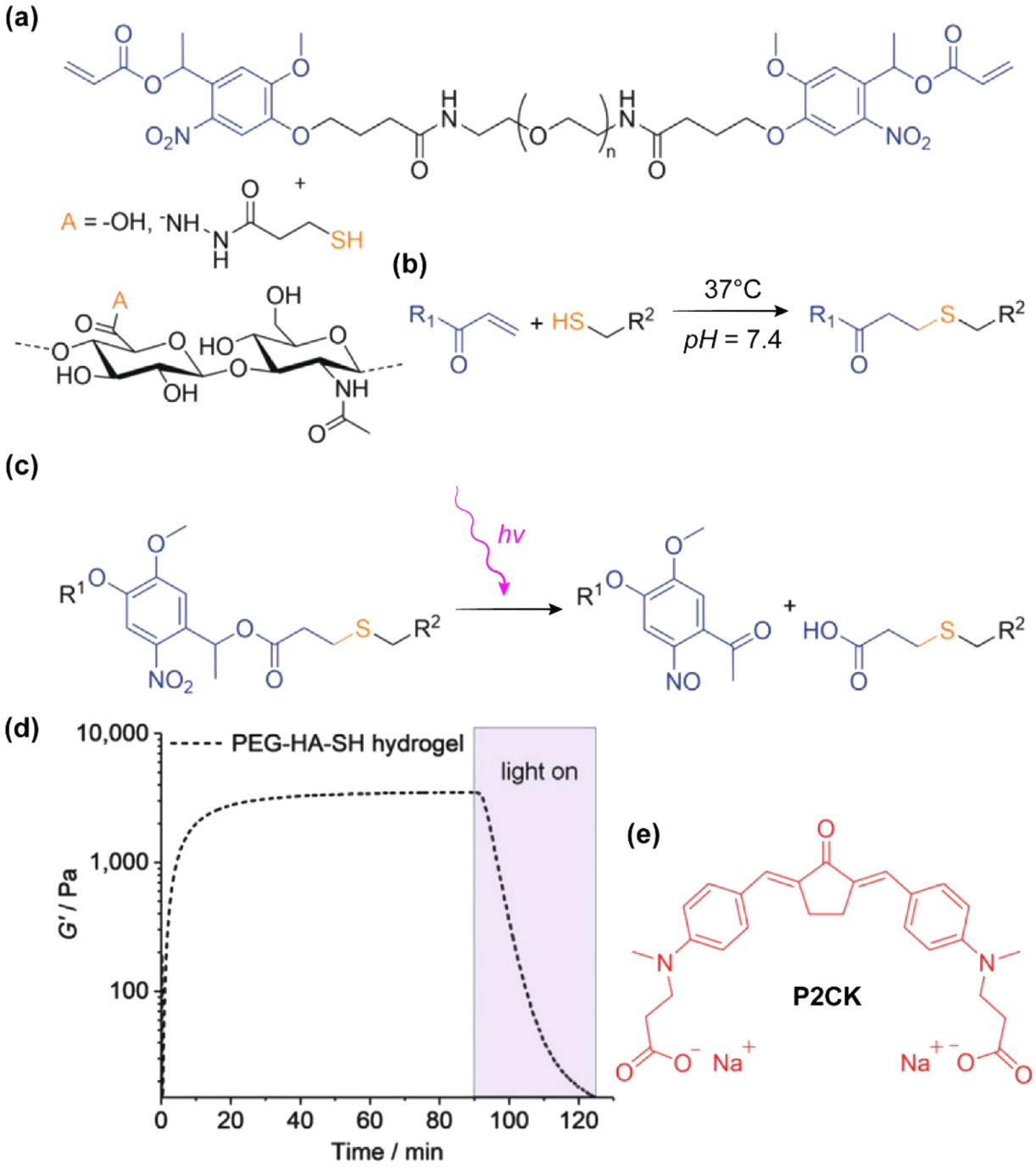


Fig. 4. Synthetic procedure and degradation behavior of HA-based photosensitive hydrogel. (a) Chemical structure of a photosensitive PEG-based crosslinker. (b) Chemical structure of thiolated HA (HA-SH) and its reaction with a PEG-based crosslinker. Crosslinking occurs via thiol-ene click reaction through a Michael addition pathway under physiological conditions. (c) o-NB ester linkages in PEG-HA-SH hydrogel are cleaved upon UV irradiation or two-photon excitation. (d) Oscillatory measurements of G′ during gelation (< 90 min) and UV/Vis-induced degradation (≥ 90 min). (e) Chemical structure of a highly sensitive two-photon sensitizer P2CK used in PDT. [173], Copyright 2018. Adapted with permission from John Wiley and Sons Inc.

functionalized with a thiolated compound containing a hydrazide linkage (Fig. 4). PEG6000, which has two functional end groups, i.e., ortho-nitrobenzyl (o-NB) conjugated to acrylate, serves as macromolecular crosslinker. The Michael-type thiol-ene addition between HA-HS and o-NB-functionalized PEG resulted in crosslinking and hydrogel formation. However, UV exposure or two-photon excitation can result in o-NB ester bond cleavage [173]. As shown in Fig. 4d, during the click-mediated polymerization, G' increased quickly and plateaued at approximately 30 min. Contrarily, upon light exposure the mechanical integrity of the gel system was rapidly compromised, resulting in a dramatic drop of G' within 30 min.

The degradation of hydrogels is critical to mimic the dynamic microenvironmental conditions of cells to induce the intended cell behavior [174]. The mechanical properties of hydrogels affect cell fate via mechanotransduction. In other words, cells continually sense their surroundings such that the stiffness of scaffolds affect their focal adhesion and spreading. Furthermore, adjustable degradation of hydrogels is favorable for controlled drug/cell release and to promote cell infiltration into the scaffolds from surrounding tis-

sues. Degradation can be induced by enzymes (e.g., protease), light (e.g., UV), hydrolysis and ion exchange [175].

The degradation of a hydrogel indicates the biodegradation of polymer backbones, side chains, or crosslinkers. Degradable clickable crosslinkers should possess degradable or cleavable linkages in their structure, regardless of clickable functionalities. For example, a macromolecular crosslinker such as P2CK with photocleavable groups can degrade upon light irradiation (Fig. 4e) [173]. Click-functionalized crosslinkers containing linkages which are labile to biological cues (e.g., enzymes), are important to the manufacture of clickable biodegradable hydrogels for *in vivo* applications.

Ionically crosslinked alginate hydrogels degrade under physiological conditions because of ion exchange. In other words, ion expulsion from the hydrogel network results in PSA chain dissociation. On the other hand, click-crosslinked alginate does not degrade under physiological conditions, unless crosslinked using clickable crosslinkers with degradable linkages in their structure [176]. Moreover, research studies have shown that oxidation of alginate chains endows them with hydrolytic degradation [177,178]. This indicates that clickable oxidized alginate-based hydrogels can be passively degraded in contact with water. These clues prompted Mooney's group to fabricate hydrolytically degradable hydrogels based on oxidized alginate [179]. Sequential oxidations and selective reduction of alginate chains using sodium periodate and ammonia borane, respectively, were used to make partially oxidized alginate chains. Partially oxidized aldehyde groups were converted to hydroxyl groups. Carbodiimide chemistry was then utilized to introduce clickable groups on oxidized alginate chains. They obtained an injectable hydrogel, *in situ* crosslinked by norbornene tetrazine (Nb-Tz) click reaction. In another study, an injectable hydrogel was designed based on carboxymethyl chitosan (CMC) [79]. In this work, PEG, used as a macromolecular crosslinker, was end-functionalized with propiolate groups. Spontaneous amino-yne click reactions between primary amines from CMC and activated alkynes on PEG resulted in an injectable hydrogel. The crosslinking resulted in the formation of β-aminoacrylate linkages between PEG and CMS which happen to be cleavable by singlet oxygen ($^1O_2$) and in weak acidic environments. Furthermore, a pH-induced sol-gel transition was observed for these hydrogels, indicating their potential as a pH-responsive carrier for drug delivery. Rheological measurements showed that the gelation time occurs quickly, within 7 min under physiological conditions. Such injectable and degradable hydrogels can be utilized in various biomedical applications. Although there is considerable potential for scientists to design materials with amino- and activated alkyne-containing PSA, there are only very few reports to date using this strategy.

Complementary to click chemistry, clip chemistry, which enables bond cleavage, is equally important for making diverse (bio)(macro)molecular structures [172]. Precise bond cleavage, similar to gene editing using CRISPR/Cas9, enables trimming at the molecular level.

5. Clickable polysaccharides: applications

5.1. Drug delivery

DDS and theranostic platforms have attracted much attention in management and therapy of various diseases such as cancer [180–183]. In this regard, many natural-derived polymers, especially PSA and their derivatives, have been widely used in the formulation of DDS as shown in Table 3 [184,185]. Targeted DDS, which can target specific cells with high specificity and release the payload at a controlled rate, continue to receive increased attention [186]. A general overview on the development of DDS makes it clear that they have been gradually becoming more complex, both in molecular structure and architectural features, to fulfill the ever-increasing requirements of advanced DDS [187–189].

However, uncontrolled chemical reactions, which proceed under harsh conditions and non-specific physical interactions, have limited the fabrication of well-defined, complex molecular/architectural constructs that can interact with other moieties with high specificity. Development of CRP strategies have enabled materials scientist to design more structurally defined polymers *in vitro,* though *in vivo* polymerization in mammalian cells remains elusive. Nevertheless, minor changes in the structure of biomacromolecules made by cells are possible. For example, MGE methods have allowed the making of modified PSA bearing clickable functionalities [101,103,190]. On the other hand, bioorthogonal click chemistry strategies enable the performing of chemical reactions in living systems with minor chemical interference and appropriate rates [65].

Most traditional, smart DDS depend on non-specific physical interactions that result in assembly (usually *in vitro*) of drug carriers when constructing materials come into contact or disassembly (usually *in vivo*) when carriers are exposed to endogenous (e.g., pH of endosome microenvironment) or exogenous stimuli such as near-infrared (NIR) irradiation. Non-specific interactions result in assemblies with a wide range of shape and size distributions that depend on fabrication conditions. On the other hand, some smart DDS utilize cleavable covalent bonds which can be dissociated using special stimuli such as UV exposure or redox species [188]. However, both conjugation and cleavage are limited by requirements or outcomes such as relatively harsh environmental conditions, low reaction rates, production of toxic side products, and probable chemical reactions with other biomolecules in the complex intra/extracellular microenvironment. These highlight the importance of bioorthogonal reactions that can be carried out at high rate under physiological conditions. Devaraj and co-workers discussed the merits of various bioorthogonal reactions that have revolutionized chemical biology, and they provided some insights and future perspectives to further develop novel click chemistries [3]. The click chemistry concept, which overlaps with orthogonality in several aspects, has made such stringent conditions possible during the last two decades of its development. Clickable molecules constitute invaluable building blocks for making unprecedented and unparallel DDS. Moreover, CRP and MGE strategies have enabled us to make clickable polymers, clickable OSA/PSA, and other glycoconjugates having clickable functional groups. MGE using clickable building blocks makes it possible to construct macromolecules, vesicles, and cells bearing clickable functionalities [101,112,118].

Non-covalent and covalent click chemistries greatly enhance the design modalities for DDS that can assemble/disassemble and conjugate/cleave *in vivo*. Clickable PSA that can be synthesized via biosynthetic pathways and can be modified with clickable groups *in situ* hold great potential for making novel DDS. Clickable PSA may play a major role in the DDS or be present as a minor component of the formulation. For example, they can be utilized for making hydrogels through *in situ* crosslinking of clickable groups. In this regard, an injectable hydrogel was manufactured based on a thiol-X click-crosslinking strategy for sustained drug release [191]. In this DDS, thiolated CS (CS-HS) and alkyne-functionalized β-cyclodextrin (alkyne-β-CD) constitutes the major components of the injectable hydrogel, which are conjugated via a thiol-yne click reaction. Hydrogel properties can be adjusted through changing the ratio of CS-HS to alkyne-β-CD moieties. Moreover, β-CD serves as host for guest drug molecules. This injectable hydrogel utilizes two different PSA with complementary click groups, though it is possible to introduce click functionalities on similar PSA chains [192]. In the CS-HS/alkyne-β-CD hydrogel, the host–guest interactions prevent burst release. Many injectable hydrogels suffer from burst release, or release in a short period of time, because of in-

Table 3
Clickable PSA-based drug delivery platforms.

Materials	Click reaction	Outcome	Type of delivery system	Payload	Drug release behavior	Refs.
CS-HS, alkylated-β-CD	Thiol-ene	*In vivo* gelling	MITCH	Bendamustine hydrochloride	n/a	[191]
HA-HS, HP-PEG	Thiol-ene	*In vivo* gelling	MITCH	Stem cells	n/a	[165]
Alg-Nor, Alg-Tz, Laponite	iEDDA	Crosslinking	Injectable cryogel	Protein	n/a	[210]
HA-CHO	Hydrazone formation	Grafting (coating of liposome with HA)	Liposome	Porphyrin (photosensitizer)	CD44 targeted cancer PDT	[229]
MAL-PPO-PEG-PPO-MAL, Alg-furan	iEDDA	Crosslinking	Heat sensitive MITCH	Vanillin (model drug)	n/a	[230]
HA-MA, HA-HS, RFP (photo-initiator)	Thiol-ene photoclick	*In vitro* crosslinking	MITCH	Protein	n/a	[231]
HA-Cys-MA, HA-Lys-Tet	Tetrazole-alkene photo-click	Crosslinking, photoinduced fluorescence (emanating from pyrazoline cycloadducts)	Fluorescent bioresponsive nanogel	Cytochrome C and granzyme B (therapeutic proteins)	CD44 targeted cancer theranostic platform	[232]
HA-*g*-AMA, HA-*g*-Lys-MTet, Cy5	Tetrazole-alkene photoclick	Photoinduced, fluorogenic gelation	Fluorescent microgel (25-50 μm)	Cy5-labeled Herceptin (mAb)	Theranostic platform for ovarian tumor	[233]
HA-furan, HA-tyramine	Diels–Alder	Microfluidics-assisted crosslinking (click and/or enzymatic crosslinking)	Cell-laden microgels	Cell	n/a	[234]
HA-CHO, HA-HS, HA-HS-ADH heterobifunctional light-sensitive crosslinker	Hydrazone formation	*In situ* crosslinking, prodrug activation	Hydrogel	Dopamine (free encapsulated form or prodrug forms)	On-demand drug photorelease (emanating from hydrogel photodegradation or prodrug activation)	[235]
HA-BCN, JR2EK-Az peptide	SPACC	Conjugation	Supramolecular hydrogel	Hydrolyzing enzyme	n/a	[236]
Propargylated HA, Rfv, PEG-N_3	CuAAC	Conjugation (HA-c-Rfv), PEGylation	Nanogel	Hydrophobic drugs (e.g., dexamethasone, piroxicam and paclitaxel)	n/a	[237]
Dextran (hydrophilic), acetalated dextran (hydrophobic)	CuAAC	PSA-based amphiphilic block copolymer	pH-responsive self-assembled NP (~70 nm)	Hydrophobic drug (curcumin)	n/a	[238]
HA-HS, D-Ene	Thiol-ene	Gelation	Injectable theranostic hydrogel	D-Dexa, D-Cy5	Sustained delivery of corticosteroids and D-Cy5 for corneal inflammation	[239]
Radiolabeled ADIBO-PEG_4-NOTA-^{64}Cu, N_3-HGP21	SPAAC	Radiolabeling (conjugation)	HA-GO	Cy3-PNA (fluorescent probes)	Theranostic nanoplatform for targeted cancer therapy	[240]
HA-*g*-Cys-MA, HA-*g*-Lys-Tz/GALA	Photoclick	Crosslinking and nanogel creation	Nanogel	Saporin	Intracellular protein delivery	[223]
DBCO-modified heparin-quercetin conjugates	SPAAC	Azide-modified glycoconjugates (on T cells), DBCO-modified nano-assembly interactions	Nanogel 1: containing clickable sugar; Nanogel 2: DBCO-modified nano-assemblies encapsulating DOX and ZnPc	DOX (drug) and zinc phthalocyanine (photosensitizer)	Two-step click pre-targeting	[216]

sufficient interactions between drug molecules and hydrogel walls and insufficient mass transfer resistance due to the interconnected porous structure of the hydrogel [193].

A general strategy for prolonged release of drugs is to prevent convection mass transfer and to add extra resistances against molecular diffusion. For example, loading drugs in micro- or nanocapsules and dispersing them in a hydrogel add an extra resistance to diffusion and prevent burst release while also fixing them in place [194]. Similarly, coating the capsules with extra layers (e.g., via layer-by-layer assembly) increases the mass transfer resistance [195,196]. For example, an appropriate solution to prevent burst release from biodegradable poly(lactic-*co*-glycolic acid) (PLGA) microcapsules is to coat them using hydrophilic polymers such as PEG or to embed them into an injectable hydrogel before administration into the body [197].

Bioorthogonal click reactions enable *in vivo* crosslinking of a hydrogel with no (or inoffensive) side products. In addition, the bioorthogonality of click reactions means that clickable groups are inert to therapeutics, an essential quality for safe delivery of payloads with preserved bioactivities. In related research, a clickable HA, modified with trans-cyclooctene (TCO) and Tz, was utilized to make an injectable hydrogel for dexamethasone delivery [198]. It was observed that dexamethasone-loaded PLGA microspheres dispersed in click-crosslinked HA-based hydrogels outperformed in sustained delivery, compared to free PLGA or PLGA in injectable thermosensitive hydrogels based on Pluronic. In fact, physical interactions in thermosensitive hydrogels are more easily broken under mechanical stresses in the body [199]. Accordingly, injectable hydrogels based on physical crosslinking usually cannot be utilized as DDS for prolonged release, as they may disassemble under physiological conditions (e.g., under mechanical loads or biofluids). Therefore, utilization of injectable hydrogels with covalent crosslinking, which greatly improves hydrogel tolerance to environmental conditions, is of enormous importance. The first solution to address this problem is to use injectable hydrogels with both physical and chemical bonds. Physical bonds are created under physiological conditions at high rates, while covalent linkages form with low kinetics. Physical bonds may be cleaved gradually, while covalent bonds remain intact, allowing the hydrogel to preserve its integrity—a prerequisite for sustained drug release. The second approach to address this challenge draws on the merits of click reactions. Click chemistry greatly widens the scope of injectable MITCH by making it possible to create covalent bonds under mild conditions without the need of using a photoinitiator or catalyst [200]. An injectable hydrogel was fabricated based on acrylated hyaluronic acid (HA-acrylate) and HA-HS as components of MITCH [164]. The thiol-ene click reaction (Michael addition) between HS and alkene moieties on adjacent HA chains creates covalent bonds which constitute the primary crosslinked network. In addition to these covalent bonds, the gradual spontaneous oxidation of pendant sulfhydryl groups on HA chains results in disulfide bond formation. This secondary crosslinked network provides the primary network with higher stability and enhanced mechanical strength. Disulfide linkages are cleavable, denoting degradation and dynamic behavior of the hydrogel, in combination with degradability of the base HA matrix. This dual crosslinked hydrogel showed excellent cell protection *in vivo* and supported the growth of mouse fibroblast cells (L929). Indeed, injectable MITCH based on click chemistry are highly potent for therapeutics and cell delivery applications, because they do not require toxic crosslinkers, catalysts, photoinitiators/UV irradiation, or immunogenic enzymes to trigger crosslinking; furthermore, no harmful byproducts are produced during the gelation process [201].

As described in Section 3, iEDDA has emerged as a unique click reaction with exceptionally high rate and high orthogonality. Hydrogel crosslinking based on iEDDA is a highly biocompatible strategy for making injectable hydrogels for delivery of cells, therapeutics, and imaging probes. Introduction of appropriate dienes and strained dienophiles on PSA and/or crosslinkers enables the fabrication of MITCH with high biocompatibility and sufficiently low gelation times. For example, an injectable hydrogel was designed based on norbornene-modified alginate, as shown in Fig. 5 [202]. Hydrogel crosslinking was accomplished using diselenide linkers as well as crosslinkers containing tetrazine functionality. NIR irradiation generates reactive oxygen species (ROS) which cleaves the crosslinkers' diselenide bonds. This bond cleavage facilitates the on-demand release of anticancer drugs such as doxorubicin (DOX). Using crosslinkers containing clickable end groups and stimuli-responsive linkages is a general strategy for designing degradable and injectable hydrogels as well as smart DDS with on-demand drug release behavior. Stimulus-cleavable linkages combined with clickable groups provide a vast playground for material scientists to fabricate a variety of injectable hydrogels and *in situ* self-assembled nanostructures [203,204]. It should be noted that amino-yne click reactions usually result in the fabrication of polymers with stimuli-responsive linkages such as $^1O_2$-sensitive and acid-labile β-aminoacrylates [76]. These cleavable linkages can be leveraged for designing on-demand drug release systems that respond to acidic environments such as tumors or to $^1O_2$ produced by photosensitizers during PDT.

Another approach is to introduce clickable groups in the structure of therapeutic species such as drugs and proteins. Azide-functionalized alginate was clicked using an alkyne-modified quorum sensing inhibitor (QSI) to make alginate nanoparticles [205]. pH-sensitive hydrazine linkers between alginate chains and QSI result in pH-responsive drug release. Moreover, an antibiotic drug (ciprofloxacin) was incorporated into the nanogel via electrostatic interaction of ciprofloxacin with anionic side groups on alginate chains. pH alteration, which results in change in electrostatic charges and hydrazine bond cleavage, leads to co-delivery of ciprofloxacin and QSI, respectively. Biofilm formation is greatly diminished via this co-delivery nanoplatform. Furthermore, it was observed that alginate nanoparticles can penetrate deep into biofilms, indicating the effectiveness of this method for previously formed biofilms [205].

On the other hand, clickable PSA that constitute a nanogel formulation or present as coating layers on nanoparticles can be clicked with other clickable molecules for various purposes such as installing tumor-targeting moieties, polymeric shielding to evade the immune system, and introduction of stimuli-responsive linkages. Modified-PSA-coated gold (Au) nanorod was used as a pH-sensitive nanoplatform for combined photothermal chemotherapy to combat breast cancer as shown in Fig. 6 [206]. The carbodiimide chemistry and click reactions were applied sequentially to graft catechol and hydrazide functional groups on hydroxyethyl chitosan (HECS). A maleimide-containing molecule was grafted on the HECS chain followed by a click reaction between the maleimide and thiol groups of mercaptopropionylhydrazide. Catechol functionalities of HECS enable conjugation on the Au surface while hydrazide functionality provides DOX conjugation via acid-labile hydrazone linkages (Fig. 6a). Oxidized HA was further deposited via electrostatic interactions on HECS-coated (polycationic) Au nanorods, for targeting CD44 receptors that are overexpressed on cancer cells. pH-responsive release of DOX (via clipping of hydrazone linkage), good stability in aqueous solution at neutral pH, longer circulation time, and enhanced cellular internalization were observed for these nanoplatforms, interpreted as surface charge reversal phenomenon for electrostatic complexation of polyanions and polycations.

Clickable crosslinkers or other molecules can have two or more clickable functionalities that undergo click reactions under special triggers, while others are inert to those triggers. Such molecules

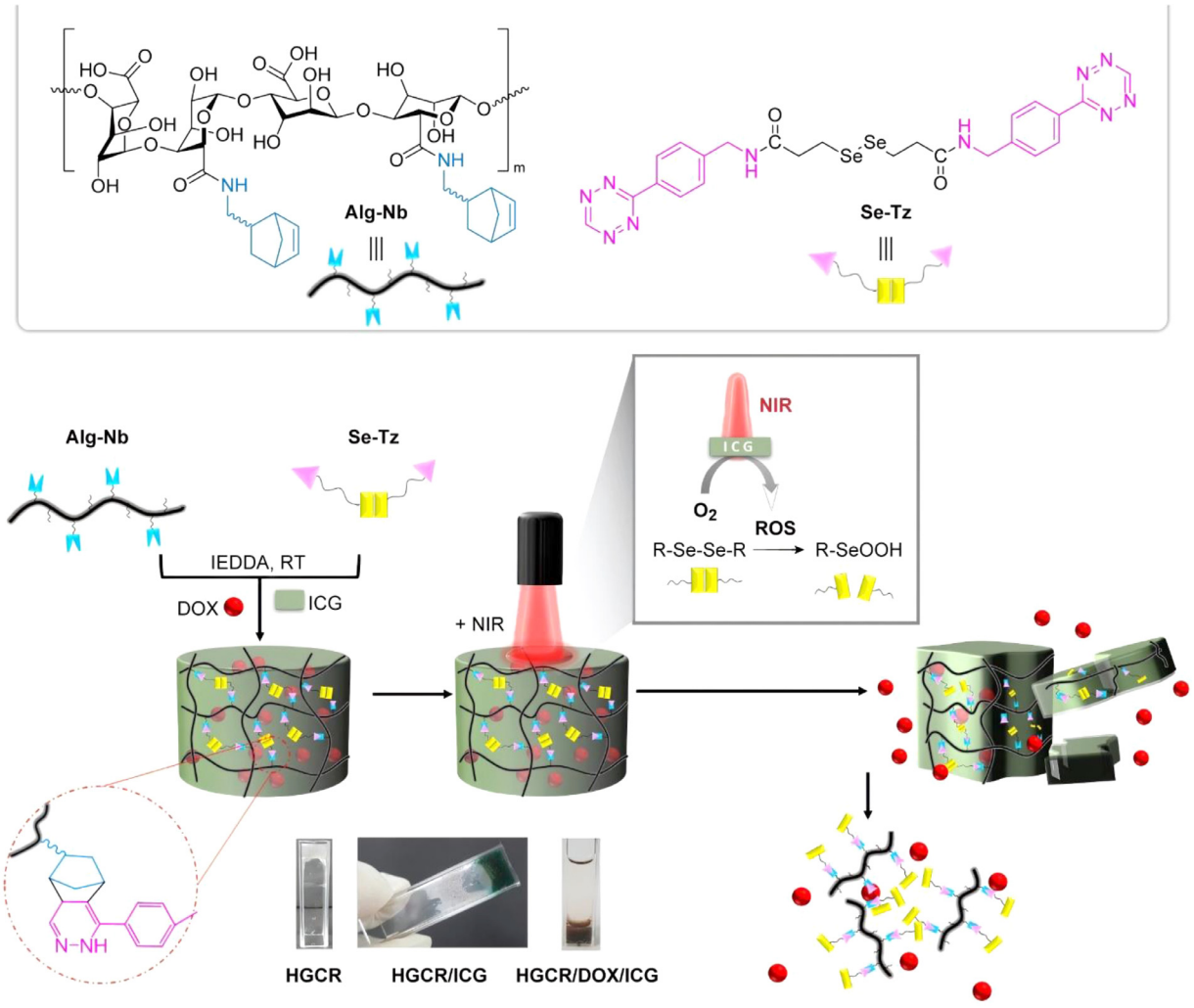


Fig. 5. Preparation process for injectable NIR-responsive hydrogels based on norbornene-modified alginate and bifunctional crosslinkers containing redox-sensitive diselenide linkages. [202], Copyright 2019. Adapted with permission from Elsevier Science Ltd.

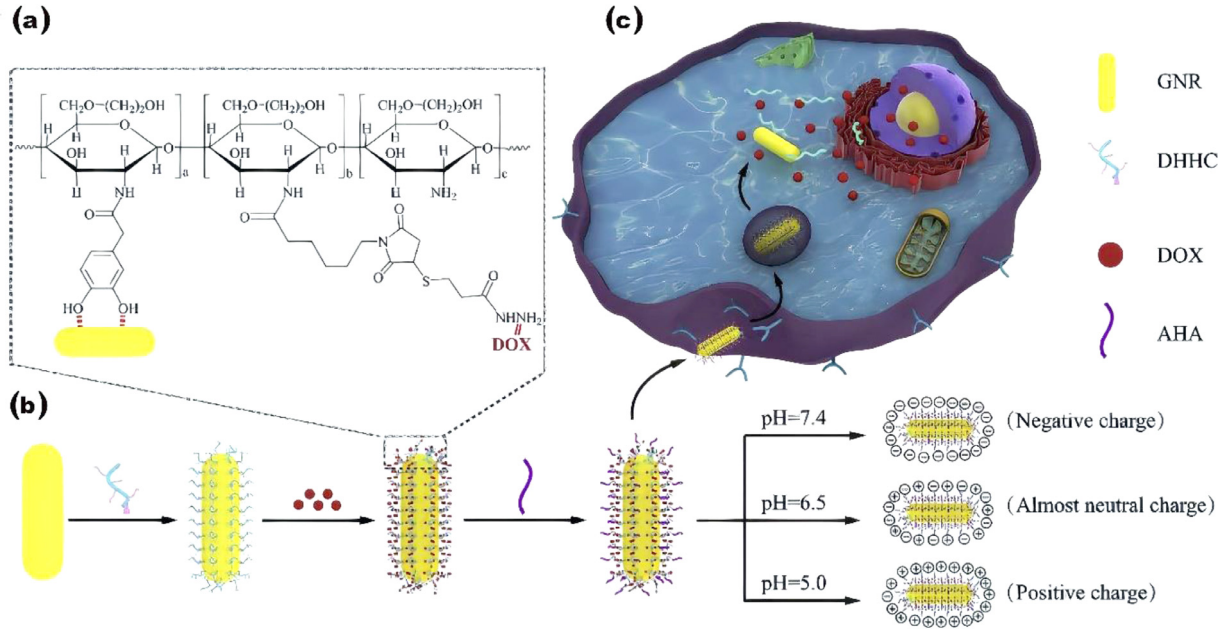


Fig. 6. Schematic illustrating the manufacturing process and application of a delivery platform. (a) Chemical structure of bifunctionalized hydroxyethyl chitosan (HECS); (b) the manufacturing pathway for HECS and oxidized hyaluronic acid (AHA) double-layer-coated Au nanorods (ANR) which are loaded with the anticancer drug; (c) DOX release and the photothermal effect in combined chemo-photothermal cancer therapy. [206], Copyright 2019. Adapted with permission from Elsevier Science Ltd.

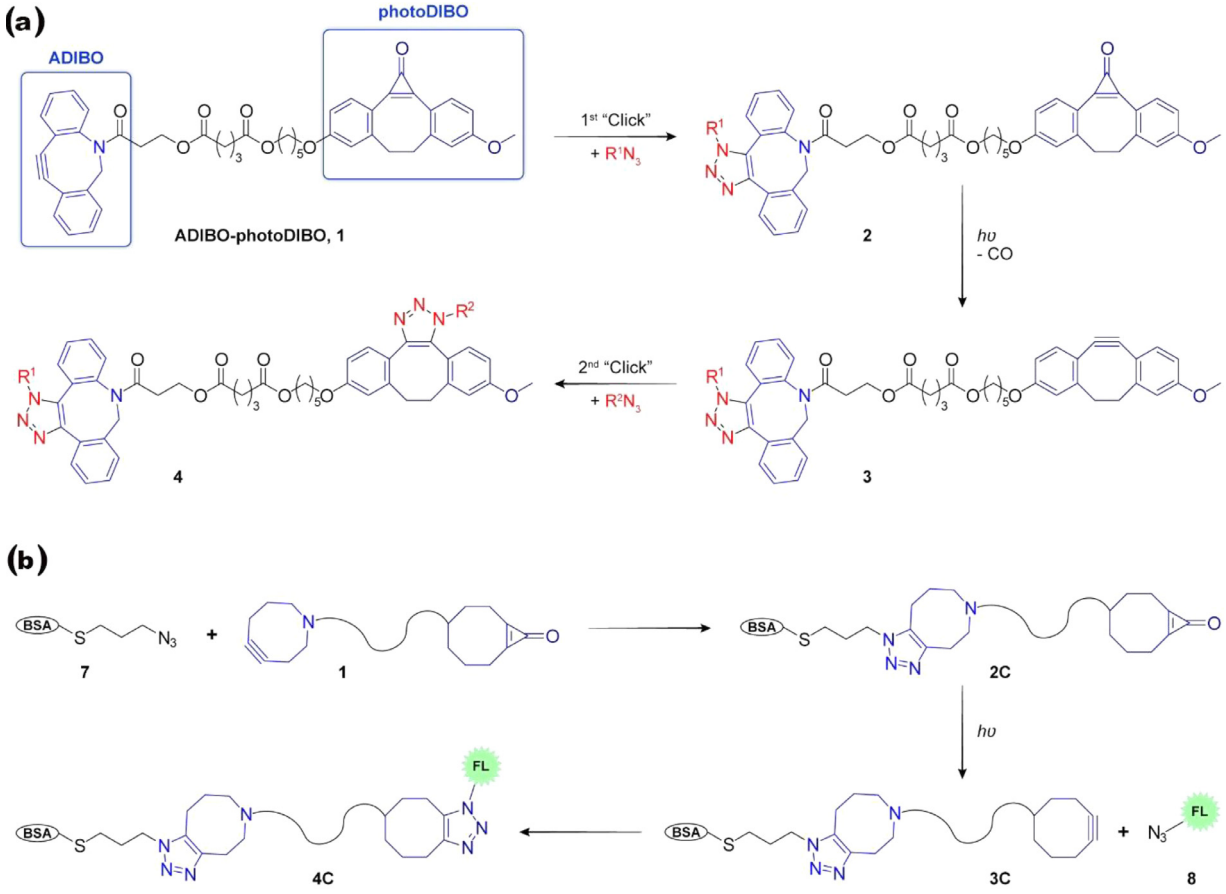


Fig. 7. Orthogonal click reactions of a heterobifunctional molecule to bind two substrates. (a) Sequential and orthogonal click and photoclick reactions on a double-clickable molecule; (b) sequential conjugation of azide-modified BSA and azide-modified fluorescent probe to a double-clickable molecule. [207], Copyright 2014. Adapted with permission from the American Chemical Society.

with multiple clickable groups can undergo sequential and orthogonal click reactions which allow complex architectural constructs to be fabricated. Such hetero(bi/tri)functional crosslinkers such as maleimide, diarylcyclooctyne, DBCO, and end-clickable groups show great promise for these applications.

For example, a heterobifunctional crosslinker containing azadibenzocyclooctyne (ADIBO) and cyclopropenone-masked dibenzocyclooctyne (photo-DIBO) end groups was used to attach two different substrates with azide functionalities via sequential click reactions, as schematically illustrated in Fig. 7 [207]. First the azide-functionalized substrate (e.g., bovine serum albumin, BSA) reacts with ADIBO through SPAAC, while photo-DIBO remains intact because of its azide-inert nature. In the second step, UV exposure triggers the photo-click reaction between photo-DIBO and the second azide-functionalized substrate (e.g., fluorescein). Such photo-click reactions allow unparallel spatiotemporal control over the coupling process [80,81,83].

It should be emphasized that using clickable crosslinker-bearing protecting groups (e.g., N-(t-butoxycarbonyl)-N-(2-(maleinimido)ethyl)glycine N-hydroxysuccinimidyl ester) or using clickable molecules containing functional groups that can undergo carbodiimide chemistry (e.g., 4-(maleimidomethyl)-benzoic acid-N-hydroxysuccinimide (NHS) ester) further enhances our ability to make clickable PSA *in vitro*. Prolonged release of therapeutic proteins in targeted sites is very important to enhance therapeutic efficiency. However, many hydrogel-based delivery systems fail to prevent burst release or preserve the bioactivity of proteins, a consequence of having an interconnected porous structure or incompatible crosslinking strategies, respectively. In this regard, development of more architecturally and chemically appropriate crosslinking strategies is critical for preserving the bioactivity of therapeutic proteins and guaranteeing their sustained release. Macromolecular crosslinkers which can create linkages through bioorthogonal chemistry are potentially the most promising candidates for delivery of proteins and other biological entities. Bioorthogonal click reactions can be used for fast crosslinking under mild reaction conditions, preventing protein denaturization and preserving the bioactivity of molecules. However, when designing delivery vehicles using click chemistry, chemical kinetics as well as *in vivo* stability of clickable groups should be carefully considered for proper safety and increased efficiency. The intracellular stability of different clickable groups differs significantly. For instance, azides are stable in the cytoplasm and remain reactive for over a day, whereas the reactivity of bicyclononynes decreases dramatically due to their limited stability in biological environments [208]. Furthermore, a number of tetrazine derivatives have been shown to retain reactivity even after 10 h of incubation in fetal bovine serum (FBS), indicating their potential stability when employed with a dienophile through the iEDDA reaction in such an environment [209]. While less stable in IEDDA bioorthogonal reactions, TCO derivatives are more reactive than tetrazines; therefore, the development of TCO derivatives with higher stability would substantially increase their utility in cells and living organisms. With that in mind, particular attention should be paid to the substituted groups as they may change the water solubility and kinetics of cycloaddition reactions of the initial reagent [209]. Karver and co-workers have shown that introducing strong EWG on tetrazines resulted in higher reaction rates while compromising

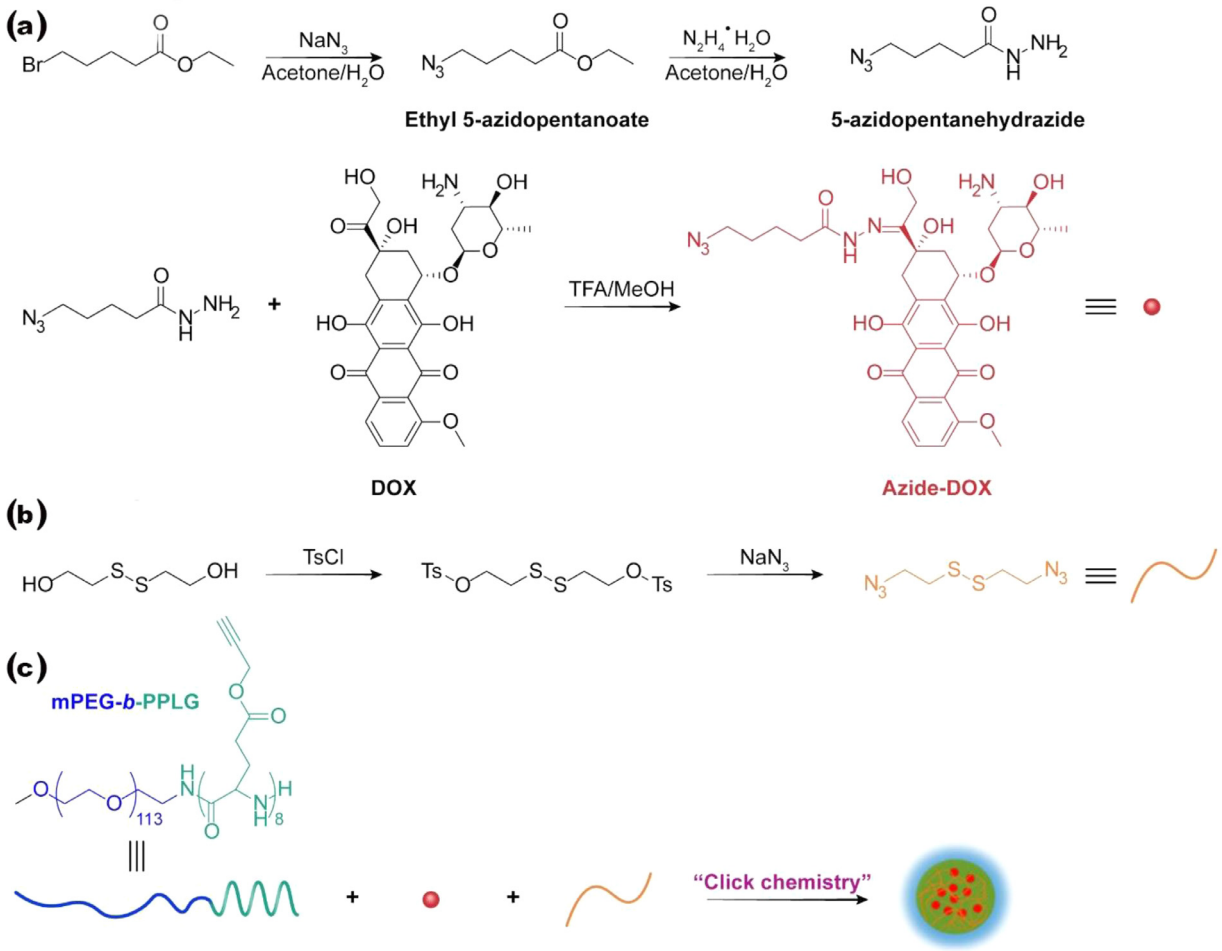


Fig. 8. Click chemistry for designing a dual-responsive delivery system. Sequential reactions for synthesis of (a) conjugated DOX molecules containing an azide end group and hydrazide linkage; (b) disulfide crosslinker; (c) nanogel formation via click reaction. [211], Copyright 2016. Adapted with permission from the American Chemical Society.

their stability and/or water solubility. Contrastingly, EDG enhanced the stability and water solubility of tetrazines while decreasing iEDDA reaction kinetics.

An injectable cryogel was manufactured based on clickable alginate for delivery of proteins [210]. In this context, an iEDDA reaction between alginate-norbornene and alginate-tetrazine creates covalent linkages between alginate chains in the cryogel structure. Proteins were adsorbed onto Laponite nanoplatelets before incorporation into the cryogel. Furthermore, the electrostatic interactions between Laponite (negatively charged) and proteins (positively charged domains) prevented the fast release of proteins. It was observed that the rate of protein release is adjustable via altering Laponite concentration. In fact, the adsorption on a substrate and embedding into nano- and microgels is a general strategy to prevent fast release of therapeutics from hydrogel-based delivery systems.

Prodrug activation is another fascinating application of click chemistry for the safe delivery of drugs with minimal side effects. The maximum tolerated dose (MTD) can be substantially increased with a prodrug, a biologically inactive substance that is metabolized in the body to produce a drug. In this method, pro-dyes (or pro-photosensitizers) or inactive forms of other therapeutics are administered instead of delivering the drug directly. After reaching the site of action (such as the tumor microenvironment or intracellular medium), the prodrug is activated using another system (free molecules, nanogels, or cells) containing complementary clickable groups. As shown in Fig. 8, a DOX-based polymeric prodrug containing a hydrazone functional group was synthesized and embedded into nanogels [211]. The nanogels were prepared via click reaction between alkyne functionalities of methoxy poly(ethylene glycol)-*b*-poly(γ-propargyl-L-glutamate) (mPEG-*b*-PPLG) with azide functional groups of 2-azidoethyl disulfide, which serve as click crosslinker. Nanogels with uniform diameter of around 60 nm were passively accumulated in the tumor site based on the EPR effect. Redox-sensitive properties of the disulfide linkage in the crosslinker structure result in reduction sensitivity of the nanogel. It was observed that this dual-responsive delivery nanoplatform was effectively internalized by HeLa and MCF-7 cancer cells, inhibiting their growth. DOX release was significantly higher in the presence of GSH and under endosomal pH (5.5) compared to physiological pH (7.4).

Another click-activatable prodrug was used for the systemic administration of chemotherapeutics with a significantly higher MTD and the possibility of multiple administrations with minimal systemic toxicity [212]. As depicted in Fig. 9, this click activated protodrugs against cancer (CAPAC) platform includes an injectable hydrogel based on tetrazine-modified HA, which is injected at the tumor site, and a prodrug administered systematically through injection. The prodrug is activated after reaction with tetrazine moieties in the hydrogel structure through an iEDDA click reaction. This *in situ* activation indicates a targeted DDS is superior to traditional targeted DDS and can significantly enhance the specificity of the delivery platforms. A phase I clinical trial of SQ3370, a new therapeutic modality consisting of a protodrug of DOX and a protodrug-

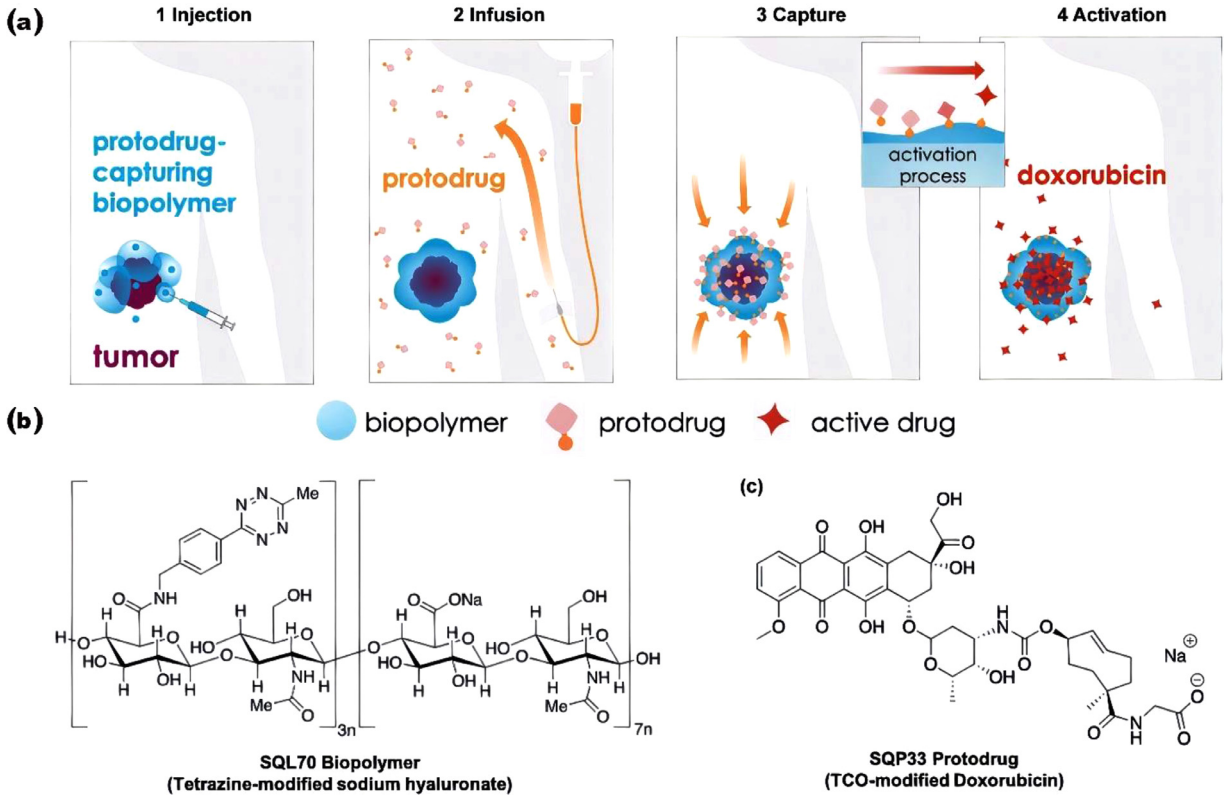


Fig. 9. Chemical structure and method of operation for a two-step delivery system based on click-activatable prodrugs and clickable PSA. (a) The mechanism of action for the CAPAC platform: (1) intratumoral injection of tetrazine-modified HA hydrogel; (2) systemic administration of protodrug; (3) protodrug capture by hydrogel followed by fast iEDDA reaction; (4) release of active drug from hydrogel after click reaction; (b) the chemical structure of tetrazine-modified HA (SQL70); (c) chemical structure of protodrug (SQP33). [215], Copyright 2021. Adapted with permission from John Wiley and Sons Inc.

activating PSA, has recently been approved by the food and drug administration (FDA) and is currently being tested in 110 participants with advanced solid tumors [213]. Furthermore, another clinical trial on 7 patients had previously shown encouraging results, indicating the potential of the CAPAC platform [214]. To date, all FDA-approved nanomedicines are based on passive targeting approaches as active targeting nanoplatforms still show limited efficacy. However, CAPAC platforms offer great promise as a highly efficient targeted delivery strategy for various therapeutics. In this approach, passive targeting via EPR is primarily used for introducing artificial clickable receptors on plasma membranes of cancer cells, which can be clicked using complementary clickable species such as prodrugs. As clickable groups are absent in natural systems, this strategy provides an effective targeting method. In contrast, traditional targeted delivery systems are based on biomarkers that can vary across patients or across tumor types, and these biomarkers may be present on both cancerous and healthy cells, indicating that traditional targeted delivery systems may also destroy healthy cells. Recently, in a similar work, the SQ3370 therapeutic, a class of CAPAC, was used to evaluate cancer treatment outcomes in a mouse model of colorectal cancer bearing two tumors [215]. These are examples of recently developed two-step click-targeting strategies.

Two-step click-targeting strategies for effective targeting of cancer cells have been widely utilized in recent years. The first step is to introduce clickable sugar units to glycoconjugates on the cellular surface using MGE. These unnatural sugars can be delivered via intratumoral injection or through systemic administration of a delivery nanoplatform containing unnatural monosaccharides, which accumulate in the tumor microenvironment based on the EPR effect. For example, Qiao and co-workers injected $Ac_4ManNAz$ intratumorally in surficial breast cancers [216]. MGE resulted in receptor-like azide functionalities on cancer cells with high density (Stage I, Fig. 10).

On the other hand, the self-assembly of DBCO-modified heparin–quercetin conjugates (DLQ) in the presence of DOX and zinc phthalocyanine (ZnPc, a photothermal therapy agent) resulted in nano-assemblies (DOX/ZnPc@DLQ) encapsulating both DOX and ZnPc. Intravenous injection of DOX/ZnPc@DLQ resulted in efficient tumor targeting as a result of the click reactions between DBCO of the nano-assemblies and azide functionalities on cancer cell surfaces (Stage II, Fig. 10). This combined chemo-photothermal therapy enhanced tumor targeting, leading to highly efficient tumor ablation.

Click-activatable photosensitizers can also be used for cancer treatment applications. For example, in a recent paper, cancer cells with artificial labels containing azide groups have been utilized to activate chlorin e6 (Ce6) [217]. In this work, artificial receptors were installed on cancer cells using an MGE approach followed by administration of DBCO and Ce6-modified nanoparticles. EPR phenomenon aid the NP to accumulate in the tumor site which enables pretargeting of tumor cells. The second part of the tumor targeting platform includes pH-sensitive NP that disassemble in the tumor microenvironment, releasing polymer chains modified with DBCO and Ce6. A click reaction between DBCO and azide groups results in anchored Ce6 on cell membranes. PDT using laser irradiation leads to ROS production and plasma membrane damage which induces cell apoptosis.

Immunotherapy and recruitment of immune system cells is a promising strategy to combat cancer cells [218]. To this end, MGE was utilized to install azide-bearing moieties onto the surfaces of cancer cells and T cells. Then the azide functional groups on cancer cells and T cells were modified with β-CD and adamantyl trimers, respectively, using the click reaction (Fig. 11). Furthermore, an MGE

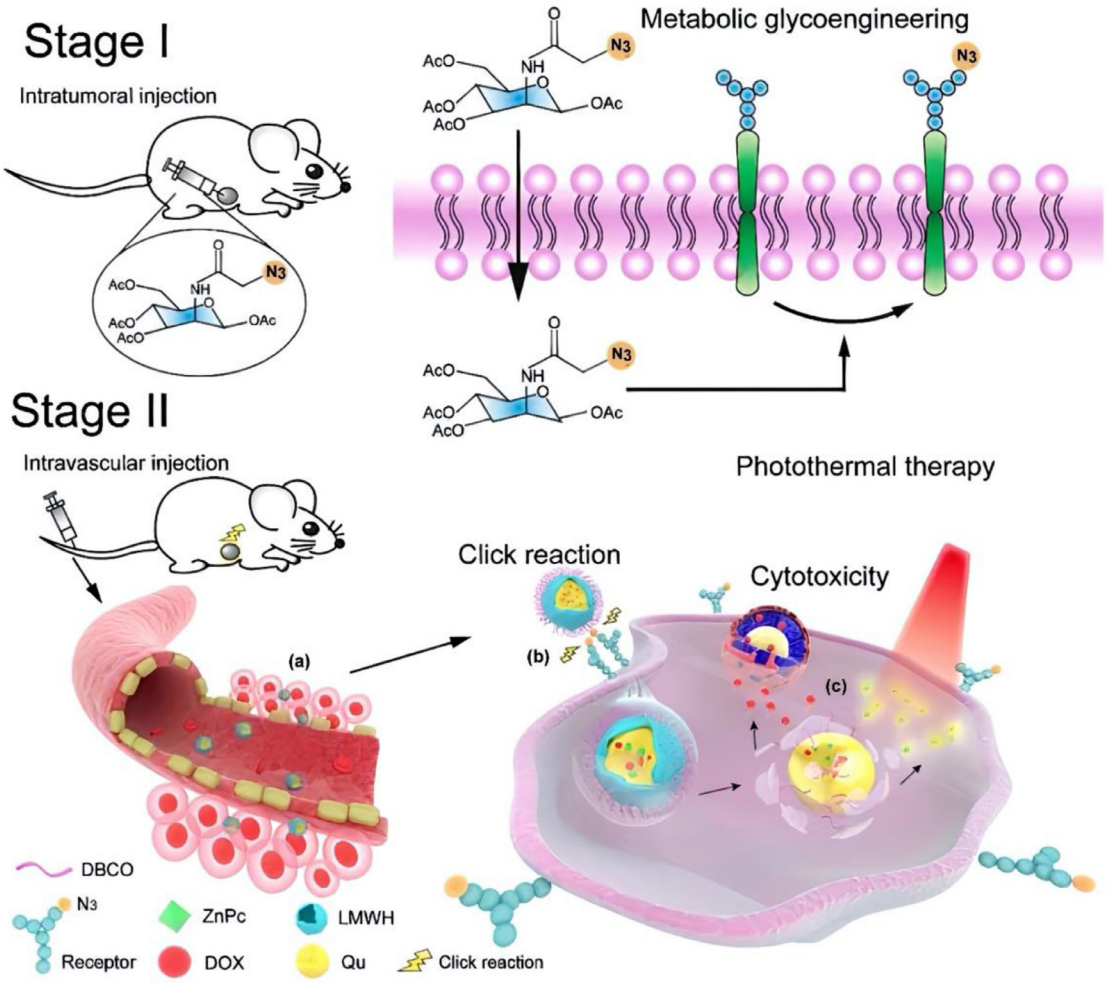


Fig. 10. The mechanism of action of a two-step click-targeting delivery system. Stage I: introducing azide groups on plasma membranes of cancer cells by MGE. Stage II: (A) passive targeting of a delivery nanoplatform containing unnatural sugars at the tumor site via the EPR effect; (B) active chemical targeting of clickable artificial receptors on cancer cells by clickable nano-assemblies containing DOX and synergistic chemo-phototherapy of tumors under NIR irradiation. [216], Copyright 2020. Reproduced with permission. Open Access distributed under the terms of the Creative Commons Attribution License CC BY 4.0.

approach can be utilized to install tumor-targeting ligands on natural killer cells [219].

In a similar work, specific non-covalent click interactions between complementary trimers on cancer and T cells increased interleukin-2 (IL-2) cytokine secretion, which activated natural killer (NK) cells and resulted in cancer cell lysis [220]. As illustrated in Fig. 11, MGE was utilized to functionalize complementary clickable groups on tumor and T cells. This shows a general strategy to manipulate cell–cell interactions via a synthetic, non-covalent chemistry with high specificity similar to the interactions between cell membrane receptors and their specific ligands.

Bioorthogonal chemistry was also used for *in vivo* tracking of transplanted cells via click-mediated labeling using fluorescent dyes. For example, T cells labeled with NIR fluorescent dye offered a direct labeling method for monitoring T cell trafficking, and ultimately, immunotherapeutic outcome [109]. Moreover, other cells such as EPCs have been labeled and subsequently tracked using a similar strategy [110].

Delivery of heat shock protein 70-targeting siRNA (siHSP70) in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) can combat TRAIL-resistant tumor. However, co-delivery using a single DDS can be challenging because they should be released in different locations—on the cell surface for TRAIL and in the cytosol for siHSP70. This cascade delivery of different therapeutics requires a more complex formulation, architecturally and chemically. Disassembly of physical interactions (e.g., charge neutralization or reversal) or bond-cleavage in chemical interactions play pivotal roles in these hierarchical assemblies.

Various endogenous and exogenous stimuli should be considered when designing hierarchical assemblies. Physicochemical and biological cues in the inter- and intracellular microenvironment (e.g., pH, redox species, temperature, enzymes, and signaling molecules) and exposure to exogenous stimulants (e.g., light, ultrasonic waves, electric or magnetic fields) affect the physical and chemical interactions [222]. Accordingly, assembly/disassembly of such structures is affected. Chemical bonds are more difficult to cleave than physical bonds, indicating that harsher conditions are needed to clip ordinary covalent bonds. However, bond cleavage in dynamic covalent bonds and reversible click reactions is more feasible [172]. A hierarchical modular assembly was designed and fabricated for co-delivery of positively charged TRAIL and negatively charged siHSP70 [221]. As shown in Fig. 12, the nanoplatform core includes cationic liposomes decorated with clickable hooks, i.e., ADIBO functionalities.

Clickable ADIBO was installed on distearyl phosphatidyl ethanolamine (DSPE) and cholesterol (Chol) molecules. Clickable HA was grafted onto liposomes, containing siHSP70 and decorated with TRAIL, through a SPAAC reaction where HA serves as the tumor targeting species. The tumor microenvironment is rich with enzymes such as matrix metalloproteinase 2 (MMP2) and

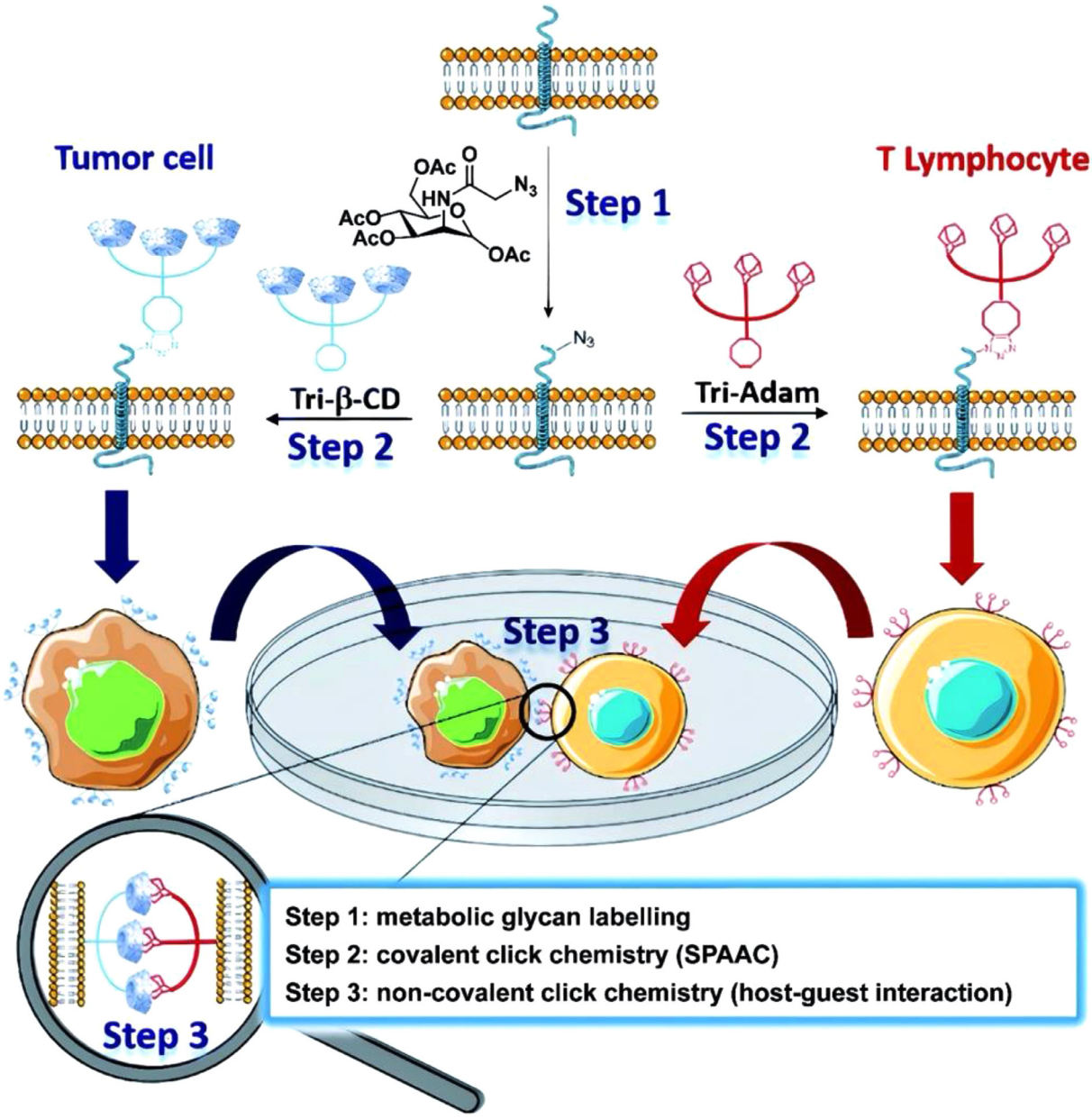


Fig. 11. Cell membrane engineering to install host–guest molecular recognition moieties. Step 1: MGE to label cell membranes with clickable azide functional groups. Step 2: immobilization of clickable markers, containing β-CD and adamantyl trimers, on plasma membranes of tumor and immune cells, respectively, via click conjugation. Step 3: Cell–cell interactions via multivalent host–guest interactions. [220], Copyright 2010. Reproduced with permission from the Royal Society of Chemistry.

hyaluronidase. MMP2 cleaves the sensitive peptide linkage, releasing the TRAIL and resulting in cell death via affecting death receptors. Moreover, CD44 receptors mediate liposome internalization.

In addition to cell targeting and internalization for the management of various diseases, cytosolic transportation of therapeutic proteins is also crucial. However, cellular uptake of large proteins is limited, and endosomal escape presents a challenge. As shown in Fig. 13, a microfluidic apparatus followed by photo-click crosslinking was used to fabricate an HA-based click nanogel for the intracellular delivery of large proteins [223]. The microfluidic setup enabled efficient mixing of HA-*g*-cystamine-methacrylate (HA-*g*-Cys-MA), HA-*g*-lysine-tetrazole (HA-*g*-Lys-Tz)/GALA, and saporin. These HA-based nanogels displayed higher cellular uptake and endosomal escape.

To control the release of Au NP from alginate hydrogel, a coiled coil affinity-based system based on two peptides was designed in which the polymer chains were chemically modified through an azide-alkyne Huisgen cycloaddition (AAC) [224]. In this system, pre-click strategies were utilized to graft K-coil onto alginate. Alkyne-modified alginate was clicked by azido-homoalanine K-coil. Moreover, complementary E-coil peptides in the cysteine-tagged E-coil–epidermal growth factor (EGF) conjugates were immobilized onto Au NP through thiol-Au interactions.

Specific coiled coil affinity interactions between complementary peptides, a type of multivalent interaction, enable effective stabilization of Au NP in polymer matrix and prevent their uncontrolled release. These NP also behave as nanoscale crosslinkers or compatibilizers that lead to gelling. Another ingenious application of click chemistry in delivery of therapeutics is its ability to induce *in situ* self-assembly of clickable polymers, enabling deep penetration into solid tumors [225]. In other words, in this approach, macromolecules are delivered to the tumor site, in contrast to tradi-

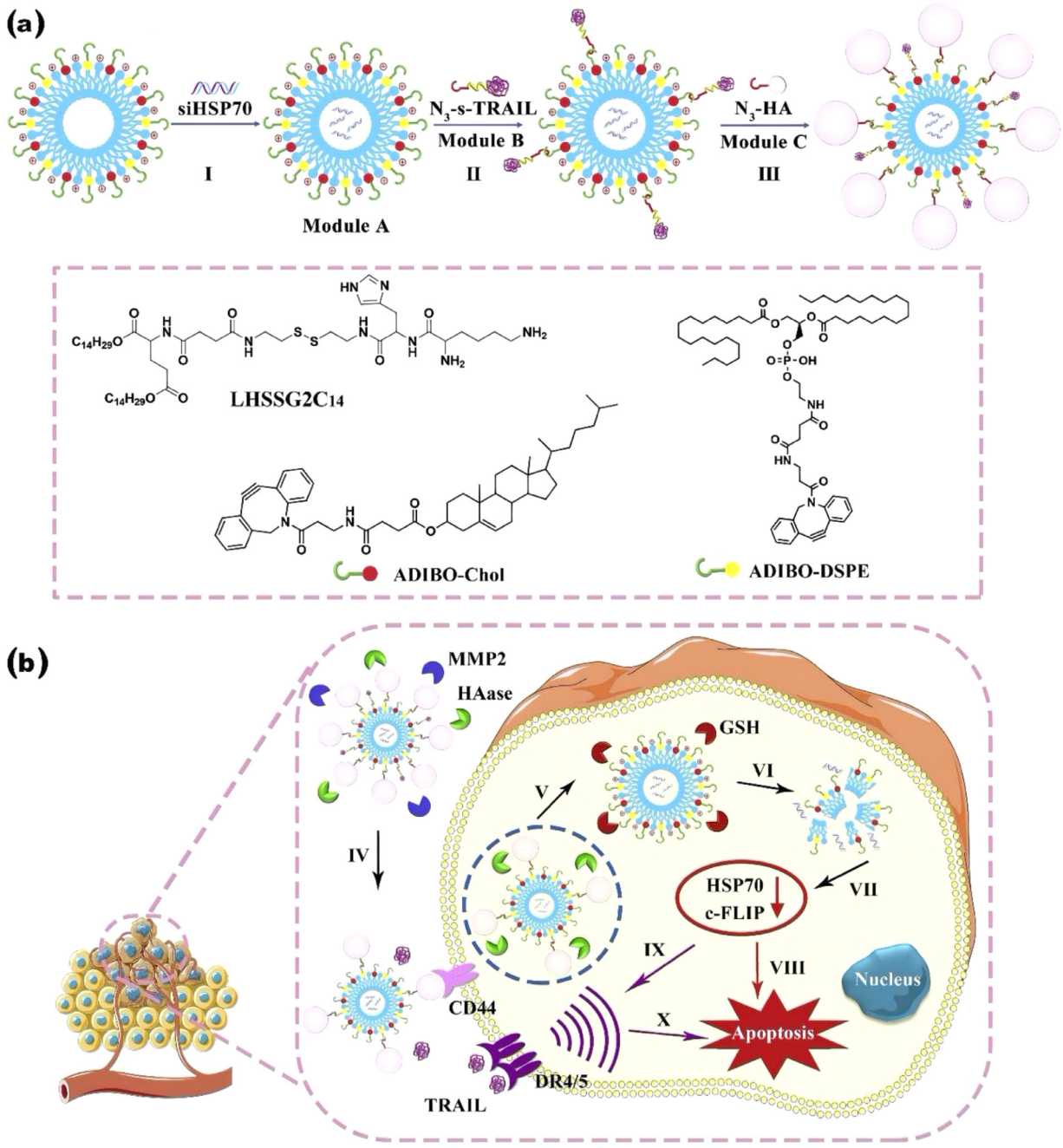


Fig. 12. Schematic representing the manufacturing process of a targeted delivery system. (a) The synthesis pathway for modular nano-assembly with hierarchical structure. (b) TRAIL release (IV) triggered by enzymes in tumor microenvironment, followed by active targeting and cell internalization; endosomal escape (V) after enzymatic degradation of the HA shell and redox-mediated release of siHSP70 (VI) in intracellular space; RNAi (VII) and gene downregulation (VIII) resulting in apoptosis; enhanced TRAIL-induced cancer cell apoptosis (IX) and enhanced apoptosis induced by synergy between TRAIL and siHSP70. [221], Copyright 2019. Adapted with permission from Elsevier Science Ltd.

tional delivery systems based on nanoplatforms (e.g., nanoparticles or nanogels) which enables enhanced diffusion into solid tumors. This strategy allows the creation of dynamic nanomedicines with morphology-adaptable features that enhance our ability to provide more robust drug delivery and imaging platforms *in situ* [226,227]. For example, an iEDDA click reaction in cooperation with an enzymatic reaction resulted in *in situ* self-assembly which enabled pretargeted multimodality imaging [204]. Both covalent and non-covalent click chemistry are invaluable tools for *in situ* fabrication of nano-assemblies. Controlled morphology transformation (CMT), *in vivo*, would revolutionize our ability to make novel delivery and bioimaging platforms [228]. *In vivo* CMT approaches based on click chemistry originate from association/dissociation or bond formation/cleavage. Clickable PSA can potentially be key polymers for making *in situ* self-assembled nanostructures.

5.2. Tissue engineering

Polymeric scaffolds, and especially injectable hydrogels, are promising candidates for non-invasive TE applications [241,242]. As mentioned earlier, bioorthogonal click chemistry enables the production of diverse forms of MITCH from just a few click reactions between various clickable (bio)(macro)molecules. End- or side-chain functionalization of PSA/OSA (or their derivatives) with

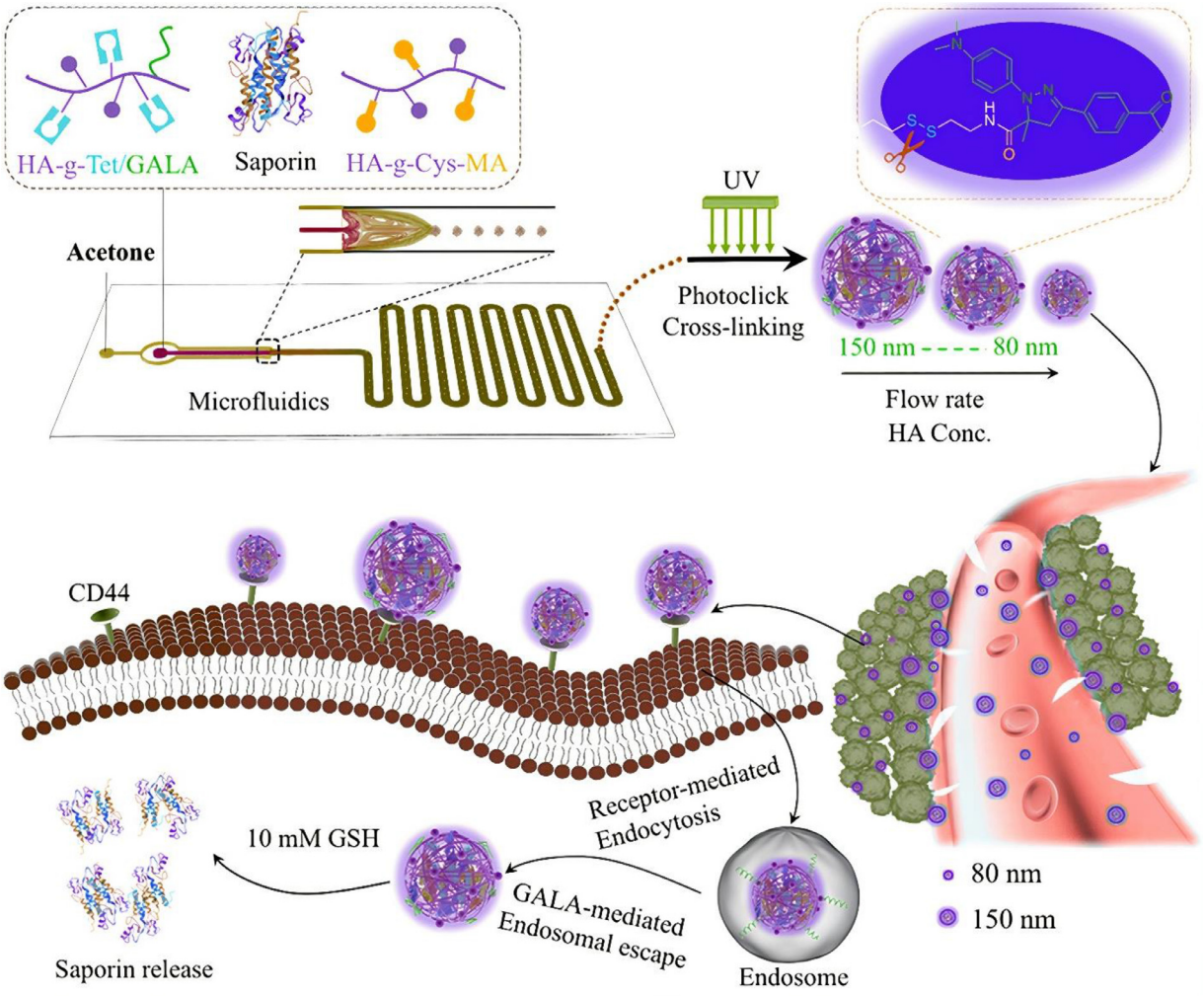


Fig. 13. Schematic illustrating the manufacturing process for photo-induced click-crosslinked nanogels in a microfluidic device. Nanogels fabricated from HA derivatives (HA-*g*-cystamine-methacrylate (HA-*g*-Cys-MA) and HA-*g*-Tet/GALA) by employing a microfluidic approach and catalyst-free photo-click reaction (top). Active targeting of cancer cells via CD44 receptors. GALA (pH-sensitive fusogenic peptide)-mediated endosomal escape followed by intracellular delivery of therapeutic proteins (bottom). [223], Copyright 2019. Adapted with permission from the American Chemical Society.

clickable groups results in diverse types of injectable hydrogels. In addition, versatile clickable crosslinking agents further enhance our ability to design hydrogels with a wide range of physicochemical properties and biological activities. Clickable crosslinkers can have two or more similar (bi- or trifunctional) or different (heterobifunctional) clickable groups, stimuli-cleavable linkages (e.g., disulfide), biodegradable fragments (oligo- or polyesters and peptides), and hydrophilic or amphiphilic segments (e.g., oligo(ethylene glycol), poloxamers). They may also have special (macro)molecular architectures (e.g., 4-arm PEG, star-shaped polymers, dendrimers, or bottlebrush polymers) or may have functional groups enabling carbodiimide chemistry. On the other hand, organic/inorganic clickable additives such as nanofibers, nanotubes, nanoplatelets, nanoclusters, and nanoparticles can be incorporated to adjust mechanical, electrical, or morphological features of the obtained hydrogel. These features enable the design of a wide variety of injectable hydrogels with tailor-made properties like gelation time, dynamic mechanical properties, morphological transformation, and degradation rate. Such hydrogels with diverse properties create an invaluable source of biomaterials for TE applications. This section overviews the clickable PSA that have been utilized for such applications.

Many clickable polymers based on both synthetic and naturally derived polymers have been used to fabricate TE constructs [9]. However, clickable PSA, which offer several advantages such as versatility, biocompatibility, biodegradation, and non-immunogenicity, are especially important for such applications. Bioorthogonal click reactions, contrary to traditional chemical crosslinking strategies, enable the immobilization of bioactive molecules such as growth factors (GF) and the effective encapsulation of cells, while preserving their bioactivity and viability. In these systems, the release of bioactive molecules from the cell secretome in diseased or damaged tissues has been shown to be beneficial by stimulating tissue regeneration [243–245]. In addition to delivering cells and spheroids, click-crosslinked scaffolds have been shown to control cell fate due to their biomechanical properties [175,246].

An HA-based injectable hydrogel was crosslinked via iEDDA and utilized for cartilage TE [247]. This hydrogel contains a chondrogenic differentiation factor and human periodontal ligament stem cells. The mechanical properties of this MITCH are significantly higher than those of neat HA because of covalent bonding, which is highly important for cartilage TE. On the other hand, the hydrogel has a high biocompatibility, suitable biodegradation, and preserves the bioactivity of the chondrogenic differentiation factor for an extended time. This work highlights the importance of the bioorthogonal iEDDA reaction, which does not disturb the bioactivity of bi-

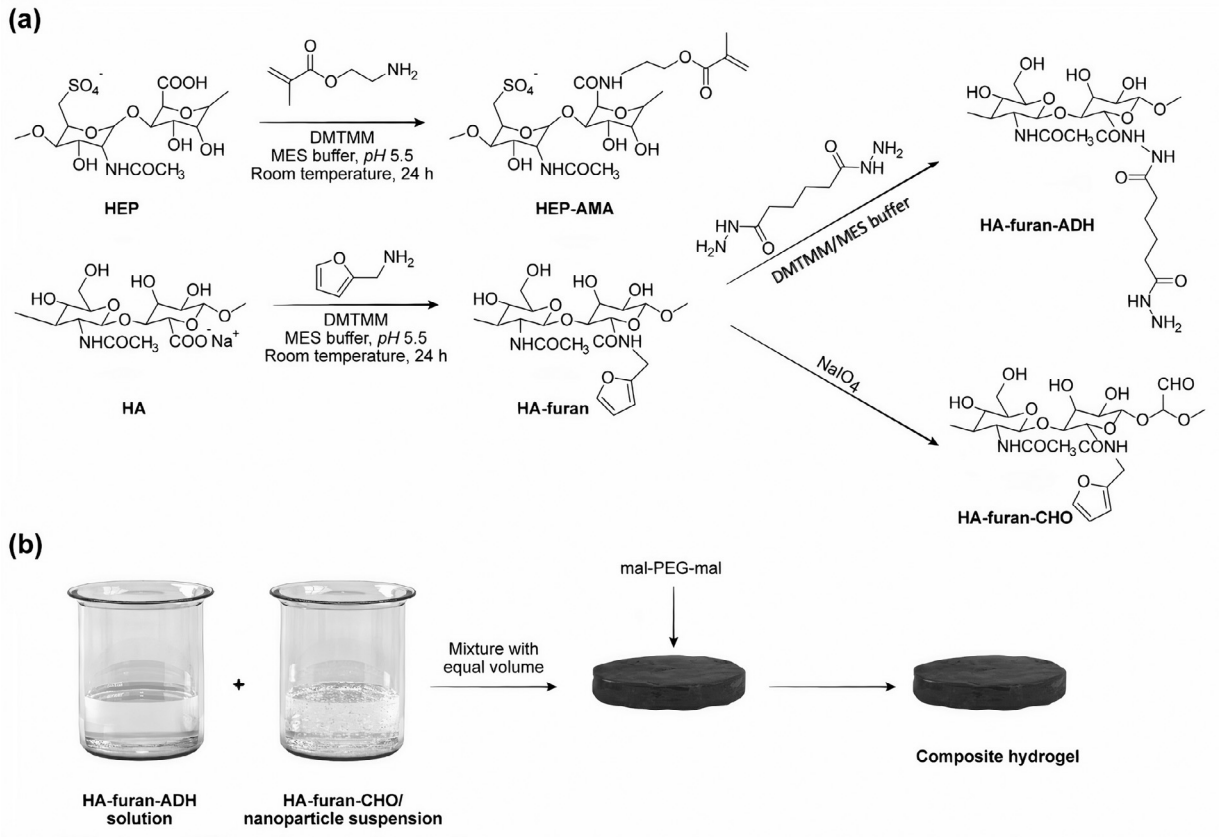


Fig. 14. Manufacturing process for the fabrication of a pH-responsive and injectable composite hydrogel based on click-modified HA and PEG. (a) Synthetic pathway to make HA derivatives. (b) Mixing-induced gelation to create a composite hydrogel. [248], Copyright 2019. Adapted with permission. Open Access distributed under the terms of the Creative Commons Attribution License CC BY 4.0.

ological entities while enabling appropriate mechanical properties for TE.

Multifunctional hydrogels show great promise in a wide range of biomedical applications. For instance, a multifunctional composite hydrogel containing pH-sensitive nanogels was designed for TE [248]. pH-responsive nanogels were fabricated with acetylated β-cyclodextrin (Ac-β-CD). However, a small fraction of methacrylate-modified heparin was used in this formulation to preserve the bioactivity of the loaded GF. Polymerizable methacrylate groups were introduced in the heparin structure to make a semi-interpenetrating polymer network (semi-IPN) nanogel. The injectable hydrogel was manufactured based on click-crosslinked HA. Furan-modified HA (HA-furan) was separately modified to introduce hydrazide and aldehyde functional groups, resulting in HA-furan-ADH and HA-furan-CHO, respectively (Fig. 14). Nanogels were dispersed in aqueous solution containing HA-furan-CHO. Upon contact of aqueous solution containing HA-furan-CHO and HA-furan-ADH, the aldehyde and hydrazide groups on adjacent HA chains reacted to produce pH-sensitive acyl-hydrazone linkages, i.e., the first crosslinked network. Prior to HA gelation (i.e., before or at the start of acyl-hydrazone linkage creation) maleimide-modified PEG was added to the reaction mixture to make a composite hydrogel. Accordingly, the DA click reaction between furan functional groups of HA and maleimide-modified PEG resulted in the second crosslinking mechanism. This reversible DA click chemistry allowed creation of dynamic covalent bonds, indicating the dynamic behavior of the double-crosslinked hydrogel. Double crosslinking (via non-covalent, covalent, or a combination of both) is a general strategy to enhance mechanical properties (stiffness, toughness, tensile strength, etc.) and introduce dynamic features (stimuli-responsiveness, self-healing, and degradation) into the hydrogel.

Biomaterial implants are widely utilized in TE applications where biofilm formation can often result in serious problems [249]. Accordingly, antimicrobial coatings are important in the development of biomaterials implants. CuAAC in aqueous medium was utilized to conjugate clickable CS with AMP [250]. AMP are important constituents of the innate immune system and affect the lipid membranes of microbes, with remarkable specificity even at low concentration [251,252]. This makes the immobilization of AMP on medical devices an interesting strategy to prevent biofilm formation. Stallmann and co-workers have shown that Dhvar-5, a synthetic AMP that mimics the salivary histatins (rich in histidine), can effectively kill various bacterial species while being safe to mammalian cells [253]. This group previously managed to synthesize Dhvar-5–CS conjugates via the chemoselective CuAAC reaction [254]. The resulting Dhvar-5–CS conjugates were spin-coated on Au substrates to produce thin coatings with excellent antimicrobial properties [250].

Cell-instructive biomaterials play a pivotal role in the regulation of cell behavior and morphogenesis [255,256]. In fact, regardless of bioactive molecules such as GF, the dynamic fibrous architecture of the ECM greatly affects the behavior (e.g., spreading, growth, migration, differentiation, and proliferation) and fate of cells. Therefore, biomimetic fibrous scaffolds and multiphasic gel-in-gel materials with tunable viscoelastic properties have been shown to be capable of adjusting cell behavior [257]. Clickable PSA can be used to fabricate cell-instructive hydrogels with tunable gelation and degradation properties, both *in vitro* and *in vivo*. In this context, clickable alginate was utilized to make an IPN, using a combina-

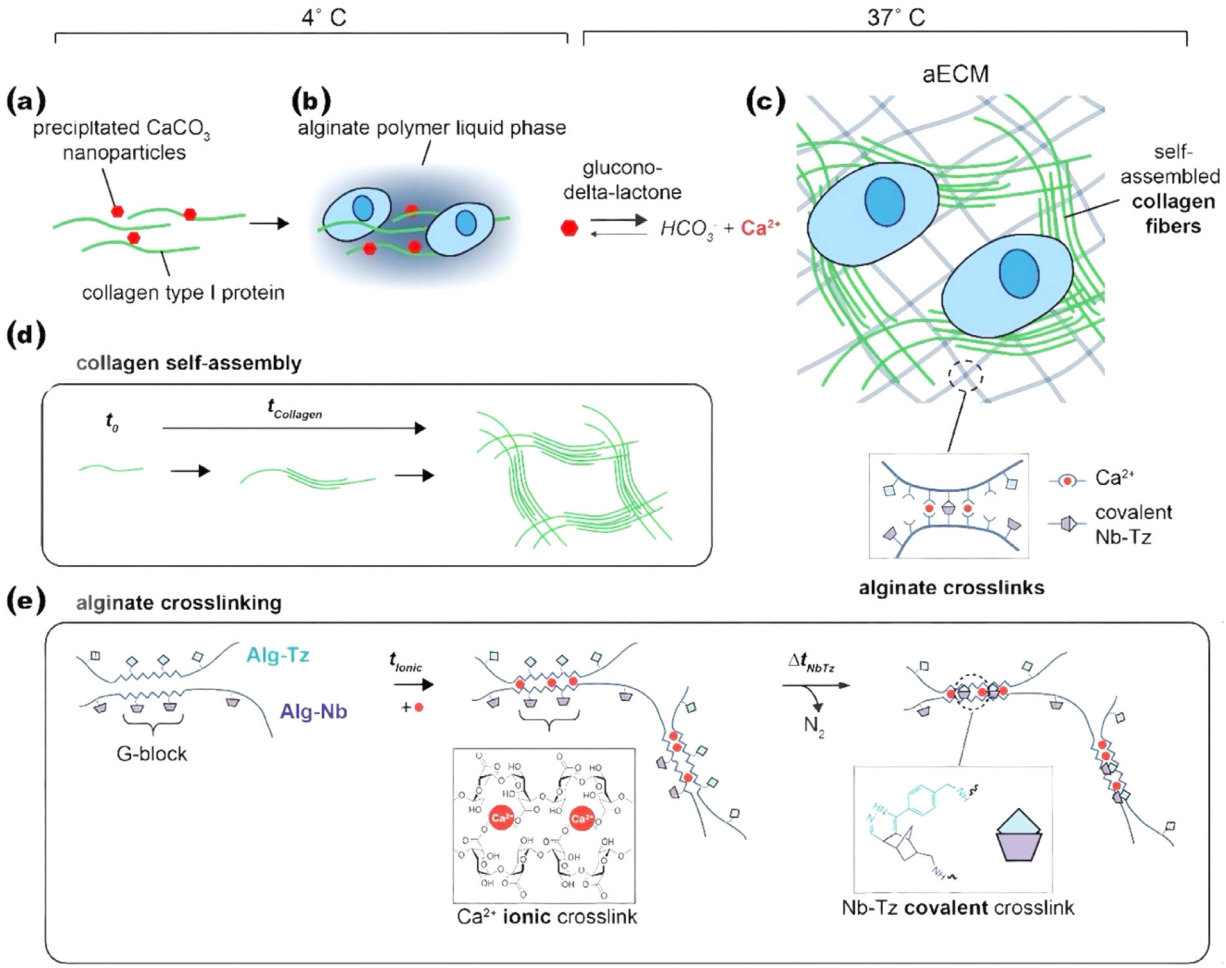


Fig. 15. Semi-synthetic ECM with a hierarchical structure. (a) Mixing collagen type I with $CaCO_3$ nanoparticles at 4°C and (b) introducing cell-laden alginate hydrogel to the mixture at 4°C and pH 7.4. (c) By adding a weak acid that triggers the release of calcium ions from nanoparticles, the ionic crosslinking of alginate starts creating a fibrous hydrogel. Regardless of ionic crosslinking (red dots), there are click-mediated covalent crosslinks between alginate chains that create a double-crosslinked network. (d) Self-assembly process for collagen fibers over time. (e) Sequential ionic (physical, faster) and click (covalent, slower) crosslinking of alginate hydrogel. [170], Copyright 2019. Adapted with permission from Elsevier Science Ltd.

tion of ionic (responsible for the dynamic viscoelasticity) and covalent (responsible for the stiffness and elastic behavior) crosslinking strategies to adjust the mechanical properties of the hydrogel and mimic the mechanical features of the native ECM [170]. In addition, fibrillar collagen type I (Col 1) was also embedded in the hydrogel to further imitate the structural features of the native ECM. This sequential crosslinking, which starts with ionic crosslinking and continues with click reactions, gradually increases the mechanical properties of the hydrogel while its mass transfer mechanism gradually changes from convection to diffusion (Fig. 15). Specifically, at an early stage, where minor ionic crosslinking is created, convection mass transfer is possible with relative ease (i.e., viscous fluid flow). But gradually, with formation of the hydrogel system (i.e., crosslinked network), fluid flow is significantly prohibited, and molecular diffusion becomes the prevailing mass transfer mechanism through the hydrogel. The motility and assembly of bulky Col 1 macromolecules can be carried out at early stages, but their movement is nearly impossible at later stages after the crosslinked network has been formed. Ionic crosslinking of alginate chains proceeds relatively quickly; after that, norbornene and tetrazine functionalities on adjacent alginate chains react with the relatively slower kinetics of iEDDA. However, care must be taken when introducing clickable groups to avoid high functional group density which may prevent ionic crosslinking of alginate. Immunomodulatory paracrine markers were measured in human MSC-laden hydrogels to evaluate the effect of mechanical cues on gene expression of cells, i.e., cell-instructive behavior. The results showed that the viscoelastic properties and stiffness of hydrogels induce various effects on the encapsulated cells as confirmed by gene expression analysis. This two-step crosslinking strategy for injectable hydrogels can be beneficial as physical and relatively loose crosslinkers can be replaced by more robust covalent linkages, preventing hydrogels from fracturing under physiological conditions. For example, ionic crosslinking is gradually cleaved because of ion release to the hydrogel environment; in other words, they serve as sacrificial crosslinkers over a long period of time. Other crosslinkers with appropriate biocompatibility, such as genipin, have been previously used for making ionic/covalent dual-crosslinked hydrogels [258]. However, click chemistry outperforms these strategies because of its excellent biocompatibility, bioorthogonality, and range of crosslinkers.

Another cell-instructive hydrogel was used for viable cell encapsulation based on methylfuran-modified HA [259]. Methylfuran-modified HA is a more electron-rich clickable HA derivative compared to furan-modified HA, such that the rate of DA click is significantly enhanced under physiological conditions. Moreover, the rate of retro DA was also enhanced. The DA reaction between methylfuran-HA and maleimide-modified PEG enables three dimensional (3D) cell encapsulation. Computational analysis on the click reaction revealed that the geometry of the transition state

Table 4
Clickable PSA-based systems for TE.

Base materials	Click reaction	Click aim	Type of tissue scaffolds	Payload	(Potential) application	Refs.
Alg-Nor, Alg-Tz	iEDDA, thiol-ene	Covalent crosslinking (iEDDA), post-gelation modification using cell adhesive peptides (thiol-ene)	Injectable hydrogels (MITCH)	Cells	Cell culture, cell delivery	[176]
Gel-furan, HA- furan CnS, MAL-PEG-MAL	DA	Crosslinking	Semi-IPN hydrogel	n/a	Cartilage TE	[270]
HA-Tz, 4-arm PEG-TCO	iEDDA	Crosslinking	MITCH	BMP2, BMSC	Bone TE, bioinks	[271]
HA-furan, MAL-PEG-MAL	DA	Crosslinking	Double-crosslinked hydrogel	Cells	Cartilage TE	[272]
HA-furan-ADH, HA-furan-CHO	DA	Crosslinking	Double-crosslinked hydrogel	n/a	Cartilage TE	[273]
HA-CBT, HA- D-Cys	Click condensation reaction	Crosslinking	Injectable hydrogel	Keratinocytes	n/a	[274]
Aminooxy-PEG- aminooxy, HA-CHO	Oxime ligation	Crosslinking	Injectable hydrogel	Schwann cells	CNS, PNS	[275]
HA-furan, MAL-PEG-MAL	DA	Crosslinking	Injectable hydrogel	BDNF	Spinal cord injury	[276]
CS-N_3, alkylated-PEG	Copper free AAC	Crosslinking	Injectable hydrogel	MSC culture	TE	[277]
Dex-DBCO, azide-Dex-PA	SPAAC	Crosslinking	Injectable hydrogel	Cells	Cell encapsulation and TE	[278]
HA-furan, MAL-PEG-MAL	DA	Crosslinking	Injectable hydrogel	Cell culture	Soft TE	[279]

and unexpected interactions associated with hydrogen bonding are contributing factors in the kinetics of the DA reaction.

Cell-based therapies are important strategies for tissue regeneration and wound healing applications [201,260]. Cells possess highly efficient sensors that can recognize various physicochemical, mechanical, and biological cues in their immediate microenvironment. Accordingly, designing biomimetic delivery platforms greatly affects the efficacy of cell-based therapies. Injectable hydrogels with high biocompatibility and tunable mechanical properties can enhance the viability of encapsulated cells and affect their secretome. Click reactions provide a robust alternative crosslinking strategy over conventional methods that utilize toxic crosslinkers.

Thiolated HA was used to make an injectable and electroactive hydrogel for subcutaneous delivery of adipose-derived mesenchymal stem cells (ADSC) [261,262]. The base polymer, a hyperbranched poly(β-amino ester) (PBAE) containing alkene end groups, was modified with electroconductive tetra-aniline (TA) moieties to make conductive PBAE-TA. Hyperbranched PBAE was fabricated using the reaction between PEGDA and cystamine bearing disulfide linkages which can trigger and adjust the biodegradation of the hydrogel. PBAE-TA possesses good water solubility, and self-assembly of hydrophobic AT moieties creates AT cores coated by PBAE shells. After injection of HA-HS and PBAE-TA, the thiol-ene click reaction resulted in crosslinking and gelling of the hydrogel. Compared to oligoanilines, polyaniline possesses higher electrical conductivity [263–265]. Accordingly, polyaniline and its nanocomposites have been widely used in various applications [266–268]. However, compared to polyaniline, oligoanilines benefit from greater biocompatibility which makes them more appealing for *in vivo* applications [269]. Other clickable PSA-based systems that have been used in TE are summarized in Table 4.

5.3. Wound healing

Skin is the first line of defense against infection and dehydration, and is crucial to wound management [280]. Polymeric scaffolds and hydrogels have been widely utilized to manage acute and chronic wounds [281]. Furthermore, some of the hydrogels have been incorporated with bioactive molecules and/or cells or spheroids to enhance the therapeutic outcome [260]. In fact, wound dressings are experiencing a gradual shift from passive to active dressings containing bioactive and cell-instructive cues. Physicochemical, mechanical, biological, and structural features of scaffolds (e.g., water content, exudate absorption, stiffness, antimicrobial properties, porosity for gas exchange) and release behavior of encapsulated or produced bioactive molecules greatly affect the efficacy of the wound management dressing [282,283]. An injectable hydrogel that can fill wounds, preserve the moist environments in the wound area, absorb wound exudates, combat bacteria, and release bioactive molecules on demand is a powerful, novel strategy for wound management. Moreover, naturally derived PSA, such as alginate and CS and their derivatives, have been widely utilized as biomaterials for making wound management hydrogels [284].

Click chemistry, which enables *in situ* crosslinking of injectable hydrogels with no byproducts (or minor inoffensive ones, like nitrogen), safe immobilization of biological cues into scaffolds, viable encapsulation of cells, and on-demand release of payloads would greatly affect the development of novel wound dressings. Click reactions provide a vast playground for designing injectable hydrogels with tailor-made properties. As clickable groups are absent in biological systems, they do not interfere with biological processes. Clickable OSA/PSA, representing major components of wound dressings, can be used to fabricate MITCH and coat clickable fibrous scaffolds under mild conditions without the use of toxic crosslinkers.

HA is an important biomaterial that plays a key role in the natural healing process of wounds, and this has resulted in extensive utilization of HA in wound dressing formulation [285]. To promote diabetic wound healing, injectable hydrogels based on clickable HA and containing thiol moieties have been used to controllably deliver ADSC [165]. Macromolecular hyperbranched PEG, containing multiple acrylate functionalities (HP-PEG) were utilized as crosslinker for *in situ* crosslinking of the HA-HS, using thiol-ene click chemistry (Fig. 16). The HP-PEG was manufactured via *in situ* reversible addition fragmentation chain-transfer polymerization (RAFT). The obtained hydrogel showed adjustable mechanical and good anti-fouling and non-swelling features. Moreover, it was observed that these clicked hydrogels preserve the stemness of ADSC and do not adversely affect their secretome. The hydrogels prevent inflammation in the diabetic wound site and aid the processes of angiogenesis and re-epithelialization. These features can be interpreted based on the superior features of hydrogel dressings and bioorthogonal click reactions that do not trigger inflammatory reactions.

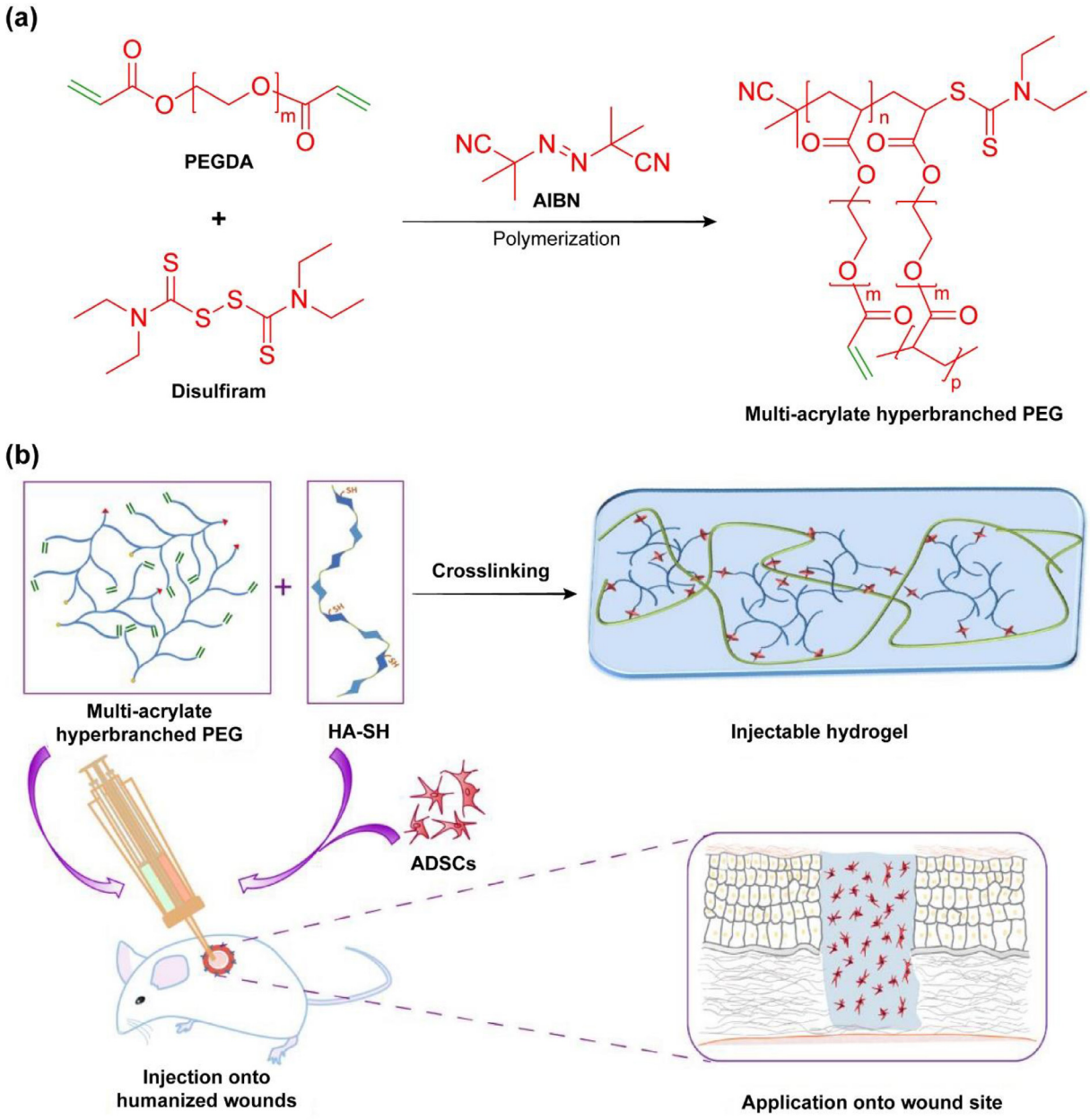


Fig. 16. Injectable hydrogels for stem cell delivery based on click chemistry. (a) The polymerization process for synthesis of macromolecular HP-PEG with acrylate side chains. (b) *In situ* crosslinking of HA-SH and acrylated HP-PEG via click chemistry for delivery of stem cells in the diabetic wound area. [165], Copyright 2018. Adapted with permission from Elsevier Science Ltd.

A moldable hydrogel was manufactured based on clickable HA for wound healing applications [286]. Maleimide-modified HA was synthesized using carbodiimide-mediated chemistry for further functionalization with bisphosphonate (BP) groups via a thiol-maleimide click reaction, leading to HA-BP. Using metal ions, HA-BP can crosslink and form a supramolecular hydrogel since BP residues on the polymer chains possess a high ion chelation strength.

Dynamic metal–ligand coordination bonds are created after addition of silver (Ag) ions, resulting in moldable supramolecular hydrogels that can be used for filling wound cavities. Moreover, the gradual release of Ag^{+} ions from supramolecular hydrogels results in antimicrobial properties and gradual dissociation of the hydrogel, which is important for managing infected wounds. Modification of HA chains via click chemistry ensures no byproducts and mild reaction conditions. Accordingly, no costly purification step is required and there is no risk of harmful or toxic trace reagents that might result in inflammation. In fact, click reactions that produce reduced byproducts are highly valuable tools for modification or *in situ* gelation of biomaterials for *in vivo* applications.

As discussed earlier in Section 5.2, Wei and co-workers have constructed a conductive injectable hydrogel based on PBAE-TA and HA-HS for cell delivery applications [261]. As shown in Fig. 17, they also utilized MITCH for diabetic wound healing applications but with some modifications [287]. In this work, sequential crosslinking of the hydrogel was carried out using a click (i.e., thiol-ene) and an enzymatic reaction with fast and slow kinetics, respectively. The fast thiol-ene reaction between PBAE-TA and HA-HS created the first crosslinked network with electroconductive properties that originated from TA moieties. Fast gelation via click reaction ensured integrity and appropriate mechanical properties for the injected hydrogel under physiological conditions, i.e., in the presence of biofluids, enzymes, and mechanical loads. Vanillin (Van) was grafted onto gelatin chains and was incorporated into

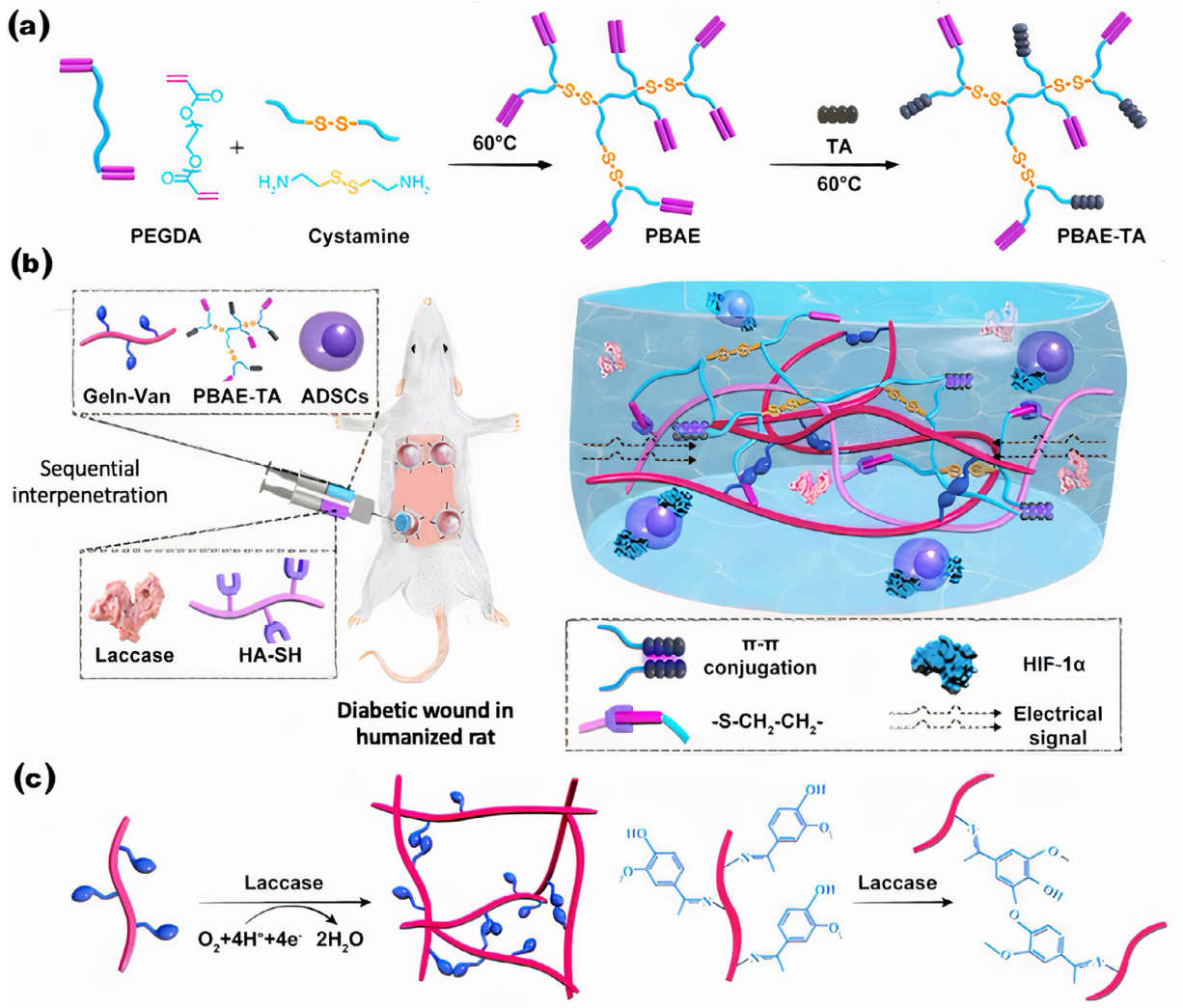


Fig. 17. Synthesis pathways and mechanism of operation of a cell-laden injectable hydrogel based on clickable polysaccharides. (a) Synthetic pathway for the synthesis of a conductive PBAE-TA macromolecular structure containing multiple acrylate and TA chain-end functionalities and several redox-sensitive disulfide linkages. (b) Sequential click (fast) and enzymatic (slow) crosslinking of an injectable hydrogel containing stem cells. (c) Mechanism for formation of hypoxic microenvironment. [287], Copyright 2020. Adapted with permission from the American Chemical Society.

the PBAE-TA formulation; ADSC were also embedded into a PBAE-AT/Gel-Van solution. Cell-laden hydrogels for wound healing not only affect the wound microenvironment but also impact the cellular behavior as cells can absorb or release radicals or other biological cues that play an important role in different stages of healing. Redox species such as TA can scavenge excessive ROS created during early stages of healing, which is beneficial to the healing process. O_2 shortage, known as hypoxia, also affects cell behavior, such that hypoxia-inducible hydrogels have gained much attention in TE [288]. In fact, hypoxia is involved in poor vascularization, development of cancer, and diabetic wound occurrence [289–291]. Laccase was embedded into HA-HS to mediate an enzymatic reaction for O_2 consumption resulting in an oxygen-deficient microenvironment. In fact, laccase and Gel-Van undergo a slow reaction, resulting in the creation of a hypoxic microenvironment. In contrast to click reactions, the slow enzymatic reaction ensures sustained hypoxia for a long period of time. It was previously reported that hypoxia enhances the proliferation and gene expression of ADSC, resulting in an improved wound healing process [292]. Induced hypoxia enhances the expression of hypoxia-inducible factor-1α (HIF-1α) and connexin 43 (Cx43) secreted by ADSC. HIF-1α expression, which plays a critical role in inflammation, cell metabolism, cell migration, wound hemostasis and remodeling, and angiogenesis, is stable only if the hypoxia condition is met [290,293,294]. In addition, it affects the expression of vascular endothelial growth factor (VEGF). Fast gelation via a bioorthogonal reaction carried out under mild conditions does not affect the enzymatic O_2 consumption reaction and its reagents. In other words, clickable functional groups do not interfere chemically with enzymes and other biological species present in the wound area.

Another MITCH based on thiolated HA and a multifunctional branched PEG-based copolymer was used as antimicrobial wound dressing [295]. The thiol-ene click reaction resulted in *in situ* gelation of the composite hydrogel containing an antimicrobial agent (silver sulfadiazine, SSD) and human ADSC. Sustained delivery of SSD enabled enduring antibacterial activity for the hydrogel dressing. Bioorthogonal click crosslinking ensures the viability of encapsulated cells and does not interfere with biological processes in the wound area.

5.4. 3D printing and bioprinting

Bioinks must fulfill the requirements of 3D printing, have appropriate mechanical and physicochemical properties, and be bio-

compatible. Chemistry of the base materials plays a critical role in designing bioinks for 3D printing applications [296]. The gelation features and time-dependent viscoelastic properties of bioinks, which arise from physical and/or chemical interactions, have intense effects on both the static and the dynamic behavior of the resulting constructs. Chemical crosslinking makes robust, usually irreversible, covalent bonds with strong mechanical properties while physical interactions are generally weaker and dynamic in nature and are created and destroyed much faster. Therefore, using a combination of physical and chemical crosslinking strategies is recommended when designing bioinks with tailor-made properties that may be time dependent [297]. Stimuli that trigger the association/dissociation of physical bonds (change in temperature or acidity) or the creation/cleavage of covalent bonds (light irradiation or water) govern the formation process as well as the properties, dynamic features, and degradation of the obtained 3D-printed constructs.

The bioorthogonality and fast kinetics of click reactions, which produce covalent bonds under mild conditions, are especially important for creating robust bioinks with high mechanical properties. Compared to conventional methods requiring photoinitiators and UV exposure to trigger covalent bond formation, copper-free click reactions require no toxic initiators or harmful light exposure [298,299]. Moreover, these orthogonal reactions produce no byproducts (or only non-toxic byproducts) indicating minor interferences with biological entities in the cell microenvironment. Furthermore, a few click reactions enable us to design a wide variety of clickable bioinks with tunable physicochemical and mechanical properties originating from versatility in crosslinkers. In other words, various small molecules, or macromolecules (possibly of various chemical structures) containing homo/heterogeneous click functional groups can be designed to adjust click reaction kinetics and physicochemical and mechanical properties of the resulting constructs. Clickable bioinks, therefore, represent a significant opportunity in bioprinting applications.

Polymeric hydrogels, especially PSA-based hydrogels, have been a major component of bioink formulations [300,301]. Clickable PSA are promising candidates for making novel bioinks with adjustable viscoelastic properties because they can constitute a major component in injectable hydrogels. They can be crosslinked either by another PSA bearing complementary clickable groups or a (macromolecular) crosslinker having clickable functionalities. For example, a two-component bioink based on HA was used for 3D extrusion printing [302]. The gelation mechanism for this system includes a click reaction between hydrazide and aldehyde resulting in hydrazone linkage formation while water is produced as a byproduct. In another work, 4-arm PEG containing 4 acrylate end groups (ene moieties) was used as the macromolecular clickable crosslinker for thiolated HA and gelatin for bioprinting vessel-like constructs [303]. The quad-functional clickable groups on 4-arm PEG significantly enhanced the mechanical properties of the hydrogel compared to difunctional PEG diacrylate (PEGDA) crosslinkers. This clickable hydrogel enables bioprinting of high-cell-density suspensions.

Clickable HA-based hydrogels were designed and manufactured for 3D bioprinting applications [192]. Part of HA was modified with a thiol-containing molecule while the other part was functionalized with methacrylic anhydride to obtain HA-HS and HA-MA polymers. The mechanical properties and degradation rate of the hydrogel is precisely adjustable with the thiol:ene ratio. Methacrylate polymerization and thiol-ene click coupling results in a double-crosslinked hydrogel network suitable for 3D bioprinting.

Photoactivated materials can be instrumental in 3D bioprinting [304]. Photo-triggered reactions benefit from unparalleled spatiotemporal control over architectural features of the 3D-printed constructs. Click chemistry also enables the safe and viable encapsulation of various cell lines. Collectively, photoactivated click reactions will likely play critical roles in manufacturing 3D-(bio)printed constructs with high resolution and finely tuned properties. We encourage advanced readers to look at a review article that was recently published on the applications of hydrogels crosslinked by click chemistry for extrusion-based bioprinting [305]. This article discusses how click chemistry can be exploited to make novel bioinks with tailored properties such as adjusted gelation time and degradation rate.

6. Concluding remarks and future perspective

The emergence of click chemistry has fundamentally changed our perspective on chemical reactions. Due to its extremely selective, versatile, and biocompatible nature, bioorthogonal click chemistry has been employed with great success in complex biological systems such as living cells and tissues. Click chemistry allows chemical reactions to occur in biological environments (e.g., blood vessels, ECM) and cellular compartments (e.g., in mitochondria or endosome) and greatly broadens the potential capabilities of biomaterials for *in vivo* applications. Biomaterials can be synthesized and manipulated and used in biological systems—in contrast to conventional systems in which biomaterials may be manufactured *in vitro* under harsh reaction conditions and requiring costly purification processes.

Currently, the idea of making biomaterials *in vivo* with the aid of cellular synthetic machinery is comparable to the emergence of mRNA-based vaccines (e.g., for COVID-19) on the global market [262]. In these vaccines, spike proteins are made using the machinery of the host cells, in contrast to conventional vaccines in which spike proteins are made *in vitro* and then administered into the body. Furthermore, MGE strategies enable the construction of artificial glycans, or glycoconjugates, which have unnatural monosaccharides with special functionalities that facilitate click reactions. This *in situ* fabrication of clickable PSA and clickable glycoconjugates allows chemistry to be performed inside living organisms, with high specificity and no offensive byproducts. Furthermore, naturally derived PSA can be modified using chemical modification strategies to prepare a wide spectrum of clickable OSA/PSA.

Bioorthogonal click reactions using clickable PSA comprise a versatile platform for designing complex molecular and architectural constructs for the biomedical field. Clickable PSA and glycoconjugates have brought about the design of novel targeted DDS for cancer therapy relying on orthogonal click interactions instead of physiological biomarkers. In other words, artificial receptors, based on click-functionalized glycoconjugates, are used to coat the outer surfaces of cancerous cells. These clickable receptors can only interact with deliberately administered complementary clickable groups since no native clickable functional groups exist in biological molecules in mammalian cells or ECM. This strategy can revolutionize targeted delivery platforms and the future of cancer treatment. The versatility of clickable OSA/PSA allows for the construction of a wide variety of macromolecular and architectural structures that are invaluable biomaterials sources for designing novel DDS. Various DDS with morphological transformation features can be made using covalent and non-covalent click chemistry and reversible click chemistry. In other words, clickable PSA make possible the creation and disassembly of nanoplatforms at the site of action (e.g., the tumor microenvironment). Added to this is the potential to harness both endo- and exogenous stimuli to adjust the degradation and release of the click-crosslinked PSA-based hydrogels at multiple scales.

Above all, an extensive variety of injectable hydrogels with finely tunable physicochemical, mechanical, and biological properties can be fabricated for minimally invasive TE, wound healing, and 3D bioprinting applications. The major advantages of click

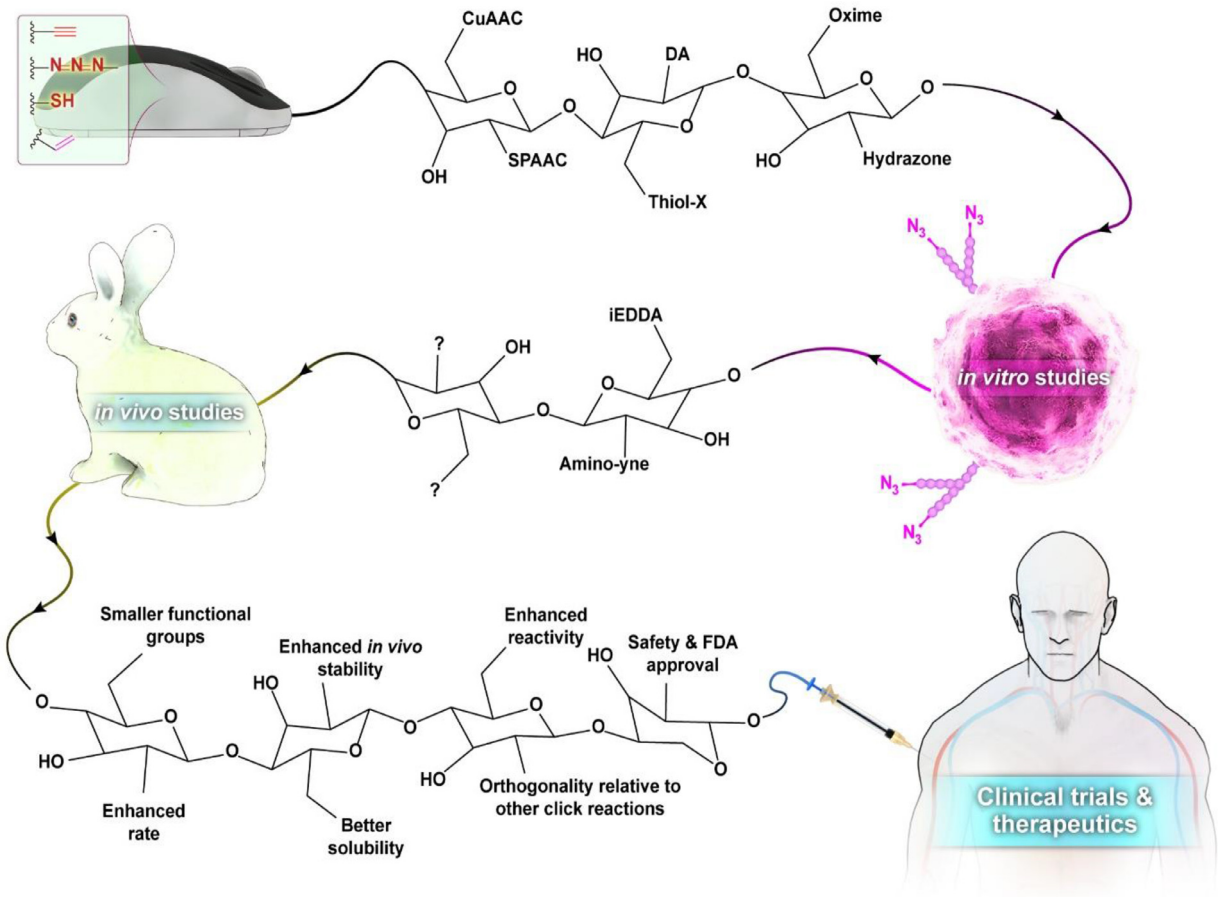


Fig. 18. Current status and future directions. Schematic depicting an overview of the current state and key requirements for PSA-based click chemistries, especially metal-free, in the biomedical field (TE, bioimaging, drug delivery/development, etc.) prior to being translated into clinical practices. Well-established and emerging click reactions (e.g., CuAAC, SPAAC, Oxime, Hydrazone) can be leveraged to design functional, biocompatible, and biodegradable clickable PSA with improved solubility, specificity, and higher reaction rates. The selection, optimization, and biological evaluations of PSA-based materials, including *in vitro* and biostability studies, are crucial to move to preclinical testing and demonstrate their safety and efficacy. Once preclinical research is complete, PSA-based material candidates must move on to clinical trials according to rigorous standards set forth by the FDA.

modification of PSA when designing PSA-based injectable hydrogels are chemical reactions performed under mild conditions with high yield and the generation of safe byproducts without costly and laborious purification processes. *In situ* gelation of such hydrogels should not interfere with other biological processes nor result in severe immune responses, unlike conventional crosslinking agents that can be cytotoxic even at low concentrations. Furthermore, clickable PSA-based bioinks with tailored rheological properties and gelation time are invaluable biomaterials for 3D bioprinting applications.

While clickable PSA-based biomaterials are exceptionally valuable building blocks in the biomedical field, many applications yet remain to be explored. We predict a paradigm shift from PSA-based to clickable-PSA-based biomaterials in the future, as these functionalities have the potential to greatly expand the applicability of PSA in medicine.

Lastly, although there have been tremendous advances in click chemistry with great potential for biomedical applications (Fig. 18), a number of challenges still remain, particularly in the arena of PSA. These challenges and opportunities are described as follows:

- While click chemistry is well-established, further research is necessary for the development of new click reactions with higher selectivity, smaller clickable functional groups, and higher reaction rates under ambient or physiological conditions. Investigating more stable yet reactive, chemically versatile, inexpensive, and safer clickable groups that can react with high specificity is an open field of research. Moreover, the production process for clickable materials such as azides should be redesigned for enhanced safety and greener procedures. Additionally, the use of artificial intelligence (AI)-based methodologies can potentially help chemists and materials scientists to discover new and improved clickable groups, a field of research that has yet to be explored [306]. AI-based strategies combined with high throughput screening should allow scientists to find and select functional groups from safe and sustainable sources [307].
- Clickable PSA can be leveraged for the design of multifunctional and injectable hydrogels for TE and 3D bioprinting. However, the gelation temperature, gelation time, and rheological properties should be finely tuned to meet the physicochemical and biological requirements of such applications.
- Most click chemistry studies have been designed from a chemical perspective; however, for biomedical applications, click reactions are often performed within complex biological environments. Organic and inorganic components of cells such as enzymes and redox-active moieties can potentially alter not only the stability and reactivity of clickable groups, but also the reaction yield, kinetics, and regiospecificity. Therefore, while there is a significant body of work in such complex environments, as we build and innovate with a broader wealth of new click chemistries, more research is needed to further investigate reaction rates, bioorthogonality, and ultimately biocompatibility.

- While click chemistry is considered to be bioinert, safety evaluations should be further considered in light of new or recent chemistries such as amino-yne click reactions. Furthermore, high concentrations of clickable moieties can be potentially harmful to cells and a better understanding of the effect of functional group concentration on cell behavior should be performed. For instance, Ac_4ManNAz, an azide-containing metabolic glycoprotein labeling reagent, was shown to be safe for cells at low concentrations but harmful at higher concentrations [308].
- Any solid components and solutes produced during the degradation process of materials made with clickable PSA need to be better identified and characterized. For instance, the hydrolytic or enzymatic degradation of clicked PSA has been shown to produce harmful byproducts such as triazole, an enzyme inhibitor [309]. Additionally, from a risk assessment and regulatory perspective, comprehensive testing in biological systems and toxicokinetic studies of degradation products are required before moving to clinical trials.
- Click chemistry-based approaches have the potential to improve the utility and outcomes of various treatment modalities such as cancer therapy. For instance, a number of therapeutics (e.g., small molecules, antibodies, GF) with adverse side effects or a limited therapeutic window may benefit from utilizing a PSA-based click chemistry approach, thereby improving efficacy. Preliminary data from a recent phase I clinical trial of SQ3370 against advanced solid tumors have suggested that a click chemistry-based approach to activate DOX at the tumor sites could work in humans with improved safety and efficacy when compared to DOX alone [213,310]. This finding opens up an array of possibilities for future click chemistry-based therapies. To this end, more research is required to spur new innovative ways in which these click chemistry-based strategies could be applied to new or existing technologies for their rapid translation from bench-to-bedside, and ultimately to improve patient outcomes and reduce healthcare expenditures.

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.

CRediT authorship contribution statement

Mohsen Khodadadi Yazdi: Conceptualization, Writing – original draft. **S. Mohammad Sajadi:** Investigation. **Farzad Seidi:** Validation. **Navid Rabiee:** Methodology. **Yousef Fatahi:** Methodology. **Mohammad Rabiee:** Validation. **C.D. Midhun Dominic:** Investigation. **Payam Zarrintaj:** Data curation. **Krzysztof Formela:** Methodology. **Mohammad Reza Saeb:** Conceptualization, Supervision. **Sidi A. Bencherif:** Conceptualization, Supervision, Writing – review & editing.

Data availability

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

Acknowledgement

S.A.B. acknowledges support from the National Institutes of Health (NIH, 1R01EB027705 award) and the National Science Foundation (NSF CAREER award, DMR 1847843). The authors would like to thank Hossein Behnammanesh from Shahid Beheshti University of Medical Sciences for providing the Bioimaging photograph used in Fig. 1.

References

[1] Prescher JA, Bertozzi CR. Chemistry in living systems. Nat Chem Biol 2005;1:13–21.
[2] Borrmann A, van Hest JC. Bioorthogonal chemistry in living organisms. Chem Sci 2014;5:2123–34.
[3] Devaraj NK. The future of bioorthogonal chemistry. ACS Cent Sci 2018;4:952–9.
[4] Mohammadi Y, Saeb MR, Penlidis A, Jabbari E, Stadler FJ, Zinck P, Matyjaszewski K. Intelligent machine learning: tailor-making macromolecules. Polymers 2019;11:579 (Basel).
[5] Mohammadi Y, Saeb MR, Penlidis A, Jabbari E, Zinck P, Stadler FJ, Matyjaszewski K. Intelligent Monte Carlo: a new paradigm for inverse polymerization engineering. Macromol Theory Simul 2018;27:1700106.
[6] Saeb MR, Mohammadi Y, Kermaniyan TS, Zinck P, Stadler FJ. Unspoken aspects of chain shuttling reactions: patterning the molecular landscape of olefin multi-block copolymers. Polymer 2017;116:55–75.
[7] Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew Chem Int Ed Engl 2002;41:2596–9.
[8] Patterson DM, Nazarova LA, Prescher JA. Finding the right (bioorthogonal) chemistry. ACS Chem Biol 2014;9:592–605.
[9] Zou Y, Zhang L, Yang L, Zhu F, Ding M, Lin F, et al. “Click” chemistry in polymeric scaffolds: bioactive materials for tissue engineering. J Control Release 2018;273:160–79.
[10] Ganz D, Harijan D, Wagenknecht HA. Labelling of DNA and RNA in the cellular environment by means of bioorthogonal cycloaddition chemistry. RSC Chem Biol 2020;1:86–97.
[11] Tsarevsky NV, Bencherif SA, Matyjaszewski K. Graft copolymers by a combination of ATRP and two different consecutive click reactions. Macromolecules 2007;40:4439–45.
[12] Jia HR, Zhu YX, Duan QY, Wu FG. Cell surface-localized imaging and sensing. Chem Soc Rev 2021;50:6240–77.
[13] Carillo S, Casillo A, Pieretti G, Parrilli E, Sannino F, Bayer-Giraldi M, Cosconati S, Novellino E, Ewert M, Deming JW. A unique capsular polysaccharide structure from the psychrophilic marine bacterium Colwellia psychrerythraea 34H that mimics antifreeze (glyco) proteins. J Am Chem Soc 2015;137:179–89.
[14] Jiang K, Chaimov D, Patel SN, Liang JP, Wiggins SC, Samojlik MM, Rubiano A, Simmons CS, Stabler CL. 3-D physiomimetic extracellular matrix hydrogels provide a supportive microenvironment for rodent and human islet culture. Biomaterials 2019;198:37–48.
[15] Colombani T, Eggermont LJ, Rogers ZJ, McKay LG, Avena LE, Johnson RI, Storm N, Griffiths A, Bencherif SA. Biomaterials and oxygen join forces to shape the immune response and boost COVID-19 vaccines. Adv Sci 2021;8:2100316.
[16] Colombani T, Eggermont LJ, Hatfield SM, Rogers ZJ, Rezaeeyazdi M, Memic A, Sitkovsky MV, Bencherif SA. Oxygen-generating cryogels restore T cell mediated cytotoxicity in hypoxic tumors. Adv Funct Mater 2021;31:2102234.
[17] He T, Li B, Colombani T, Joshi-Navare K, Mehta S, Kisiday J, Bencherif SA, Bajpayee AG. Hyaluronic acid-based shape-memory cryogel scaffolds for focal cartilage defect repair. Tissue Eng Part A 2021;27:748–60.
[18] Navare KJ, Colombani T, Rezaeeyazdi M, Bassous N, Rana D, Webster T, Memic A, Bencherif SA. Needle-injectable microcomposite cryogel scaffolds with antimicrobial properties. Sci Rep 2020;10:1–16.
[19] Memic A, Rezaeeyazdi M, Villard P, Rogers ZJ, Abudula T, Colombani T, Bencherif SA. Effect of polymer concentration on autoclaved cryogel properties. Macromol Mater Eng 2020;305:1900824.
[20] Villard P, Rezaeeyazdi M, Colombani T, Joshi-Navare K, Rana D, Memic A, Bencherif SA. Autoclavable and Injectable Cryogels for Biomedical Applications. Adv Healthc Mater 2019;8:e1900679.
[21] Rezaeeyazdi M, Colombani T, Memic A, Bencherif SA. Injectable hyaluronic acid-co-gelatin cryogels for tissue-engineering applications. Materials 2018;11:1374 (Basel).
[22] Prata JE, Barth TA, Bencherif SA, Washburn NR. Complex fluids based on methacrylated hyaluronic acid. Biomacromolecules 2010;11:769–75.
[23] Reily C, Stewart TJ, Renfrow MB, Novak J. Glycosylation in health and disease. Nat Rev Nephrol 2019;15:346–66.
[24] Slámová K, Kapešová J, Valentová K. “Sweet flavonoids”: Glycosidase-catalyzed modifications. Int J Mol Sci 2018;19:2126.
[25] Disney MD. A glimpse at the glycoRNA world. Cell 2021;184:3080–1.
[26] Ban I, Markuš S, Gyergyek S, Drofenik M, Korenak J, Helix-Nielsen C, Petrinić I. Synthesis of poly-sodium-acrylate (PSA)-coated magnetic nanoparticles for use in forward osmosis draw solutions. Nanomaterials 2019;9:1238.
[27] Ramakrishna C, Kujawski M, Chu H, Li L, Mazmanian SK, Cantin EM. Bacteroides fragilis polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. Nat Commun 2019;10:1–13.
[28] Yang L, Gao MX, Zou HY, Li YF, Huang CZ. Plasmonic Cu2–x S y Se1–y nanoparticles catalyzed click chemistry reaction for SERS immunoassay of cancer biomarker. Anal Chem 2018;90:11728–33.
[29] Zhao X, Wang N, Chen H, Bai L, Xu H, Wang W, Yang H, Wei D, Yang L, Cheng Z. Preparation of a novel sandwich-type electrochemical immunosensor for AFP detection based on an ATRP and click chemistry technique. Polym Chem 2020;11:900–8.
[30] Hong T, Liu W, Li M, Chen C. Click chemistry at the microscale. Analyst 2019;144:1492–512.

[31] Elchinger PH, Faugeras PA, Boëns B, Brouillette F, Montplaisir D, Zerrouki R, Lucas R. Polysaccharides: The “click” chemistry impact. Polymers 2011;3:1607–51.
[32] Solberg A, Mo IV, Omtvedt LA, Strand BL, Aachmann FL, Schatz C, et al. Carbohydr polym special issue invited contribution: click chemistry for block polysaccharides with dihydrazide and dioxyamine linkers-a review. Carbohydr Polym 2021;15:118840.
[33] Meng X, Edgar KJ. “Click” reactions in polysaccharide modification. Prog Polym Sci 2016;53:52–85.
[34] Deng Y, Shavandi A, Okoro OV, Nie L. Alginate modification via click chemistry for biomedical applications. Carbohydr Polym 2021;270:118360.
[35] Kaur J, Saxena M, Rishi N. An overview of recent advances in biomedical applications of click chemistry. Bioconjug Chem 2021;32:1455–71.
[36] Wu D, Yang K, Zhang Z, Feng Y, Rao L, Chen X, Yu G. Metal-free bioorthogonal click chemistry in cancer theranostics. Chem Soc Rev 2022;51:1336–76.
[37] Agrahari AK, Bose P, Jaiswal MK, Rajkhowa S, Singh AS, Hotha S, Mishra N, Tiwari VK. Cu (I)-catalyzed click chemistry in glycoscience and their diverse applications. Chem Rev 2021;121:7638–956.
[38] Fantoni NZ, El-Sagheer AH, Brown T. A Hitchhiker's guide to click-chemistry with nucleic acids. Chem Rev 2021;121:7122–54.
[39] Kumar GS, Lin Q. Light-triggered click chemistry. Chem Rev 2021;121:6991–7031.
[40] Sarrett SM, Keinanen O, Dayts EJ, Dewaele-Le Roi G, Rodriguez C, Carnazza KE, Zeglis BM. Inverse electron demand Diels-Alder click chemistry for pretargeted PET imaging and radioimmunotherapy. Nat Protoc 2021;16:3348–81.
[41] Huisgen R, Knorr R, Moebius L, Szeimies G. 1, 3-Dipolar cycloadditions. XXIII. Addition of organic azides to C–C triple bonds. Chem Ber 1965;98:4014–21.
[42] Tornoe CW, Christensen C, Meldal M. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(i)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. J Org Chem 2002;67:3057–64.
[43] Kolb HC, Finn MG, Sharpless KB. Click chemistry: diverse chemical function from a few good reactions. Angew Chem Int Ed Engl 2001;40:2004–21.
[44] Kolb HC, Sharpless KB. The growing impact of click chemistry on drug discovery. Drug Discov Today 2003;8:1128–37.
[45] Krebs A., Wilke J. Angle strained cycloalkynes. Wittig Chemistry 1983:189-233.
[46] Harris T, Alabugin IV. Strain and stereoelectronics in cycloalkyne click chemistry. Mendeleev Commun 2019;29:237–48.
[47] Agard NJ, Prescher JA, Bertozzi CR. A strain-promoted [3 + 2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems. J Am Chem Soc 2004;126:15046–7.
[48] Baskin JM, Prescher JA, Laughlin ST, Agard NJ, Chang PV, Miller IA, Lo A, Codelli JA, Bertozzi CR. Copper-free click chemistry for dynamic *in vivo* imaging. Proc Natl Acad Sci USA 2007;104:16793–7.
[49] Laughlin ST, Baskin JM, Amacher SL, Bertozzi CR. *In vivo* imaging of membrane-associated glycans in developing zebrafish. Science 2008;320:664–7.
[50] Sletten EM, Bertozzi CR. A hydrophilic azacyclooctyne for Cu-free click chemistry. Org Lett 2008;10:3097–9.
[51] Rideout DC, Breslow R. Hydrophobic acceleration of Diels-Alder reactions. J Am Chem Soc 1980;102:7816–17.
[52] Debets MF, van Hest JC, Rutjes FP. Bioorthogonal labelling of biomolecules: new functional handles and ligation methods. Org Biomol Chem 2013;11:6439–55.
[53] Hoyle CE, Lowe AB, Bowman CN. Thiol-click chemistry: a multifaceted toolbox for small molecule and polymer synthesis. Chem Soc Rev 2010;39:1355–87.
[54] Lowe AB. Thiol–ene “click” reactions and recent applications in polymer and materials synthesis: a first update. Polym Chem 2014;5:4820–70.
[55] Nair DP, Podgorski M, Chatani S, Gong T, Xi W, Fenoli CR, Bowman CN. The thiol-Michael addition click reaction: a powerful and widely used tool in materials chemistry. Chem Mater 2014;26:724–44.
[56] Tasdelen MA. Diels–Alder “click” reactions: recent applications in polymer and material science. Polym Chem 2011;2:2133–45.
[57] Nicolaou KC, Snyder SA, Montagnon T, Vassilikogiannakis G. The Diels–Alder reaction in total synthesis. Angew Chem Int Ed Engl 2002;41:1668–98.
[58] Zhang Y, Broekhuis AA, Picchioni F. Thermally self-healing polymeric materials: the next step to recycling thermoset polymers? Macromolecules 2009;42:1906–12.
[59] Orozco F, Niyazov Z, Garnier T, Migliore N, Zdvizhkov AT, Raffa P, Moreno-Villoslada I, Picchioni F, Bose RK. Maleimide self-reaction in furan/maleimide-based reversibly crosslinked polyketones: processing limitation or potential advantage? Molecules 2021;26:2230.
[60] Schäfer S, Kickelbick G. Double reversible networks: improvement of self--healing in hybrid materials via combination of diels–alder cross-linking and hydrogen bonds. Macromolecules 2018;51:6099–110.
[61] Truong TT, Thai SH, Nguyen HT, Phung DTT, Nguyen LT, Pham HQ, Nguyen LTT. Tailoring the hard–soft interface with dynamic diels–alder linkages in polyurethanes: toward superior mechanical properties and healability at mild temperature. Chem Mater 2019;31:2347–57.
[62] Cookson R, Gilani S, Stevens I. 4-Phenyl-1, 2, 4-triazolin-3, 5-dione: a powerful dienophile. Tetrahedron Lett 1962;3:615–18.
[63] De Bruycker K, Billiet S, Houck HA, Chattopadhyay S, Winne JM. Du Prez FE. Triazolinediones as highly enabling synthetic tools. Chem Rev 2016;116:3919–74.
[64] Mondal P, Jana G, Behera PK, Chattaraj PK, Singha NK. Fast “ES-Click” reaction involving furfuryl and triazolinedione functionalities toward designing a healable polymethacrylate. Macromolecules 2020;53:8313–23.
[65] Mondal P, Behera PK, Singha NK. Macromolecular engineering in functional polymers via ‘click chemistry’ using triazolinedione derivatives. Prog Polym Sci 2020;113:101343.
[66] Kalia J, Raines RT. Hydrolytic stability of hydrazones and oximes. Angew Chem Int Ed Engl 2008;47:7523–6.
[67] Collins J, Xiao Z, Müllner M, Connal LA. The emergence of oxime click chemistry and its utility in polymer science. Polym Chem 2016;7:3812–26.
[68] Ulrich S, Boturyn D, Marra A, Renaudet O, Dumy P. Oxime ligation: a chemoselective click-type reaction for accessing multifunctional biomolecular constructs. Chemistry 2014;20:34–41.
[69] Knall AC, Slugovc C. Inverse electron demand Diels-Alder (iEDDA)-initiated conjugation: a (high) potential click chemistry scheme. Chem Soc Rev 2013;42:5131–42.
[70] Oliveira BL, Guo Z, Bernardes GJL. Inverse electron demand Diels-Alder reactions in chemical biology. Chem Soc Rev 2017;46:4895–950.
[71] Dong J, Krasnova L, Finn M, Sharpless KB. Sulfur (VI) fluoride exchange (SuFEx): another good reaction for click chemistry. Angew Chem Int Ed 2014;53:9430–48.
[72] Barrow AS, Smedley CJ, Zheng Q, Li S, Dong J, Moses JE. The growing applications of SuFEx click chemistry. Chem Soc Rev 2019;48:4731–58.
[73] Yang C, Flynn JP, Niu J. Facile synthesis of sequence-regulated synthetic polymers using orthogonal SuFEx and CuAAC click reactions. Angew Chem Int Ed 2018;57:16194–9.
[74] Xu L, Wu P, Dong J. New polymers from SuFEx click chemistry: syntheses and perspectives. In: Zheng Zhao RH, Anjun Q, Ben ZT, editors. Synthetic polymer chemistry: innovations and outlook. London: Royal Society of Chemistry; 2019. p. 1–31.
[75] He B, Su H, Bai T, Wu Y, Li S, Gao M, Hu R, Zhao Z, Qin A, Ling J, Tang BZ. Spontaneous amino-yne click polymerization: a powerful tool toward regio- and stereospecific poly(beta-aminoacrylate)s. J Am Chem Soc 2017;139:5437–43.
[76] Zhang J, Zhang Z, Wang J, Zang Q, Sun JZ, Tang BZ. Recent progress in the applications of amino–yne click chemistry. Polym Chem 2021;12:2978–86.
[77] Zhang Y, Shen J, Hu R, Shi X, Hu X, He B, Qin A, Tang BZ. Fast surface immobilization of native proteins through catalyst-free amino-yne click bioconjugation. Chem Sci 2020;11:3931–5.
[78] He B, Huang J, Liu X, Zhang J, Lam JW, Tang BZ. Polymerizations of activated alkynes. Prog Polym Sci 2022;126:101503.
[79] Huang J, Jiang X. Injectable and degradable pH-responsive hydrogels via spontaneous amino-yne click reaction. ACS Appl Mater Interfaces 2018;10:361–70.
[80] Fairbanks BD, Macdougall LJ, Mavila S, Sinha J, Kirkpatrick BE, Anseth KS, Bowman CN. Photoclick chemistry: a bright idea. Chem Rev 2021;121:6915–90.
[81] Ahangarpour M, Kavianinia I, Harris PWR, Brimble MA. Photo-induced radical thiol-ene chemistry: a versatile toolbox for peptide-based drug design. Chem Soc Rev 2021;50:898–944.
[82] Akeroyd N, Klumperman B. The combination of living radical polymerization and click chemistry for the synthesis of advanced macromolecular architectures. Eur Polym J 2011;47:1207–31.
[83] Kaur G, Singh G, Singh J. Photochemical tuning of materials: a click chemistry perspective. Mater Today Chem 2018;8:56–84.
[84] Mansfeld U, Pietsch C, Hoogenboom R, Becer CR, Schubert US. Clickable initiators, monomers and polymers in controlled radical polymerizations–a prospective combination in polymer science. Polym Chem 2010;1:1560–98.
[85] Chatterjee S, Anslyn EV, Bandyopadhyay A. Boronic acid based dynamic click chemistry: recent advances and emergent applications. Chem Sci 2020;12:1585–99.
[86] Schreiber CL, Smith BD. Molecular conjugation using non-covalent click chemistry. Nat Rev Chem 2019;3:393–400.
[87] Xu Z, Wang L, Fang F, Fu Y, Yin Z. A review on colloidal self-assembly and their applications. Curr Nanosci 2016;12:725–46.
[88] Jain A, Cheng K. The principles and applications of avidin-based nanoparticles in drug delivery and diagnosis. J Control Release 2017;245:27–40.
[89] Guryanov I, Fiorucci S, Tennikova T. Receptor-ligand interactions: Advanced biomedical applications. Mater Sci Eng C Mater Biol Appl 2016;68:890–903.
[90] Rutten MG, Vaandrager FW, Elemans JA, Nolte RJ. Encoding information into polymers. Nat Rev Chem 2018;2:365–81.
[91] Budhathoki-Uprety J, Shah J, Korsen JA, Wayne AE, Galassi TV, Cohen JR, Harvey JD, Jena PV, Ramanathan LV, Jaimes EA, Heller DA. Synthetic molecular recognition nanosensor paint for microalbuminuria. Nat Commun 2019;10:3605.
[92] Chen N, Qin S, Yang X, Wang Q, Huang J, Wang K. "Sense-and-treat" DNA nanodevice for synergetic destruction of circulating tumor cells. ACS Appl Mater Interfaces 2016;8:26552–8.
[93] Li Z, Ren X, Sun P, Ding H, Li S, Zhao Y, Zhang K. Protecting-group-free iterative exponential growth method for synthesizing sequence-defined polymers. ACS Macro Lett 2021;10:223–30.
[94] Battigelli A, Almeida B, Shukla A. Recent advances in bioorthogonal click chemistry for biomedical applications. Bioconjug Chem 2022;33:263–71.
[95] Yoon HY, Lee D, Lim DK, Koo H, Kim K. Copper-free click chemistry: applications in drug delivery, cell tracking, and tissue engineering. Adv Mater 2022;34:e2107192.

[96] Jewett JC, Bertozzi CR. Cu-free click cycloaddition reactions in chemical biology. Chem Soc Rev 2010;39:1272–9.
[97] Cioc RC, Lutz M, Pidko EA, Crockatt M, Van Der Waal JC, Bruijnincx PC. Direct Diels–Alder reactions of furfural derivatives with maleimides. Green Chem 2021;23:367–73.
[98] Blackman ML, Royzen M, Fox JM. Tetrazine ligation: fast bioconjugation based on inverse-electron-demand Diels-Alder reactivity. J Am Chem Soc 2008;130:13518–19.
[99] Gahtory D, Sen R, Pujari S, Li S, Zheng Q, Moses JE, Sharpless KB, Zuilhof H. Quantitative and orthogonal formation and reactivity of SuFEx platforms. Chemistry 2018;24:10550–6.
[100] Du J, Meledeo MA, Wang Z, Khanna HS, Paruchuri VD, Yarema KJ. Metabolic glycoengineering: sialic acid and beyond. Glycobiology 2009;19:1382–401.
[101] Wang H, Mooney DJ. Metabolic glycan labelling for cancer-targeted therapy. Nat Chem 2020;12:1102–14.
[102] Agatemor C, Buettner MJ, Ariss R, Muthiah K, Saeui CT, Yarema KJ. Exploiting metabolic glycoengineering to advance healthcare. Nat Rev Chem 2019;3:605–20.
[103] Dold JE, Wittmann V. Metabolic glycoengineering with azide-and alkene–modified hexosamines: quantification of sialic acid levels. ChemBioChem 2021;22:1243.
[104] Laughlin ST, Agard NJ, Baskin JM, Carrico IS, Chang PV, Ganguli AS, Hangauer MJ, Lo A, Prescher JA, Bertozzi CR. Metabolic labeling of glycans with azido sugars for visualization and glycoproteomics. Methods Enzymol 2006;415:230–50.
[105] Fernandez MV, Freed EO. “Expand and Click”: a new method for labeling HIV-1 envelope glycoproteins. Cell Chem Biol 2017;24:548–50.
[106] Zhang X, Zhang Y. Applications of azide-based bioorthogonal click chemistry in glycobiology. Molecules 2013;18:7145–59.
[107] Liu B. Bio-orthogonal click chemistry for *in vivo* bioimaging. Trends Chem 2019;1:763–78.
[108] Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J. Degradation and turnover of glycans. Essentials of glycobiology. Cold Spring Harbor Laboratory Press; 1999.
[109] Kim W, Yoon HY, Lim S, Stayton PS, Kim IS, Kim K, Kwon IC. *In vivo* tracking of bioorthogonally labeled T-cells for predicting therapeutic efficacy of adoptive T-cell therapy. J Control Release 2021;329:223–36.
[110] Lim S, Yoon HY, Park SJ, Song S, Shim MK, Yang S, Kang SW, Lim DK, Kim BS, Moon SH, Kim K. Predicting *in vivo* therapeutic efficacy of bioorthogonally labeled endothelial progenitor cells in hind limb ischemia models via non-invasive fluorescence molecular tomography. Biomaterials 2021;266:120472.
[111] Koo H, Lee S, Na JH, Kim SH, Hahn SK, Choi K, Kwon IC, Jeong SY, Kim K. Bioorthogonal copper-free click chemistry *in vivo* for tumor-targeted delivery of nanoparticles. Angew Chem Int Ed Engl 2012;51:11836–40.
[112] Lee S, Koo H, Na JH, Han SJ, Min HS, Lee SJ, Kim SH, Yun SH, Jeong SY, Kwon IC, Choi K, Kim K. Chemical tumor-targeting of nanoparticles based on metabolic glycoengineering and click chemistry. ACS Nano 2014;8:2048–63.
[113] Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL, Schuetze S, Reed D, Chawla SP, Riedel RF, Krarup-Hansen A, Toulmonde M, Ray-Coquard I, Hohenberger P, Grignani G, Cranmer LD, Okuno S, Agulnik M, Read W, Ryan CW, Alcindor T, Del Muro XFG, Budd GT, Tawbi H, Pearce T, Kroll S, Reinke DK, Schoffski P. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2017;18:1089–103.
[114] Pei Y, Li M, Hou Y, Hu Y, Chu G, Dai L, Li K, Xing Y, Tao B, Yu Y, Xue C, He Y, Luo Z, Cai K. An autonomous tumor-targeted nanoprodrug for reactive oxygen species-activatable dual-cytochrome c/doxorubicin antitumor therapy. Nanoscale 2018;10:11418–29.
[115] Gong J, Yan J, Forscher C, Hendifar A. Aldoxorubicin: a tumor-targeted doxorubicin conjugate for relapsed or refractory soft tissue sarcomas. Drug Des Dev Ther 2018;12:777–86.
[116] Poreba M. Protease-activated prodrugs: strategies, challenges, and future directions. FEBS J 2020;287:1936–69.
[117] Tu Y, Dong Y, Wang K, Shen S, Yuan Y, Wang J. Intercellular delivery of bioorthogonal chemical receptors for enhanced tumor targeting and penetration. Biomaterials 2020;259:120298.
[118] Lim GT, You DG, Han HS, Lee H, Shin S, Oh BH, Kumar EKP, Um W, Kim CH, Han S, Lee S, Lim S, Yoon HY, Kim K, Kwon IC, Jo DG, Cho YW, Park JH. Bioorthogonally surface-edited extracellular vesicles based on metabolic glycoengineering for CD44-mediated targeting of inflammatory diseases. J Extracell Vesicles 2021;10:e12077.
[119] Nikić I, Kang JH, Girona GE, Aramburu IV, Lemke EA. Labeling proteins on live mammalian cells using click chemistry. Nat Protoc 2015;10:780–91.
[120] Omtvedt LA, Dalheim MO, Nielsen TT, Larsen KL, Strand BL, Aachmann FL. Efficient grafting of cyclodextrin to alginate and performance of the hydrogel for release of model drug. Sci Rep 2019;9:9325.
[121] Joseph AA, Pardo-Vargas A, Seeberger PH. Total synthesis of polysaccharides by automated glycan assembly. J Am Chem Soc 2020;142:8561–4.
[122] Wu Y, Xiong DC, Chen SC, Wang YS, Ye XS. Total synthesis of mycobacterial arabinogalactan containing 92 monosaccharide units. Nat Commun 2017;8:14851.
[123] Wu M, Qu J, Tian X, Zhao X, Shen Y, Shi Z, Chen P, Li G, Ma T. Tailor–made polysaccharides containing uniformly distributed repeating units based on the xanthan gum skeleton. Int J Biol Macromol 2019;131:646–53.
[124] Soliman SMA, El Founi M, Vanderesse R, Acherar S, Ferji K, Babin J, Six JL. Light-sensitive dextran-covered PNBA nanoparticles to continuously or discontinuously improve the drug release. Colloids Surf B Biointerfaces 2019;182:110393.
[125] Liaqat F, Eltem R. Chitooligosaccharides and their biological activities: a comprehensive review. Carbohydr Polym 2018;184:243–59.
[126] Li K, Xing R, Liu S, Li P. Advances in preparation, analysis and biological activities of single chitooligosaccharides. Carbohydr Polym 2016;139:178–90.
[127] Moussa A, Crepet A, Ladaviere C, Trombotto S. Reducing-end "clickable" functionalizations of chitosan oligomers for the synthesis of chitosan-based diblock copolymers. Carbohydr Polym 2019;219:387–94.
[128] Guerry A, Bernard J, Samain E, Fleury E, Cottaz S, Halila S. Aniline-catalyzed reductive amination as a powerful method for the preparation of reducing end-“clickable” chitooligosaccharides. Bioconj Chem 2013;24:544–9.
[129] Chen Y, Yu L, Zhang B, Feng W, Xu M, Gao L, Liu N, Wang Q, Huang X, Li P, Huang W. Design and synthesis of biocompatible, hemocompatible, and highly selective antimicrobial cationic peptidopolysaccharides via click chemistry. Biomacromolecules 2019;20:2230–40.
[130] Diget JS, Lund R, Nyström B, Wintgens V, Amiel C, Wimmer R, Nielsen TT. Self-assembled nanoparticles based on cyclodextrin-modified pullulan: Synthesis, and structural characterization using SAXS. Carbohydr Polym 2019;213:403–10.
[131] Sitterli A, Heinze T. Studies about reactive ene-functionalized dextran derivatives for Thiol-ene click reactions. React Funct Polym 2019;136:66–74.
[132] Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006;24:1551–7.
[133] Li W, Separovic F, O'Brien-Simpson NM, Wade JD. Chemically modified and conjugated antimicrobial peptides against superbugs. Chem Soc Rev 2021;50:4932–73.
[134] Kim M, Mun W, Jung WH, Lee J, Cho G, Kwon J, Ahn DJ, Mitchell RJ, Kim BS. Antimicrobial PEGtides: a modular poly(ethylene glycol)-based peptidomimetic approach to combat bacteria. ACS Nano 2021;15:9143–53.
[135] Yu L, Li K, Zhang J, Jin H, Saleem A, Song Q, Jia Q, Li P. Antimicrobial peptides and macromolecules for combating microbial infections: from agents to interfaces. ACS Appl Bio Mater 2022;5:366–93.
[136] Pranantyo D, Xu LQ, Kang ET, Chen Park MB. Chitosan-based peptidopolysaccharides as cationic antimicrobial agents and antibacterial coatings. Biomacromolecules 2018;19:2156–65.
[137] Su Y, Tian L, Yu M, Gao Q, Wang D, Xi Y, Yang P, Lei B, Ma PX, Li P. Cationic peptidopolysaccharides synthesized by ‘click’chemistry with enhanced broad--spectrum antimicrobial activities. Polym Chem 2017;8:3788–800.
[138] Zampano G, Bertoldo M, Ciardelli F. Defined Chitosan-based networks by C-6-Azide–alkyne “click” reaction. React Funct Polym 2010;70:272–81.
[139] Han J, Wang X, Liu L, Li D, Suyaola S, Wang T, et al. “Click" chemistry mediated construction of cationic curdlan nanocarriers for efficient gene delivery. Carbohydr Polym 2017;163:191–8.
[140] Yazdi MK, Vatanpour V, Taghizadeh A, Taghizadeh M, Ganjali MR, Munir MT, Habibzadeh S, Saeb MR, Ghaedi M. Hydrogel membranes: a review. Mater Sci Eng C 2020;114:111023.
[141] Garcia-Astrain C, Averous L. Synthesis and behavior of click cross-linked alginate hydrogels: Effect of cross-linker length and functionality. Int J Biol Macromol 2019;137:612–19.
[142] Tao Y, Qu D, Tian C, Huang Y, Xue L, Ju C, Hao M, Zhang C. Modular synthesis of amphiphilic chitosan derivatives based on copper-free click reaction for drug delivery. Int J Pharm 2021;605:120798.
[143] Korogiannaki M, Jones L, Sheardown H. Impact of a hyaluronic acid-grafted layer on the surface properties of model silicone hydrogel contact lenses. Langmuir 2018;35:950–61.
[144] Zandi N, Sani ES, Mostafavi E, Ibrahim DM, Saleh B, Shokrgozar MA, Tamjid E, Weiss PS, Simchi A, Annabi N. Nanoengineered shear-thinning and bioprintable hydrogel as a versatile platform for biomedical applications. Biomaterials 2021;267:120476.
[145] Taghizadeh M, Taghizadeh A, Yazdi MK, Zarrintaj P, Stadler FJ, Ramsey JD, Habibzadeh S, Rad SH, Naderi G, Saeb MR. Chitosan-based inks for 3D printing and bioprinting. Green Chem 2022;24:62–101.
[146] Yazdi MK, Sa AR, Kamyabi A, Amoli HH. Simulation of flow of short fiber suspensions through a planar contraction. Sci Iran. 2012;19:579–84.
[147] Yazdi MK, SA AR, Amoli H, Kamyabi A. Experimental investigation and theoretical prediction of extrudate swell using conformational rheological models. Int Polym Process 2012;27:478–85.
[148] Kim J, Bencherif SA, Li WA, Mooney DJ. Cell-friendly inverse opal-like hydrogels for a spatially separated co-culture system. Macromol Rapid Commun 2014;35:1578–86.
[149] Colombani T, Rogers ZJ, Eggermont LJ, Bencherif SA. Harnessing biomaterials for therapeutic strategies against COVID-19. Emergent Mater 2021;4:9–18.
[150] Gsib O, Eggermont LJ, Egles C, Bencherif SA. Engineering a macroporous fibrin-based sequential interpenetrating polymer network for dermal tissue engineering. Biomater Sci 2020;8:7106–16.
[151] Amirsadeghi A, Jafari A, Eggermont LJ, Hashemi SS, Bencherif SA, Khorram M. Vascularization strategies for skin tissue engineering. Biomater Sci 2020;8:4073–94.
[152] Gsib O, Egles C, Bencherif S. Fibrin: An underrated biopolymer for skin tissue engineering. J Mol Biol Biotechnol 2017;2:1–4.
[153] Gsib O, Duval JL, Goczkowski M, Deneufchatel M, Fichet O, Larreta-Garde V, Bencherif SA, Egles C. Evaluation of fibrin-based interpenetrating polymer networks as potential biomaterials for tissue engineering. Nanomaterials 2017;7:436.

[154] Grover GN, Braden RL, Christman KL. Oxime cross-linked injectable hydrogels for catheter delivery. Adv Mater 2013;25:2937–42.
[155] Alonso JM, Andrade Del Olmo J, Perez Gonzalez R, Saez-Martinez V. Injectable hydrogels: from laboratory to industrialization. Polymers 2021;13:650 (Basel).
[156] Shimojo AA, Pires AM, Lichy R, Rodrigues AA, Santana MH. The crosslinking degree controls the mechanical, rheological, and swelling properties of hyaluronic acid microparticles. J Biomed Mater Res A 2015;103:730–7.
[157] Kuang L, Damayanti NP, Jiang C, Fei X, Liu W, Narayanan N, Irudayaraj J, Campanella O, Deng M. Bioinspired glycosaminoglycan hydrogels via click chemistry for 3D dynamic cell encapsulation. J Appl Polym Sci 2019;136:47212.
[158] Bell S, Terentjev EM. Focal adhesion kinase: the reversible molecular mechanosensor. Biophys J 2017;112:2439–50.
[159] Lou J, Stowers R, Nam S, Xia Y, Chaudhuri O. Stress relaxing hyaluronic acid–collagen hydrogels promote cell spreading, fiber remodeling, and focal adhesion formation in 3D cell culture. Biomaterials 2018;154:213–22.
[160] Wang X, Li Z, Shi T, Zhao P, An K, Lin C, Liu H. Injectable dextran hydrogels fabricated by metal-free click chemistry for cartilage tissue engineering. Mater Sci Eng C Mater Biol Appl 2017;73:21–30.
[161] Han SS, Yoon HY, Yhee JY, Cho MO, Shim HE, Jeong JE, Lee DE, Kim K, Guim H, Lee JH. *In situ* cross-linkable hyaluronic acid hydrogels using copper free click chemistry for cartilage tissue engineering. Polym Chem 2018;9:20–7.
[162] Lu S, Bai X, Liu H, Ning P, Wang Z, Gao C, Ni B, Liu M. An injectable and self-healing hydrogel with covalent cross-linking *in vivo* for cranial bone repair. J Mater Chem B 2017;5:3739–48.
[163] Fan M, Ma Y, Zhang Z, Mao J, Tan H, Hu X. Biodegradable hyaluronic acid hydrogels to control release of dexamethasone through aqueous Diels–Alder chemistry for adipose tissue engineering. Mater Sci Eng C 2015;56:311–17.
[164] Cao W, Sui J, Ma M, Xu Y, Lin W, Chen Y, Man Y, Sun Y, Fan Y, Zhang X. The preparation and biocompatible evaluation of injectable dual crosslinking hyaluronic acid hydrogels as cytoprotective agents. J Mater Chem B 2019;7:4413–23.
[165] Xu Q, Sigen A, Gao Y, Guo L, Creagh-Flynn J, Zhou D, Greiser U, Dong Y, Wang F, Tai H. A hybrid injectable hydrogel from hyperbranched PEG macromer as a stem cell delivery and retention platform for diabetic wound healing. Acta Biomater 2018;75:63–74.
[166] Seidi F, Khodadadi Yazdi M, Jouyandeh M, Dominic M, Naeim H, Nezhad MN, Bagheri B, Habibzadeh S, Zarrintaj P, Saeb MR, Mozafari M. Chitosan-based blends for biomedical applications. Int J Biol Macromol 2021;183:1818–50.
[167] Yazdi MK, Ganjali MR, Rezapour M, Zarrintaj P, Habibzadeh S, Saeb MR. Ionically gelled polysaccharide-based interpenetrating polymer network systems for drug delivery. In: Nayak AK, Hasnain MS, Pal D, editors. Ionically gelled biopolysaccharide based systems in drug delivery. Springer; 2021. p. 121–33.
[168] Yazdi MK, Ganjali MR, Zarrintaj P, Bagheri B, Kim YC, Saeb MR. Ionically gelled carboxymethyl polysaccharides for drug delivery. In: Nayak AK, Hasnain MS, Pal D, editors. Ionically gelled biopolysaccharide based systems in drug delivery. Springer; 2021. p. 93–103.
[169] Gao F, Zhang Y, Li Y, Xu B, Cao Z, Liu W. Sea cucumber-inspired autolytic hydrogels exhibiting tunable high mechanical performances, repairability, and reusability. ACS Appl Mater Interfaces 2016;8:8956–66.
[170] Vining KH, Stafford A, Mooney DJ. Sequential modes of crosslinking tune viscoelasticity of cell-instructive hydrogels. Biomaterials 2019;188:187–97.
[171] Oki Y, Kirita K, Ohta S, Ohba S, Horiguchi I, Sakai Y, Ito T. Switching of cell proliferation/differentiation in thiol-maleimide clickable microcapsules triggered by *in situ* conjugation of biomimetic peptides. Biomacromolecules 2019;20:2350–9.
[172] Shieh P, Hill MR, Zhang W, Kristufek SL, Johnson JA. Clip chemistry: diverse (bio)(macro) molecular and material function through breaking covalent bonds. Chem Rev 2021;121:7059–121.
[173] Lunzer M, Shi L, Andriotis OG, Gruber P, Markovic M, Thurner PJ, Ossipov D, Liska R, Ovsianikov A. A modular approach to sensitized two-photon patterning of photodegradable hydrogels. Angew Chem Int Ed Engl 2018;57:15122–7.
[174] Tibbitt MW, Anseth KS. Dynamic microenvironments: the fourth dimension. Sci Transl Med 2012;4:160ps24.
[175] Kharkar PM, Kiick KL, Kloxin AM. Designing degradable hydrogels for orthogonal control of cell microenvironments. Chem Soc Rev 2013;42:7335–72.
[176] Desai RM, Koshy ST, Hilderbrand SA, Mooney DJ, Joshi NS. Versatile click alginate hydrogels crosslinked via tetrazine-norbornene chemistry. Biomaterials 2015;50:30–7.
[177] Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ. Degradation of partially oxidized alginate and its potential application for tissue engineering. Biotechnol Prog 2001;17:945–50.
[178] Kong HJ, Alsberg E, Kaigler D, Lee KY, Mooney DJ. Controlling degradation of hydrogels via the size of crosslinked junctions. Adv Mater 2004;16:1917–21.
[179] Lueckgen A, Garske DS, Ellinghaus A, Desai RM, Stafford AG, Mooney DJ, Duda GN, Cipitria A. Hydrolytically-degradable click-crosslinked alginate hydrogels. Biomaterials 2018;181:189–98.
[180] Yazdi MK, Zarrintaj P, Ghavami M, Alizadeh R, Saeb MR. Protein and peptide-based delivery systems. In: Mozafari M, editor. Nanoengineered biomaterials for advanced drug delivery. Amsterdam: Elsevier; 2020. p. 145–61.
[181] Yazdi MK, Zarrintaj P, Bagheri B, Kim YC, Ganjali MR, Saeb MR. Nanotechnology-based biosensors in drug delivery. In: Mozafari M, editor. Nanoengineered biomaterials for advanced drug delivery. Amsterdam: Elsevier; 2020. p. 767–79.
[182] Zarrintaj P, Yazdi MK, Bagheri B, Kim YC, Ramsey JD, Saeb MR. Block copolymers for nanoscale drug and gene delivery. In: Mozafari M, editor. Nanoengineered biomaterials for advanced drug delivery. Amsterdam: Elsevier; 2020. p. 181–200.
[183] Yazdi MK, Zarrintaj P, Hosseiniamoli H, Mashhadzadeh AH, Saeb MR, Ramsey JD, Ganjali MR, Mozafari M. Zeolites for theranostic applications. J Mater Chem B 2020;8:5992–6012.
[184] Yazdi MK, Taghizadeh A, Taghizadeh M, Stadler FJ, Farokhi M, Mottaghitalab F, Zarrintaj P, Ramsey JD, Seidi F, Saeb MR. Agarose-based biomaterials for advanced drug delivery. J Control Release 2020;326:523–43.
[185] Mohammed MA, Syeda JTM, Wasan KM, Wasan EK. An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharmaceutics 2017;9:53.
[186] Yazdi MK, Zarrintaj P, Mottaghitalab F, Farokhi M, Ramsey JD, Saeb MR. Controlled/localized release and nanotechnology. In: Mozafari M, editor. Nanoengineered biomaterials for advanced drug delivery. Amsterdam: Elsevier; 2020. p. 27–36.
[187] Poon W, Kingston BR, Ouyang B, Ngo W, Chan WCW. A framework for designing delivery systems. Nat Nanotechnol 2020;15:819–29.
[188] Xue Y, Bai H, Peng B, Fang B, Baell J, Li L, Huang W, Voelcker NH. Stimulus–cleavable chemistry in the field of controlled drug delivery. Chem Soc Rev 2021;50:4872–931.
[189] Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nat Mater 2013;12:991–1003.
[190] Gutmann M, Bechold J, Seibel J, Meinel L, Luhmann T. Metabolic glycoengineering of cell-derived matrices and cell surfaces: a combination of key principles and step-by-step procedures. ACS Biomater Sci Eng 2019;5:215–33.
[191] Yang N, Wang Y, Zhang Q, Chen L, Zhao Y. *In situ* formation of poly (thiolated chitosan-co-alkylated β-cyclodextrin) hydrogels using click cross-linking for sustained drug release. J Mater Sci 2019;54:1677–91.
[192] Si H, Xing T, Ding Y, Zhang H, Yin R, Zhang W. 3D Bioprinting of the Sustained Drug Release Wound Dressing with Double-Crosslinked Hyaluronic-Acid-Based Hydrogels. Polymers 2019;11:1584 (Basel).
[193] Rizzo F, Kehr NS. Recent advances in injectable hydrogels for controlled and local drug delivery. Adv Healthc Mater 2021;10:e2001341.
[194] Constantin M, Bucatariu SM, Doroftei F, Fundueanu G. Smart composite materials based on chitosan microspheres embedded in thermosensitive hydrogel for controlled delivery of drugs. Carbohydr Polym 2017;157:493–502.
[195] Ramasamy T, Haidar ZS, Tran TH, Choi JY, Jeong JH, Shin BS, Choi HG, Yong CS, Kim JO. Layer-by-layer assembly of liposomal nanoparticles with PEGylated polyelectrolytes enhances systemic delivery of multiple anticancer drugs. Acta Biomater 2014;10:5116–27.
[196] Zhang X, Liang T, Ma Q. Layer-by-Layer assembled nano-drug delivery systems for cancer treatment. Drug Deliv 2021;28:655–69.
[197] Han FY, Thurecht KJ, Whittaker AK, Smith MT. Bioerodable PLGA-based microparticles for producing sustained-release drug formulations and strategies for improving drug loading. Front Pharmacol 2016;7:185.
[198] Heo JY, Noh JH, Park SH, Ji YB, Ju HJ, Kim DY, Lee B, Kim MS. An injectable click-crosslinked hydrogel that prolongs dexamethasone release from dexamethasone-loaded microspheres. Pharmaceutics 2019;11:438.
[199] Zarrintaj P, Ramsey JD, Samadi A, Atoufi Z, Yazdi MK, Ganjali MR, Amirabad LM, Zangene E, Farokhi M, Formela K, Saeb MR, Mozafari M, Thomas S. Poloxamer: a versatile tri-block copolymer for biomedical applications. Acta Biomater 2020;110:37–67.
[200] Foo C, Lee JS, Mulyasasmita W, Parisi-Amon A, Heilshorn SC. Two-component protein-engineered physical hydrogels for cell encapsulation. Proc Natl Acad Sci USA 2009;106:22067–72.
[201] Zarrintaj P, Khodadadi Yazdi M, Youssefi Azarfam M, Zare M, Ramsey JD, Seidi F, Reza Saeb M, Ramakrishna S, Mozafari M. Injectable cell-laden hydrogels for tissue engineering: recent advances and future opportunities. Tissue Eng Part A 2021;27:821–43.
[202] Anugrah DSB, Ramesh K, Kim M, Hyun K, Lim KT. Near-infrared light-responsive alginate hydrogels based on diselenide-containing cross-linkage for on demand degradation and drug release. Carbohydr Polym 2019;223:115070.
[203] Zhang X, Wang X, Chatani S, Bowman CN. Phosphonium tetraphenylborate: a photocatalyst for visible-light-induced, nucleophile-initiated thiol-michael addition photopolymerization. ACS Macro Lett 2021;10:84–9.
[204] Hu Y, Zhang J, Miao Y, Wen X, Wang J, Sun Y, Chen Y, Lin J, Qiu L, Guo K, Chen HY, Ye D. Enzyme-mediated *in situ* self-assembly promotes *in vivo* bioorthogonal reaction for pretargeted multimodality imaging. Angew Chem Int Ed Engl 2021;60:18082–93.
[205] Singh N, Romero M, Travanut A, Monteiro PF, Jordana-Lluch E, Hardie KR, Williams P, Alexander MR, Alexander C. Dual bioresponsive antibiotic and quorum sensing inhibitor combination nanoparticles for treatment of Pseudomonas aeruginosa biofilms *in vitro* and *ex vivo*. Biomater Sci 2019;7:4099–111.
[206] Hou G, Qian J, Xu W, Sun T, Wang Y, Wang J, Ji L, Suo A. A novel pH-sensitive targeting polysaccharide-gold nanorod conjugate for combined photothermal-chemotherapy of breast cancer. Carbohydr Polym 2019;212:334–44.
[207] Arumugam S, Popik VV. Sequential "click" - "photo-click" cross-linker for catalyst-free ligation of azide-tagged substrates. J Org Chem 2014;79:2702–8.
[208] Murrey HE, Judkins JC, Am Ende CW, Ballard TE, Fang Y, Riccardi K, Di L, Guilmette ER, Schwartz JW, Fox JM, Johnson DS. Systematic evaluation of bioorthogonal reactions in live cells with clickable halotag ligands: implications for intracellular imaging. J Am Chem Soc 2015;137:11461–75.
[209] Karver MR, Weissleder R, Hilderbrand SA. Synthesis and evaluation of a series of 1,2,4,5-tetrazines for bioorthogonal conjugation. Bioconjug Chem 2011;22:2263–70.

[210] Koshy ST, Zhang DKY, Grolman JM, Stafford AG, Mooney DJ. Injectable nanocomposite cryogels for versatile protein drug delivery. Acta Biomater 2018;65:36–43.
[211] Zhang Y, Ding J, Li M, Chen X, Xiao C, Zhuang X, Huang Y, Chen X. One-step "click chemistry"-synthesized cross-linked prodrug nanogel for highly selective intracellular drug delivery and upregulated antitumor efficacy. ACS Appl Mater Interfaces 2016;8:10673–82.
[212] Wu K, Yee NA, Srinivasan S, Mahmoodi A, Zakharian M, Oneto JMM, Royzen M. Click activated protodrugs against cancer increase the therapeutic potential of chemotherapy through local capture and activation. Chem Sci 2021;12:1259–71.
[213] NIH. Phase 1 Study of SQ3370 in Patients With Advanced Solid Tumors. 2022. https://clinicaltrials.gov/ct2/show/NCT04106492
[214] Subbiah V, Bhadri V, Bui N, Batty K, Strach M, Zakharian M, Smith S, Yee N, Srinivasan S, Saville M. 547P Early pharmacokinetic data from a phase I study of SQ3370 in patients with advanced solid tumors provides proof-of-concept for the click chemistry-based CAPAC platform. Ann Oncol 2021;32:S609.
[215] Srinivasan S, Yee NA, Wu K, Zakharian M, Mahmoodi A, Royzen M, Mejía Oneto JM. SQ3370 activates cytotoxic drug via click chemistry at tumor and elicits sustained responses in injected and non-injected lesions. Adv Ther 2021;4:2000243.
[216] Qiao J, Tian F, Deng Y, Shang Y, Chen S, Chang E, Yao J. Bio-orthogonal click-targeting nanocomposites for chemo-photothermal synergistic therapy in breast cancer. Theranostics 2020;10:5305–21.
[217] Wei R, Dong Y, Tu Y, Luo S, Pang X, Zhang W, Yao W, Tang W, Yang H, Wei X, Jiang X, Yuan Y, Yang R. Bioorthogonal pretargeting strategy for anchoring activatable photosensitizers on plasma membranes for effective photodynamic therapy. ACS Appl Mater Interfaces 2021;13:14004–14.
[218] Qin H, Zhao R, Qin Y, Zhu J, Chen L, Di C, Han X, Cheng K, Zhang Y, Zhao Y. Development of a cancer vaccine using *in vivo* click-chemistry-mediated active lymph node accumulation for improved immunotherapy. Adv Mater 2021;33:2006007.
[219] Wang X, Lang S, Tian Y, Zhang J, Yan X, Fang Z, Weng J, Lu N, Wu X, Li T, Cao H, Li Z, Huang X. Glycoengineering of natural killer cells with CD22 ligands for enhanced anticancer immunotherapy. ACS Cent Sci 2020;6:382–9.
[220] Vendeuvre T, Renoux B, Clarhaut J, Papot S. Cell-cell interactions via non-covalent click chemistry. Chem Sci 2021;12:9017–21.
[221] Zhou A, Du J, Jiao M, Xie D, Wang Q, Xue L, Ju C, Hua Z, Zhang C. Co-delivery of TRAIL and siHSP70 using hierarchically modular assembly formulations achieves enhanced TRAIL-resistant cancer therapy. J Control Release 2019;304:111–24.
[222] Yazdi MK, Zarrintaj P, Khodadadi A, Ganjali MR, Bagheri B, Habibzadeh S, Saeb MR, Mozafari M. Magnetic nanoparticles in cancer therapy. In: Ehrmann A, Nguyen TA, Ahmadi M, Farmani A, Nguyen-Tri P, editors. Magnetic nanoparticle-based hybrid materials. Woodhead Publishing; 2021. p. 425–45.
[223] Huang K, He Y, Zhu Z, Guo J, Wang G, Deng C, Zhong Z, Small T. Endosome-disrupting, and bioresponsive click nanogels fabricated via microfluidics for CD44-targeted cytoplasmic delivery of therapeutic proteins. ACS Appl Mater Interfaces 2019;11:22171–80.
[224] Roth A, Murschel F, Latreille PL, Martinez VA, Liberelle B, Banquy X, De Crescenzo G. Coiled coil affinity-based systems for the controlled release of biofunctionalized gold nanoparticles from alginate hydrogels. Biomacromolecules 2019;20:1926–36.
[225] Cong Y, Ji L, Gao YJ, Liu FH, Cheng DB, Hu Z, Qiao ZY, Wang H. Microenvironment-induced *in situ* self-assembly of polymer–peptide conjugates that attack solid tumors deeply. Angew Chem Int Ed 2019;131:4680–5.
[226] Cheng Z, Cheng Y, Chen Q, Li M, Wang J, Liu H, Li M, Ning Y, Yu Z, Wang Y. Self-assembly of pentapeptides into morphology-adaptable nanomedicines for enhanced combinatorial chemo-photodynamic therapy. Nano Today 2020;33:100878.
[227] Low LE, Wu J, Lee J, Tey BT, Goh BH, Gao J, Li F, Ling D. Tumor-responsive dynamic nanoassemblies for targeted imaging, therapy and microenvironment manipulation. J Control Release 2020;324:69–103.
[228] Zhang XH, Cheng DB, Ji L, An HW, Wang D, Yang ZX, Chen H, Qiao ZY, Wang H. Photothermal-promoted morphology transformation *in vivo* monitored by photoacoustic imaging. Nano Lett 2020;20:1286–95.
[229] Feng Q, Wang J, Song H, Zhuo L-g, Wang G, Liao W, Feng Y, Wei H, Chen Y, Yang Y. Uptake and light-induced cytotoxicity of hyaluronic acid–grafted liposomes containing porphyrin in tumor cells. J Drug Deliv Sci Technol 2018;47:137–43.
[230] Garcia-Astrain C, Averous L. Synthesis and evaluation of functional alginate hydrogels based on click chemistry for drug delivery applications. Carbohydr Polym 2018;190:271–80.
[231] Lee HJ, Fernandes-Cunha GM, Myung D. *In situ*-forming hyaluronic acid hydrogel through visible light-induced thiol-ene reaction. React Funct Polym 2018;131:29–35.
[232] Chen J, Zou Y, Deng C, Meng F, Zhang J, Zhong Z. Multifunctional click hyaluronic acid nanogels for targeted protein delivery and effective cancer treatment *in vivo*. Chem Mater 2016;28:8792–9.
[233] Chen J, Huang K, Chen Q, Deng C, Zhang J, Zhong Z. Tailor-making fluorescent hyaluronic acid microgels via combining microfluidics and photoclick chemistry for sustained and localized delivery of herceptin in tumors. ACS Appl Mater Interfaces 2018;10:3929–37.
[234] Ma T, Gao X, Dong H, He H, Cao X. High-throughput generation of hyaluronic acid microgels via microfluidics-assisted enzymatic crosslinking and/or Diels–Alder click chemistry for cell encapsulation and delivery. Appl Mater Today 2017;9:49–59.
[235] Ossipov DA, Romero AB, Ossipova E. Light-activatable prodrugs based on hyaluronic acid biomaterials. Carbohydr Polym 2018;180:145–55.
[236] Selegard R, Aronsson C, Brommesson C, Danmark S, Aili D. Folding driven self-assembly of a stimuli-responsive peptide-hyaluronan hybrid hydrogel. Sci Rep 2017;7:7013.
[237] Manzi G, Zoratto N, Matano S, Sabia R, Villani C, Coviello T, et al. "Click" hyaluronan based nanohydrogels as multifunctionalizable carriers for hydrophobic drugs. Carbohydr Polym 2017;174:706–15.
[238] Breitenbach BB, Schmid I, Wich PR. Amphiphilic polysaccharide block copolymers for pH-responsive micellar nanoparticles. Biomacromolecules 2017;18:2839–48.
[239] Soiberman U, Kambhampati SP, Wu T, Mishra MK, Oh Y, Sharma R, Wang J, Al Towerki AE, Yiu S, Stark WJ, Kannan RM. Subconjunctival injectable dendrimer-dexamethasone gel for the treatment of corneal inflammation. Biomaterials 2017;125:38–53.
[240] Kim HY, Li F, Park JY, Kim D, Park JH, Han HS, Byun JW, Lee YS, Jeong JM, Char K. *In vivo* visualization of endogenous miR-21 using hyaluronic acid-coated graphene oxide for targeted cancer therapy. Biomaterials 2017;121:144–54.
[241] Zhang YS, Khademhosseini A. Advances in engineering hydrogels. Science 2017;356:eaaf3627.
[242] Gaharwar AK, Singh I, Khademhosseini A. Engineered biomaterials for *in situ* tissue regeneration. Nat Rev Mater 2020;5:686–705.
[243] Silva CR, Babo PS, Gulino M, Costa L, Oliveira JM, Silva-Correia J, Domingues RMA, Reis RL, Gomes ME. Injectable and tunable hyaluronic acid hydrogels releasing chemotactic and angiogenic growth factors for endodontic regeneration. Acta Biomater 2018;77:155–71.
[244] Alarcin E, Lee TY, Karuthedom S, Mohammadi M, Brennan MA, Lee DH, Marrella A, Zhang J, Syla D, Zhang YS, Khademhosseini A, Jang HL. Injectable shear-thinning hydrogels for delivering osteogenic and angiogenic cells and growth factors. Biomater Sci 2018;6:1604–15.
[245] Seliktar D. Designing cell-compatible hydrogels for biomedical applications. Science 2012;336:1124–8.
[246] Nhavene EPF, da Silva WM, Junior RRT, Gastelois PL, Venâncio T, Nascimento R, Batista RJC, Machado CR, de Almeida Macedo WA, de Sousa EMB. Chitosan grafted into mesoporous silica nanoparticles as benznidazol carrier for Chagas diseases treatment. Microporous Mesoporous Mater 2018;272:265–75.
[247] Park SH, Seo JY, Park JY, Ji YB, Kim K, Choi HS, Choi S, Kim JH, Min BH, Kim MS. An injectable, click-crosslinked, cytomodulin-modified hyaluronic acid hydrogel for cartilage tissue engineering. NPG Asia Mater 2019;11:1–16.
[248] Hu X, Gao Z, Tan H, Wang H, Mao X, Pang J. An injectable hyaluronic acid-based composite hydrogel by DA click chemistry with pH sensitive nanoparticle for biomedical application. Front Chem 2019;7:477.
[249] Falanga A, Laheurte P, Vahabi H, Tran N, Khamseh S, Saeidi H, Khodadadi M, Zarrintaj P, Saeb MR, Mozafari M. Niobium-treated titanium implants with improved cellular and molecular activities at the tissue–implant interface. Materials 2019;12:3861.
[250] Barbosa M, Costa F, Monteiro C, Duarte F, Martins MCL, Gomes P. Antimicrobial coatings prepared from Dhvar-5-click-grafted chitosan powders. Acta Biomater 2019;84:242–56.
[251] Sorensen OE, Borregaard N, Cole AM. Antimicrobial peptides in innate immune responses. Contrib Microbiol 2008;15:61–77.
[252] Walker LR, Marty MT. Revealing the specificity of a range of antimicrobial peptides in lipid nanodiscs by native mass spectrometry. Biochemistry 2020;59:2135–42.
[253] Stallmann HP, Faber C, Bronckers AL, de Blieck-Hogervorst JM, Brouwer CP, Amerongen AV, Wuisman PI. Histatin and lactoferrin derived peptides: antimicrobial properties and effects on mammalian cells. Peptides 2005;26:2355–9.
[254] Barbosa M, Vale N, Costa FM, Martins MC, Gomes P. Tethering antimicrobial peptides onto chitosan: Optimization of azide-alkyne "click" reaction conditions. Carbohydr Polym 2017;165:384–93.
[255] Lutolf M, Hubbell J. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 2005;23:47–55.
[256] Magno V, Meinhardt A, Werner C. Polymer hydrogels to guide organotypic and organoid cultures. Adv Funct Mater 2020;30:2000097.
[257] Kühn S, Sievers J, Stoppa A, Träber N, Zimmermann R, Welzel PB, Werner C. Cell-instructive multiphasic Gel-in-Gel materials. Adv Funct Mater 2020;30:1908857.
[258] Fan M, Ma Y, Mao J, Zhang Z, Tan H. Cytocompatible *in situ* forming chitosan/hyaluronan hydrogels via a metal-free click chemistry for soft tissue engineering. Acta Biomater 2015;20:60–8.
[259] Smith LJ, Taimoory SM, Tam RY, Baker AEG, Binth Mohammad N, Trant JF, Shoichet MS. Diels-alder click-cross-linked hydrogels with increased reactivity enable 3D cell encapsulation. Biomacromolecules 2018;19:926–35.
[260] Nilforoushzadeh MA, Khodadadi Yazdi M, Baradaran Ghavami S, Farokhimanesh S, Mohammadi Amirabad L, Zarrintaj P, Saeb MR, Hamblin MR, Zare M, Mozafari M. Mesenchymal stem cell spheroids embedded in an injectable thermosensitive hydrogel: an *in situ* drug formation platform for accelerated wound healing. ACS Biomater Sci Eng 2020;6:5096–109.
[261] Shang Y, Liang W, Tan B, Xiao M, Zou Y, Liu W, Wang W. A conductive and

biodegradable hydrogel for minimally delivering adipose-derived stem cells. Sci China Technol Sci 2019;62:1747–54.
[262] Yazdi MK, Zarrintaj P, Khodadadi A, Arefi A, Seidi F, Shokrani H, et al. Polysaccharide-based electroconductive hydrogels: Structure, properties and applications in biomedical engineering. Carbohydr Polym 2021;278:118998.
[263] Yazdi MK, Motlagh GH, Garakani SS, Boroomand A. Effects of multiwall carbon nanotubes on the polymerization model of aniline. J Polym Res 2018;25:1–15.
[264] Zarrintaj P, Yazdi MK, Jouyandeh M, Saeb MR. PANI-based nanostructures. In: Mozafari M, Chauhan NPS, editors. Fundamentals and emerging applications of polyaniline. Elsevier; 2019. p. 121–30.
[265] Yazdi MK, Saeidi H, Zarrintaj P, Saeb MR, Mozafari M. PANI-CNT nanocomposites. In: Mozafari M, Chauhan NPS, editors. Fundamentals and emerging applications of polyaniline. Elsevier; 2019. p. 143–63.
[266] Yazdi MK, Noorbakhsh B, Nazari B, Ranjbar Z. Preparation and EMI shielding performance of epoxy/non-metallic conductive fillers nano-composites. Prog Org Coat 2020;145:105674.
[267] Aghazadeh H, Yazdi MK, Kolahi A, Yekani M, Zarrintaj P, Ramsey JD, Ganjali MR, Stadler FJ, Saeb MR, Mozafari M. Synthesis, characterization and performance enhancement of dry polyaniline-coated neuroelectrodes for electroencephalography measurement. Curr Appl Phys 2021;27:43–50.
[268] Khodadadi Yazdi M, Hashemi Motlagh G. Synthesis, characterization, and thermal aging behavior of HCl-doped polyaniline/TRGO nanocomposites. J Appl Polym Sci 2017;134:44635–44.
[269] Zarrintaj P, Bakhshandeh B, Saeb MR, Sefat F, Rezaeian I, Ganjali MR, Ramakrishna S, Mozafari M. Oligoaniline-based conductive biomaterials for tissue engineering. Acta Biomater 2018;72:16–34.
[270] Yu F, Cao X, Zeng L, Zhang Q, Chen X. An interpenetrating HA/G/CS biomimic hydrogel via Diels-Alder click chemistry for cartilage tissue engineering. Carbohydr Polym 2013;97:188–95.
[271] Zhang Y, Chen H, Zhang T, Zan Y, Ni T, Liu M, Pei R. Fast-forming BMSC-encapsulating hydrogels through bioorthogonal reaction for osteogenic differentiation. Biomater Sci 2018;6:2578–81.
[272] Wang G, Cao X, Dong H, Zeng L, Yu C, Chen X. A hyaluronic acid based injectable hydrogel formed via photo-crosslinking reaction and thermal-induced diels-alder reaction for cartilage tissue engineering. Polymers 2018;10:949 (Basel).
[273] Yu F, Cao X, Du J, Wang G, Chen X. Multifunctional hydrogel with good structure integrity, self-healing, and tissue-adhesive property formed by combining diels-alder click reaction and acylhydrazone bond. ACS Appl Mater Interfaces 2015;7:24023–31.
[274] Menegatti S, Ruocco N, Kumar S, Zakrewsky M, Sanchez De Oliveira J, Helgeson ME, Leal GL, Mitragotri S. Synthesis and characterization of a self-fluorescent hyaluronic acid-based gel for dermal applications. Adv Healthc Mater 2015;4:2297–305.
[275] Hardy JG, Lin P, Schmidt CE. Biodegradable hydrogels composed of oxime crosslinked poly(ethylene glycol), hyaluronic acid and collagen: a tunable platform for soft tissue engineering. J Biomater Sci Polym Ed 2015;26:143–61.
[276] Führmann T, Obermeyer J, Tator C, Shoichet M. Click-crosslinked injectable hyaluronic acid hydrogel is safe and biocompatible in the intrathecal space for ultimate use in regenerative strategies of the injured spinal cord. Methods 2015;84:60–9.
[277] Truong VX, Ablett MP, Gilbert HTJ, Bowen J, Richardson SM, Hoyland JA, Dove AP. *In situ*-forming robust chitosan-poly(ethylene glycol) hydrogels prepared by copper-free azide-alkyne click reaction for tissue engineering. Biomater Sci 2014;2:167–75.
[278] Jain M, Rajan R, Hyon SH, Matsumura K. Hydrogelation of dextran-based polyampholytes with cryoprotective properties via click chemistry. Biomater Sci 2014;2:308–17.
[279] Nimmo CM, Owen SC, Shoichet MS. Diels-Alder Click cross-linked hyaluronic acid hydrogels for tissue engineering. Biomacromolecules 2011;12:824–30.
[280] Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008;453:314–21.
[281] Ghobril C, Grinstaff MW. The chemistry and engineering of polymeric hydrogel adhesives for wound closure: a tutorial. Chem Soc Rev 2015;44:1820–35.
[282] Chouhan D, Dey N, Bhardwaj N, Mandal BB. Emerging and innovative approaches for wound healing and skin regeneration: current status and advances. Biomaterials 2019;216:119267.
[283] Yildirimer L, Thanh NT, Seifalian AM. Skin regeneration scaffolds: a multimodal bottom-up approach. Trends Biotechnol 2012;30:638–48.
[284] He Y, Li Y, Sun Y, Zhao S, Feng M, Xu G, Zhu H, Ji P, Mao H, He Y, Gu Z. A double-network polysaccharide-based composite hydrogel for skin wound healing. Carbohydr Polym 2021;261:117870.
[285] Price RD, Myers S, Leigh IM, Navsaria HA. The role of hyaluronic acid in wound healing: assessment of clinical evidence. Am J Clin Dermatol 2005;6:393–402.
[286] Shi L, Zhao Y, Xie Q, Fan C, Hilborn J, Dai J, Ossipov DA. Moldable hyaluronan hydrogel enabled by dynamic metal-bisphosphonate coordination chemistry for wound healing. Adv Healthc Mater 2018;7:1700973.
[287] Jin X, Shang Y, Zou Y, Xiao M, Huang H, Zhu S, Liu N, Li J, Wang W, Zhu P. Injectable hypoxia-induced conductive hydrogel to promote diabetic wound healing. ACS Appl Mater Interfaces 2020;12:56681–91.
[288] Park KM, Gerecht S. Hypoxia-inducible hydrogels. Nat Commun 2014;5:1–12.
[289] Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: good and evil. Genes Cancer 2011;2:1117–33.
[290] Hong WX, Hu MS, Esquivel M, Liang GY, Rennert RC, McArdle A, Paik KJ, Duscher D, Gurtner GC, Lorenz HP, Longaker MT. The role of hypoxia-inducible factor in wound healing. Adv Wound Care (New Rochelle) 2014;3:390–9.
[291] Jayaprakash P, Dong H, Zou M, Bhatia A, O'Brien K, Chen M, Woodley DT, Li W. Hsp90alpha and Hsp90beta together operate a hypoxia and nutrient paucity stress-response mechanism during wound healing. J Cell Sci 2015;128:1475–80.
[292] Lee EY, Xia Y, Kim WS, Kim MH, Kim TH, Kim KJ, Park BS, Sung JH. Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF. Wound Repair Regen 2009;17:540–7.
[293] Cho SH, Raybuck AL, Blagih J, Kemboi E, Haase VH, Jones RG, Boothby MR. Hypoxia-inducible factors in CD4(+) T cells promote metabolism, switch cytokine secretion, and T cell help in humoral immunity. Proc Natl Acad Sci USA 2019;116:8975–84.
[294] McBrearty BA, Clark LD, Zhang XM, Blankenhorn EP, Heber-Katz E. Genetic analysis of a mammalian wound-healing trait. Proc Natl Acad Sci USA 1998;95:11792–7.
[295] McMahon S, Kennedy R, Duffy P, Vasquez JM, Wall JG, Tai H, Wang W. Poly (ethylene glycol)-based hyperbranched polymer from RAFT and its application as a silver-sulfadiazine-loaded antibacterial hydrogel in wound care. ACS Appl Mater Interfaces 2016;8:26648–56.
[296] Valot L, Martinez J, Mehdi A, Subra G. Chemical insights into bioinks for 3D printing. Chem Soc Rev 2019;48:4049–86.
[297] GhavamiNejad A, Ashammakhi N, Wu XY, Khademhosseini A. Crosslinking strategies for 3D bioprinting of polymeric hydrogels. Small 2020;16:e2002931.
[298] Hahn L, Beudert M, Gutmann M, Keßler L, Stahlhut P, Fischer L, Lorson T, Thievessen I, Lühmann T, Luxenhofer R. Biomechanical and biological performances of Diels-Alder crosslinked thermogelling bioink. Gels 2021;7:78.
[299] Gopinathan J, Noh I. Click chemistry-based injectable hydrogels and bioprinting inks for tissue engineering applications. Tissue Eng Regen Med 2018;15:531–46.
[300] Unagolla JM, Jayasuriya AC. Hydrogel-based 3D bioprinting: A comprehensive review on cell-laden hydrogels, bioink formulations, and future perspectives. Appl Mater Today 2020;18:100479.
[301] Petta D, D'Amora U, Ambrosio L, Grijpma D, Eglin D, D'Este M. Hyaluronic acid as a bioink for extrusion-based 3D printing. Biofabrication 2020;12:032001.
[302] Wang LL, Highley CB, Yeh YC, Galarraga JH, Uman S, Burdick JA. Three-dimensional extrusion bioprinting of single- and double-network hydrogels containing dynamic covalent crosslinks. J Biomed Mater Res A 2018;106:865–75.
[303] Skardal A, Zhang J, Prestwich GD. Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates. Biomaterials 2010;31:6173–81.
[304] Lee M, Rizzo R, Surman F, Zenobi-Wong M. Guiding lights: tissue bioprinting using photoactivated materials. Chem Rev 2020;120:10950–1027.
[305] Mueller E, Poulin I, Bodnaryk WJ, Hoare T. Click chemistry hydrogels for extrusion bioprinting: progress, challenges, and opportunities. Biomacromolecules 2022;23:619–40.
[306] Levré D, Arcisto C, Mercalli V, Massarotti A. ZINClick v.18: expanding chemical space of 1, 2, 3-triazoles. J Chem Inf Model 2018;59:1697–702.
[307] Meng G, Guo T, Ma T, Zhang J, Shen Y, Sharpless KB, Dong J. Modular click chemistry libraries for functional screens using a diazotizing reagent. Nature 2019;574:86–9.
[308] Han SS, Shim HE, Park SJ, Kim BC, Lee DE, Chung HM, Moon SH, Kang SW. Safety and optimization of metabolic labeling of endothelial progenitor cells for tracking. Sci Rep 2018;8:1–13.
[309] McNulty J, Nair JJ, Vurgun N, Difrancesco BR, Brown CE, Tsoi B, Crankshaw DJ, Holloway AC. Discovery of a novel class of aldol-derived 1,2,3-triazoles: potent and selective inhibitors of human cytochrome P450 19A1 (aromatase). Bioorg Med Chem Lett 2012;22:718–22.
[310] Tromblee C. Shasqi presents initial data validating approach of click chemistry activated oncology therapeutics at ESMO Congress 2021. 2021.